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Research Centre for Ecosystem Resilience

# Resilient trees, resilient woodlands: Box Gum Woodland Tree Knowledge Infrastructure

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# Resilient trees, resilient woodlands: Box Gum Woodland Tree Knowledge Infrastructure

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## Executive summary

This report provides the findings and guidelines arising from the *Resilient trees, resilient woodlands: Box Gum Woodland Tree Knowledge Infrastructure* project, a collaboration between Research Centre of Ecosystem Resilience (ReCER), Botanic Gardens of Sydney and Taronga Conservation Society Australia. Restoration of koala habitat is a key tool in ensuring the long-term survival of koalas in the wild, especially in the face of a rapidly changing climate. Genetic information can help ensure material used in habitat restoration is of appropriate provenance and sufficiently genetically diverse. Without genetic guidance, practitioners are limited to unreliable generalisations that may underestimate appropriate seed collection areas and may lead to genetically depauperate restored populations with reduced fitness & low adaptability. These guidelines provide advice on how to source genetically diverse climate-ready seed for *Eucalyptus* species that are associated with the critically endangered Box-Gum Grassy Woodland ecological community and will help ensure the long-term self-sustainability of Koala habitat restoration being undertaken by the Taronga Conservation Society Australia.

Eleven dominant and widespread woodland eucalypt species, suitable for use in restoration of Box-Gum Grassy Woodland in the Northern Tablelands of New South Wales, were sampled from across their distribution in New South Wales. 1812 leaf samples were collected from across 332 sites. DNA extraction and DArT sequencing was undertaken for these and outgroup samples. Taxonomic and distributional boundaries were tested, and population genetic analyses undertaken. Future climate matched locations were identified and combined with genetic data to determine an optimised seed sourcing strategy for each of the 11 target eucalypts that captures high levels of genetic diversity and incorporates severe climate readiness.

The genetic analyses proved a powerful tool for highlighting and resolving taxonomic and distributional uncertainty for several target woodland species, revealing that not all target taxa may be suitable for planting at the target restoration site and that careful attention need to be paid to refined species boundaries and distributions when planning seed collection for use across the Northern Tablelands.

Population genetic analyses revealed that for all species investigated, gene flow has historically been largely continuous across the tableland and inland slope regions of NSW. While consistent evolutionary patterns are seen across the target species, this is the result of this study being limited to ecologically dominant eucalypts, which share similar patterns of gene flow and genetic diversity levels across species and cannot be generalised to other taxonomic

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groups which occur in the Box-Gum Grassy Woodlands. Only the Hunter Valley proved to be a major barrier to geneflow for four of the eleven species, and therefore the resultant evolutionary divergence needs to be considered when moving seed across this region for use in restoration. In line with previous restoration genomics studies in eastern Australian woodland plants, there was no signal of local adaptation detected using the neutral genetic markers, with geographic distance being the key driver of genetic patterns. Therefore, targeted mixing of seed from multiple sources from both local to a restoration site and from the wider region (including climate ready material) is recommended for all species. This strategy will minimise any negative genetic consequences and will increase the accessibility and genetic diversity of seed. Furthermore, while not something we tested directly in this project, it has been established that a relationship exists between source population size and genetic diversity, meaning that seed should be sourced from the largest, most intact populations possible.

Ten key steps for sourcing genetically diverse climate-ready seed for use in restoration of Box-Gum Grassy Woodland are discussed, including that the number of mother trees seed are collected from (maternal lines) be maximised and that seed from each maternal line be tracked and kept separate throughout the entire process of seed collection to restoration planting. It is also important to avoid sourcing seed from small, planted or hybridising populations. The ideal spatial distribution of seed source sites and mother plant are outlined, including that seed source sites be separated by at least 20 km and mother trees within sites should be at least 100 m apart. A planting block design that maximises the chance of establishing genetically healthy and self-sustaining restored populations is presented.

Detailed findings regarding these species' evolutionary genetics and reproductive biology, and species-specific guidelines for optimised seed collections are provided for all 11 target species. Eight species for which we present specific guidelines here have been added to the publicly available Restore and Renew webtool, allowing restoration practitioners to identify appropriate seed sourcing regions for specific restoration projects not only within Box-Gum Grassy Woodlands, but all vegetation communities in which the target species occur. The genetic datasets for three remaining species were not appropriate for the model employed by the webtool and so they could not be deployed there at this time (but will be in an upgrade of the webtool that is currently being developed).

The insight gained from this research can benefit not only the restoration of eucalypts for Box-Gum Grassy Woodland on the Northern Tablelands, but eucalypt restoration in general, thereby improving the effectiveness of on-ground Koala conservation action across NSW and beyond. We finish by highlighting the value of raising awareness regarding how genomic research can improve restoration of eucalypts and identify two key areas for future research (i) develop seed sourcing guidelines for other Box-Gum Grassy Woodland species, and (ii) Evaluate and monitor feasibility and effectiveness of recommendations.

## Acknowledgements

We acknowledge the Traditional Custodians of the land on which the plant species in this study are found on and pay respects to Elders past and present. We acknowledge the many collectors who facilitated the sampling of plant material for this project, particularly Daniel Clarke (Arcane Botanica Pty Ltd) and Stringybark Ecological Pty Ltd. We acknowledge those who have provided technical assistance in adding the species targeted in this study to the Restore and Renew Webtool, Dr Jason Bragg (BGS) and Miranda Jordan (BGS). We acknowledge Dr Mitchell O'Brien and Eilish McMaster for assistance in the development of scripts used for genetic analysis within this study.

## Key terms

**Adaptability/Adaptive potential:** The ability of a population to survive and reproduce under changing environmental conditions.

**Allele:** Alternate versions of DNA sequence at a given location within the genome.

**Allelic diversity:** The number of alleles present within a population of a species. One measure of genetic diversity (see below).

**Climate-ready:** Provenances that come from areas where current climatic conditions match those predicted to occur in a target area at a future point under a particular model of climatic change.

**Ecological restoration:** Assisting ecosystems that have been disturbed or cleared to restore ecological function.

**Gene flow:** The movement of genetic material such as differing alleles (see above) between individuals or a group of individuals by processes such as dispersal of seed and pollen.

**Genetic bottleneck:** A sharp reduction in genetic diversity caused by a reduction in population size or by sampling of seed from too few individuals.

**Genetic distinctiveness:** How different the genetic diversity of a population is compared to the genetic diversity in other populations of the same species.

**Genetic diversity:** The totality of genetic variation present in a population and a determinant of adaptability and adaptive potential.

**Genetic lineage:** a set of individuals or populations connected by a continuous line of descent from a shared ancestor.

**Genetic neighbourhood:** An area within the distribution of a species where there are no restrictions to gene flow other than the distance between individuals, such that alleles can be shared between all individuals within the area given sufficient time.

**Genetic structure:** The amount and distribution of genetic variation within and between populations across the landscape.

**Genetic swamping:** Loss of rare alleles as a result of repeated crossing with individuals from a different genetic neighbourhood of the same species or a different species via hybridisation.

**Heterozygosity:** The average proportion of DNA sites in the genome where the two copies (or alleles) are different across a sample of individuals. Heterozygous refers to having two different copies of DNA at a particular location within the genome, as opposed to homozygous (see below) where the two copies at a location are the same.

**Homozygous:** Having two identical copies of DNA at a particular location within the genome, as opposed to heterozygous where the two copies at a location are different. See above.

**Inbreeding:** The mating of individuals that are genetically closely related within a population leading to the production of progeny.

**Inbreeding depression:** a decrease in fitness of progeny from closely related parents due to an increase in homozygosity with the genome.

**Isolation by distance:** The term used to describe the change of shared genetic material across geographic space due to dispersal ability limiting the mating between individuals. Importantly this often does not lead to discrete population structure and local adaptation if populations are continuous across the landscape.

**Maternal line:** All offspring from the same mother plant.

**Maintenance of maternal lines:** The practice of keeping seed or other material collected from a particular mother plant identifiable and separate from that of other mother plants through the process of collection through to propagation and planting. Upon planting, maternal lines should be traceable to individuals, but should be interplanted to maximise interbreeding and mixing of genetic diversity in progeny.

**Mother plant:** An individual plant from which seed has been collected.

**Outbreeding:** The mating of individuals that are not genetically closely related leading to the production of progeny.

**Outbreeding depression:** A reduction of fitness of offspring resulting from the interbreeding of parents that are highly genetically distinct.

**Population:** A group of individual plants growing in the same place at the same time and interbreeding freely.

**Private alleles:** Alleles observed only in a single population, thus being private to that population.

**Progeny:** Offspring of a plant, typically seed.

**Provenance:** A source area for propagules (seed, cuttings etc.) used in restoration activities.

**Seed Production Area:** Areas where native plants of known provenance are planted to harvest seed for restoration or other purposes.

**Site:** a specific locality where genetic samples or seeds have been collected.

**Sustainability/Self-sustaining:** The ability of a planting to survive and reproduce in the long-term with minimal further active investment.

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# 1. Introduction

## 1.1 Context

One of the key threatening processes for native flora and fauna in New South Wales is the clearing and fragmentation of habitat (Environment Protection Authority 2021), with grassy woodlands being one of the community types that has seen the most extensive losses (Prober and Thiele 2005). Koalas (*Phascolarctos cinereus*) were once widespread throughout the grassy woodlands of NSW, but with land use change are now absent from much of the area previously covered by these vegetation communities (Kjeldsen *et al.* 2019). Restoration of grassy woodlands is therefore a key tool in ensuring the future survival of Koalas.

Taronga Conservation Society aims to undertake large scale ecological restoration of grassy woodland habitats in New South Wales to connect fragmented patches, provide high quality habitat for Koalas and capture carbon in natural ecosystems. To that end ReCER was asked to provide guidance on undertaking genetically informed restoration of dominant woodland eucalypt species to ensure plantings establish self-sustaining populations that include the adaptive potential to survive under changing climate regimes. This report delivers the findings of ReCER's genetic studies on a suite of target *Eucalyptus* species along with the recommendations on how to source seed and undertake plantings to help Taronga achieve their objectives. Historically, the most widespread and extensive grassy woodland plant community type was the Box-Gum Grassy Woodlands (BGGW), which occur primarily on flat terrain with moderate to high fertility soils. This plant community was primarily cleared to make way for agriculture on the productive soils it was linked to, with narrower, but still significant, impacts from mining and urban development, leading to its listing as a threatened ecological community (Beeton 2006; Department of Environment, Climate Change and Water 2010). The North-West Slopes and Northern Tablelands regions of northern NSW are home to both several areas of regional Koala significance (Rennison and Fisher 2018) and the most extensive remnants of BGGW (Department of Environment, Climate Change and Water 2010), therefore ecological restoration of the BGGW in this region has high potential to increase habitat connectivity and long-term survival of the flora and fauna (including the Koala) of the BGGW. As the dominant tree taxa and the only food source for the Koala, eucalypt species are an important target for ecological restoration of the BGGW.

## 1.2 Eucalypts of Box-Gum Grassy Woodlands

The BGGW are characterised by the combination of four dominant eucalypt species: *Eucalyptus albens* (White Box), *E. blakelyi* (Blakely's Red Gum), *E. melliodora* (Yellow Box), and *E. moluccana* (Grey Box; dominant in the Nandewar bioregion only). All four of these dominant species are considered high preferred use tree species for the Koala populations of the North-West Slopes and Northern Tablelands regions (Hawes *et al.* 2016; Office of Environment and Heritage 2018) and are therefore key target species for ecological restoration. Other eucalypt species acknowledged in the BGGW community definition (Department of Climate Change, Energy, the Environment and Water 2023) that occur in the wider landscape matrix and should also be restored alongside the key BGGW species include:

- *Angophora floribunda* (Rough Barked Apple)
- *Eucalyptus bridgesiana* (Apple Box)
- *Eucalyptus caliginosa* (New England Stringybark)
- *Eucalyptus cinerea* (Argyle Apple)
- *Eucalyptus conica* (Fuzzy Box)
- *Eucalyptus goniocalyx* (Long-leaved Box, Bundy)
- *Eucalyptus macrorhyncha* (Red Stringybark)
- *Eucalyptus mannifera* (Brittle Gum)
- *Eucalyptus microcarpa* (Western Grey Box)
- *Eucalyptus nortonii* (Long-leaved Box)
- *Eucalyptus polyanthemos* (Red Box)
- *Eucalyptus rubida* (Candlebark)
- *Eucalyptus woollsiana* (Narrow-leaved Grey Box)
- *Eucalyptus youmanii* (Youman's Stringybark)

Building both knowledge and tools for ecological restoration of these eucalypts has the potential to act as a cost-reduction and success enhancing measure for the future. To do this, targeted study of the species ecology, evolution and reproductive biology is needed, and one of the most powerful techniques at our disposal to undertake such studies is restoration genetics.

## 1.3 Relevance of genetic information to ecological restoration

One of the most important steps in any successful ecological restoration project is sourcing the correct material for planting (Harrison *et al.* 2021), particularly when it comes to restoring vegetation in heavily cleared and highly fragmented landscapes where source locations may be limited. In the past, broad generalisations have dominated in the literature and practice surrounding propagule sourcing for ecological restoration. However, as knowledge and tools available have improved, and the impacts of climate change become even more pressing, there has been a shift towards more tailored advice and practices, often at the species-specific level. Restoration genetics allows for the development of species-specific propagule sourcing strategies based upon patterns of genetic diversity and divergence across a species distribution. This has led to the realisation that such patterns are often highly variable between species, even when they occur in very similar environmental conditions, only reinforcing the need for species-specific knowledge (Fahey *et al.* In Press).

The level of genetic diversity in restored populations can have major impact on the progress of the population to a self-sustaining state (i.e., successful recruitment of future generations), even if restoration appears to be successful in the short term as measured by survival and growth rates (Figure 1a). Failure to include sufficient levels of genetic diversity in a restored population can lead to multiple negative effects including inbreeding depression, a reduction in overall population fitness due to increased genome-wide homozygosity (and hence increased impact of deleterious recessive alleles), and a lack of adaptive potential and adaptability in the face of both stochastic events (fires, disease etc.) and environmental change (i.e. changing temperature and rainfall patterns due to climate change).

Ensuring high levels of genetic diversity is especially relevant to eucalypt restoration. Eucalypt populations are renowned for their consistently high levels of genetic diversity and low levels of inbreeding (Costa e Silva *et al.* 2010; Costa e Silva *et al.* 2011; Griffin *et al.* 2019; Nickolas *et al.* 2019), a pattern observed in all common eucalypt species investigated to date (rare or range restricted species often show different patterns). This has been shown to be due to a high post-zygotic (i.e., at the germination and seedling stages of the life cycle) fitness cost associated with inbreeding and self-pollination, a typical inbreeding depression pattern. However, both inbred and self-pollinated individuals are observed in seed and seedling studies of eucalypts at rates far higher than observed in mature natural populations. The absence of these inbred individuals among adult plants in natural populations suggests genetically diverse outcrossed trees have better survival and reproduction rates than inbred trees. This means that seed sourcing is very impactful on the performance and success of restored populations, as if sufficient genetic diversity and unrelated individuals are not present, even if the planted generation performs well, the populations may fail to reach a self-sustaining state due to inbreeding depression. To avoid this, the aim of ecological restoration of eucalypts needs to be to recreate the patterns observed in natural populations: high genetic diversity and low relatedness between individuals in the population.

Genetic structuring, or the patterns of genetic relatedness between populations of a species across its distribution, varies among species and primarily reflects evolutionary history. The extent of genetic structuring among populations is an important consideration when designing propagule sourcing strategies as outbreeding depression (i.e., loss of fitness due to high level of genetic divergence between parents) may occur when different genetic lineages interbreed, and different lineages may be differentially adapted to environmental conditions (Figure 1b).

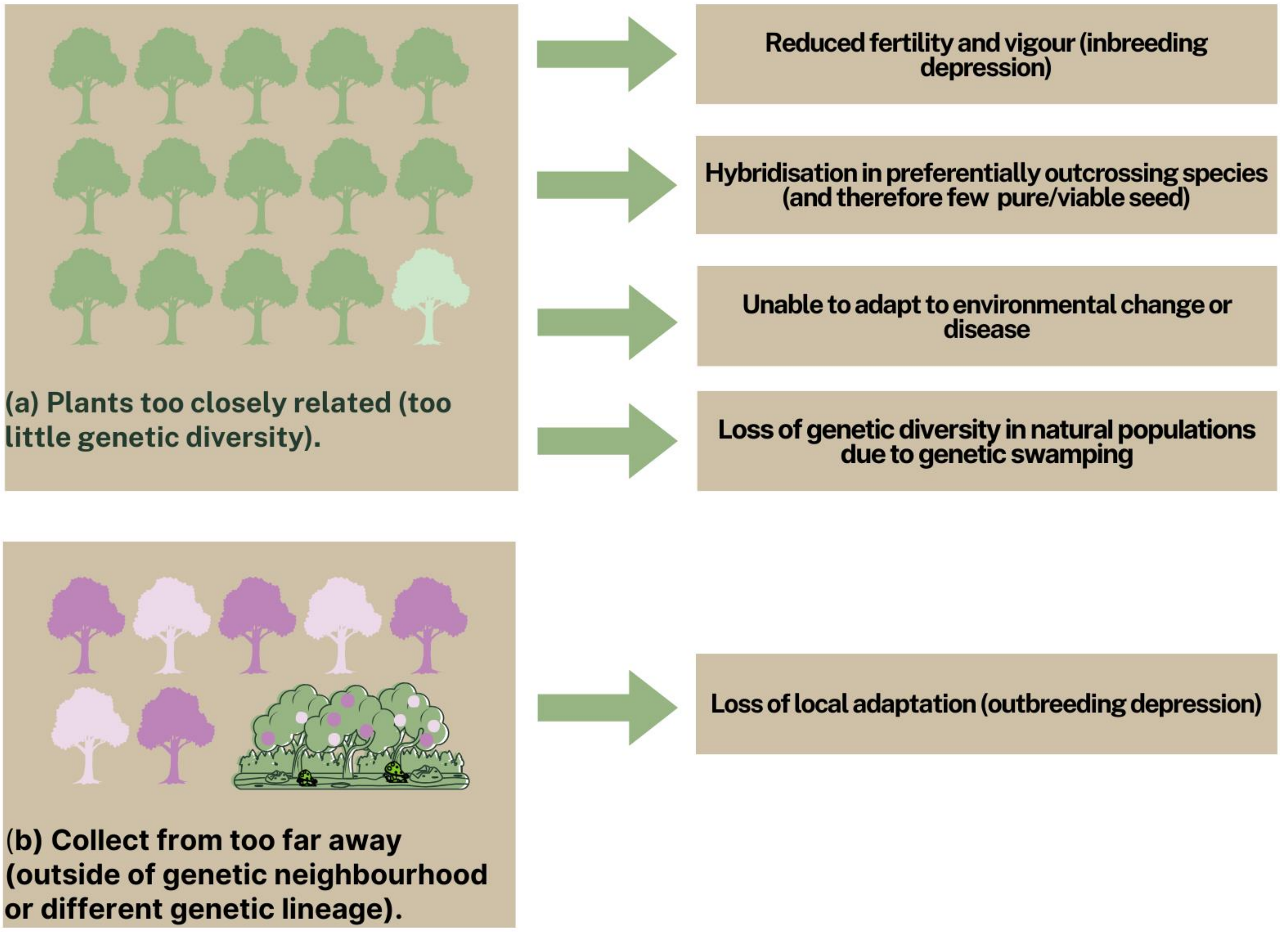


**Figure 1:** A simplified overview of the key potential genetic risks for restored populations and nearby remnant native vegetation, including consequences of sourcing seed too narrowly (a) or broadly (b).

Genetic information can also help clarify taxonomic uncertainty and define species distribution, issues of critical importance to restoration projects. Use of the incorrect species (or operational taxonomic unit (OTU) if taxonomy is incomplete) can have large negative impacts, including increasing the occurrence of hybridisation between species at the restoration site, which has the potential to impact on fitness levels of the hybridising species and can lead to the genetic swamping of a species.

Of particular importance to the restoration of eucalypts are the many outstanding questions around taxonomy (Bayly 2016) and the high levels of hybridisation that often makes the geographic boundaries between closely related species hard to determine (Larcombe *et al.* 2015). This is an area where there is substantial variation amongst different species and groups of eucalypts, with some showing geographically discreet and morphologically recognisable species while others include species that form a morphological continuum and/or have overlapping distributions. However, where species boundaries are well established, eucalypts show strong ecological differentiation, meaning planting the correct species at the correct site is important for the success of ecological restoration. Population level genetic data has been used with success in past studies to assess species boundaries and establish ecological and geographic ranges for eucalypt species.

Another important function of understanding a species genetic structuring and diversity is to increase the seed availability for restoration projects. It has been widely highlighted that the availability of seed for many species is one of the key bottlenecks for the ecological restoration sector in Australia (Hancock *et al.* 2020). One of the approaches to addressing this issue is to broaden the range of provenances that are considered appropriate for a given restoration site, as in the absence of species -specific guidance a conservative approach of only using seed sourced within a certain distance of the site is commonly adopted. By using genetic data to understand the natural genetic patterns of species, seed can often be sourced from a broader geographic area, while still being confident that outbreeding depressions and maladaptation are unlikely to occur. This is particularly true for eucalypts and other common tree taxa, where natural genetic patterns frequently suggest seed can be moved over significant distances (>100 km) without any risk of negative effects on restored populations.

## 1.4 Applying genetic information

To summarise the key genetic considerations when striving to restore self-sustaining populations of eucalypts (Figure 2), we need to:

- minimise the occurrence of inbreeding and hybridisation by maximising the number and distinctiveness of unique individuals.
- maximise genetic diversity by sourcing seed from multiple individuals from multiple populations across a broad geographic range.
- facilitate adaptation to a changing climate by supplementing, when possible, with climate-ready material.
- avoid unintended negative consequences by sourcing seed from the correct taxa (or operational taxonomic unit), within a genetic lineage, and primarily from within the same genetic neighbourhood, as the site being restored.

To achieve these objectives, we need species-specific genetic data rather than generalisations, especially when it comes to restoring threatened ecological communities, because the consequences of using inappropriate material can be dire for both restored vegetation and remnant native vegetation. Until 10-15 years ago, genetic considerations were typically inferred or generalised and obtaining genetic data was considered too complex, expensive, difficult to manage, and hard to interpret. However, technology has changed such that we can now gather relevant genomic information quickly and cost-effectively, making it feasible for restoration practitioners to access the information they need to design effective ecological restoration projects.

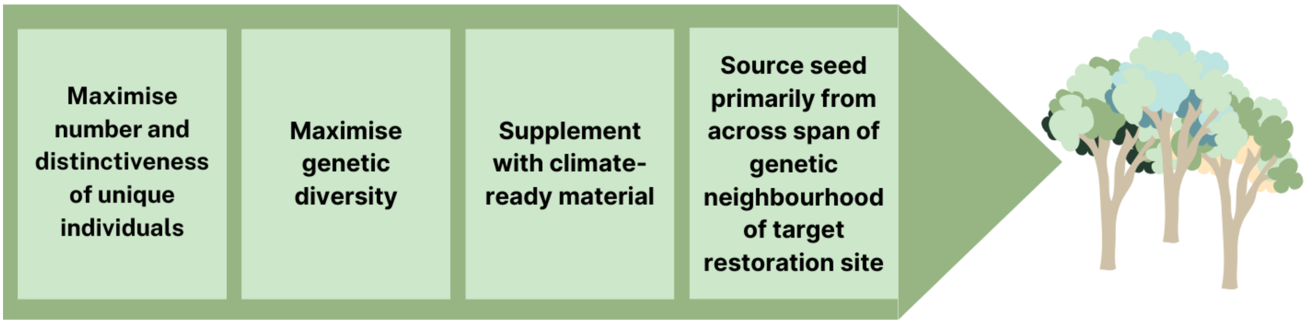


**Figure 2:** A simplified overview of the key genetic considerations when striving to restore self-sustaining populations.

## 1.5 Consideration of environmental factors influencing seed sourcing

In addition to factoring genetic and evolutionary patterns into seed sourcing strategies, it is important to consider the impacts of climate change on any restoration planting. The changes in climate over the coming decades are likely to have large impacts on the health and distribution of many plant species, both remnant and restored. One of the key methods to pre-emptively address this in ecological restoration is the use of genotypes predicted to be pre-adapted to the future climate of individual restoration sites. There are differing ways to do this, including targeted environmental impact studies, and isolating specific adapted genotypes through genetic and greenhouse studies, however these methods have large spatiotemporal costs involved and are therefore not scalable to the point they can be done at the species-specific level. The approach taken by ReCER to allow for cost-effective scaling across species is to determine if and where a species is currently experiencing the predicted future climatic conditions of a restoration site. This allows for seed to be collected from this area that is hypothesised to be pre-adapted to future climate conditions of the restoration site, assuring the persistence of the restored population under climate change. This may mean using seed that is highly genetically divergent from the remnant populations of the restoration site, however if climate change is a major threatening process, then risking any potential outbreeding depression may be necessary. For many parts of a species distribution, there may be no regions where the species currently occurs that match the future climate of the restoration site, and in these cases the best choice to maximise the chances of survival and self-sufficiency of the restored population is to ensure high levels of genetic diversity thereby capturing as many putatively adaptive alleles as possible.

It has been widely observed in the literature that many eucalypt species do not currently occupy the full climatic niche within which they can survive and reproduce (Drake *et al.* 2024), and there is differing views on how individual species will be impacted by climatic changes (González Orozco *et al.* 2016; Booth 2017). One well established factor determining a species climatic susceptibility to environmental change is the extent of its geographic range (Drake *et al.* 2024), with species with broad geographic ranges considered to be more resilient to a changing environment. As the species investigated in this project have relatively large geographic ranges, it remains likely that, while climate change will have a net negative impact on habitat availability, it is unlikely to lead to widespread loss of populations. Climate is only one of many environmental factors that influence the distribution of species, and other factors such as aspect and soil parameters are important predictors of eucalypt species distributions. This means that planting the right species in the right location within the landscape may be as important to population sustainability in the face of environmental stressors such as climate change as are genetic adaptation.

## 1.6 Restore and Renew: making genomic and climatic information freely available to restoration practitioners

The Restore and Renew project responds to the need for ecological restoration practitioners to incorporate the latest science into their toolkit, helping them to restore diverse, resilient, and adaptable ecosystems. The publicly available open access Restore and Renew webtools (https://www.restore-and-renew.org.au) share species-specific empirical knowledge on genetic diversity, climate suitability (current and future) and distributional patterns to deliver restoration guidance to practitioners in easy to use publicly available web tools. Where feasible, species-specific datasets developed by this project are added to the webtools, guiding optimised seed collections and ecological restoration across their distribution in NSW, thereby amplifying the on-ground outcomes of this research.

## 1.7 How to use these guidelines

Below we provide a brief description of each of the guideline’s sections to help you understand their use and application.

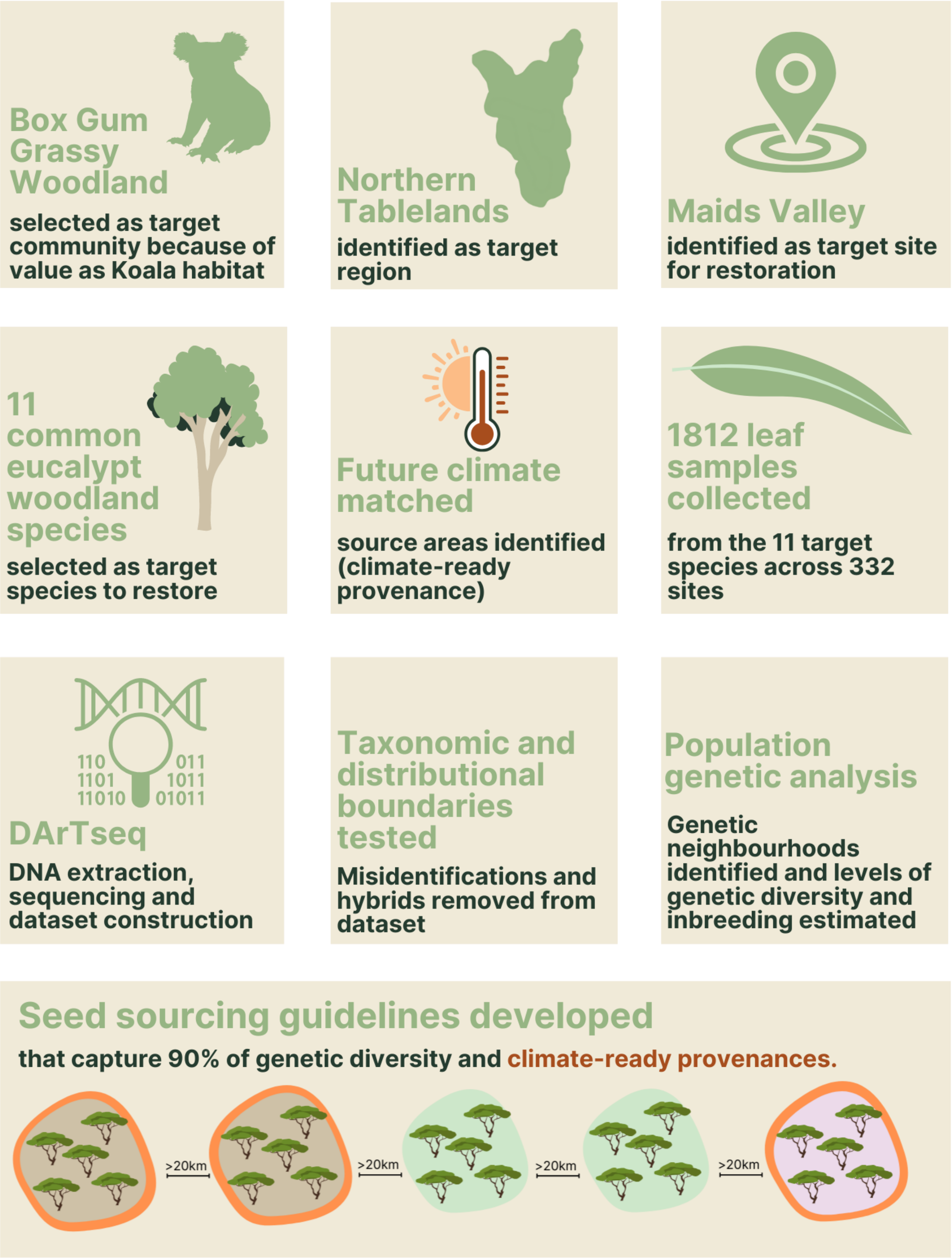
In the **introduction** we set the context of the current study and outline the relevance of genetic information to ecological restoration, highlighting why high levels of genetic diversity is especially important for eucalypts.

The **methods** section introduces the target species, provides a brief outline of the methods used for climate matching and genetic analyses, and refers to research publications for more detailed information.

In the **findings and implications section** we provide generalised recommendations for genetically informed ecological restoration, share observations on landscape patterns of diversity, identify regions that can provide climate-ready seed for use in the target regions, and outline important considerations when undertaking genetically informed restoration of eucalypt species. We then go on to present **species-specific patterns of diversity,** including definitions of taxonomic and distributional boundaries, and genetic neighbourhoods, a biologically meaningful measure of provenances that can be safely mixed in a restoration planting. We provide **guidelines for optimised seed collection** for 11 species that ensure restored plant populations are sufficiently genetically diverse to be self-sustaining in the long term, while also being sufficiently adapted to both current and future climate conditions.

And finally, a conclusions and **future directions** section summarises the value of the methodology used, and knowledge infrastructure established and describes how it can be applied to additional species to further improve ecological restoration.

## 2. Summary of methodology



**Box Gum Grassy Woodland**

selected as target community because of value as Koala habitat

**Northern Tablelands**

identified as target region

**Maids Valley**

identified as target site for restoration

**11 common eucalypt woodland species**

selected as target species to restore

**Future climate matched**

source areas identified (climate-ready provenance)

**1812 leaf samples collected**

from the 11 target species across 332 sites

**DArTseq**

DNA extraction, sequencing and dataset construction

**Taxonomic and distributional boundaries tested**

Misidentifications and hybrids removed from dataset

**Population genetic analysis**

Genetic neighbourhoods identified and levels of genetic diversity and inbreeding estimated

**Seed sourcing guidelines developed**

that capture 90% of genetic diversity and climate-ready provenances.

Figure 3: Snapshot of methodology for the Resilient trees, resilient woodlands: Box Gum Woodland Tree Knowledge Infrastructure project.

## 2.1 Selecting target species and target site

As a pilot project between Taronga Conservation Society Australia and the Research Centre for Ecosystem Resilience, Botanic Gardens of Sydney, it was decided the scope of the project would be on common woodland eucalypt species, as these would be amongst the first species planted in any restoration due to their ecological dominance and role as a food source for Koalas. As one goal of this project was to inform and improve restoration efforts of the eucalypts of Box-Gum Grassy Woodlands (BGGW), the four defining eucalypt species of the BGGW were chosen as the first target taxa. Along with these defining species, seven species that are included in the broader definition of the BGGW (Department of Environment, Climate Change and Water 2010), and that are common species in the broader landscape of the tablelands and western slopes of NSW were identified as targets. This resulted in the following set of eleven target species for the pilot project:

- *Eucalyptus albens* (White Box)
- *Eucalyptus* blakelyi (Blakely's Red Gum)
- *Eucalyptus melliodora* (Yellow Box)
- *Eucalyptus moluccana* (Grey Box)
- Eucalyptus bridgesiana (Apple Box)
- *Eucalyptus caliginosa* (New England Stringybark)
- *Eu*calyptus goniocalyx (Long-leaved Box, Bundy)
- *Eucalyptus macrorhynchya* (Red Stringybark)
- *Eucalyptus polyanthemos* (Red Box)
- *Eucalyptus rubida* (Candlebark)
- *Eucalyptus youmanii* (Youman's Stringybark)

As species that co-occur and have similar environmental niches, there is broad similarity in the distributions of the target species, which helped to make sampling of wild populations less costly to undertake genetic study (Figure 4). All species but *E. moluccana* are primarily restricted to the band of woodland vegetation on the inland side of the Great Dividing Range, as far north as the southern Darling Downs in Queensland, with only *E. melliodora* extending north to Carnarvon National Park. Greater variance exists in the southern end of the target species distribution, with some only present in central and eastern Victoria, while others extend to western Victoria. *Eucalyptus albens*, *E. macrorhyncha* and *E. goniocalyx* also have disjunct populations in the Mount Lofty Ranges of South Australia, and the cool climate adapted *E. rubida* is present in south-east Tasmania. *Eucalyptus moluccana* has the most distinct distribution, being a species primarily of coastal valley woodlands between Jervis Bay in NSW and Atherton in north Queensland. This species only forms a part of the BGGW in the Northern Tablelands and Inland Slopes of NSW where it is considered a defining species of the vegetation type (Department of Environment, Climate Change and Water 2010).

Taronga Conservation Society identified a site in the Maids Valley region of the Northern Tablelands as a likely location for initial restoration plantings. As the target species covered in this report have differing distributions, if a target species is not present in the Northern Tablelands (e.g. *E. goniocalyx* and *E. polyanthemos*), we advise against using it in restoration plantings at the Maids Valley site and provide only generalised guidance on sourcing seed for restoration for that species. This is extended to species which are currently considered to occur in the region but are found not to based upon the genetic evidence generated in this project, which is the case for *E. macrorhyncha* and *E. rubida,* and putatively for *E. moluccana*.

An important note in the context of ecological restoration of the BGGW at the Maids Valley site is that the location is towards the northern edge of NSW. This limits what guidance can be provided regarding sourcing climate ready provenances, which will largely be in Queensland, in the context of this pilot study as sampling is limited to the NSW portion of the target species distributions. Future work may present the opportunity to sample pre-adapted genotypes from Queensland for species whose distributions extent beyond the border.

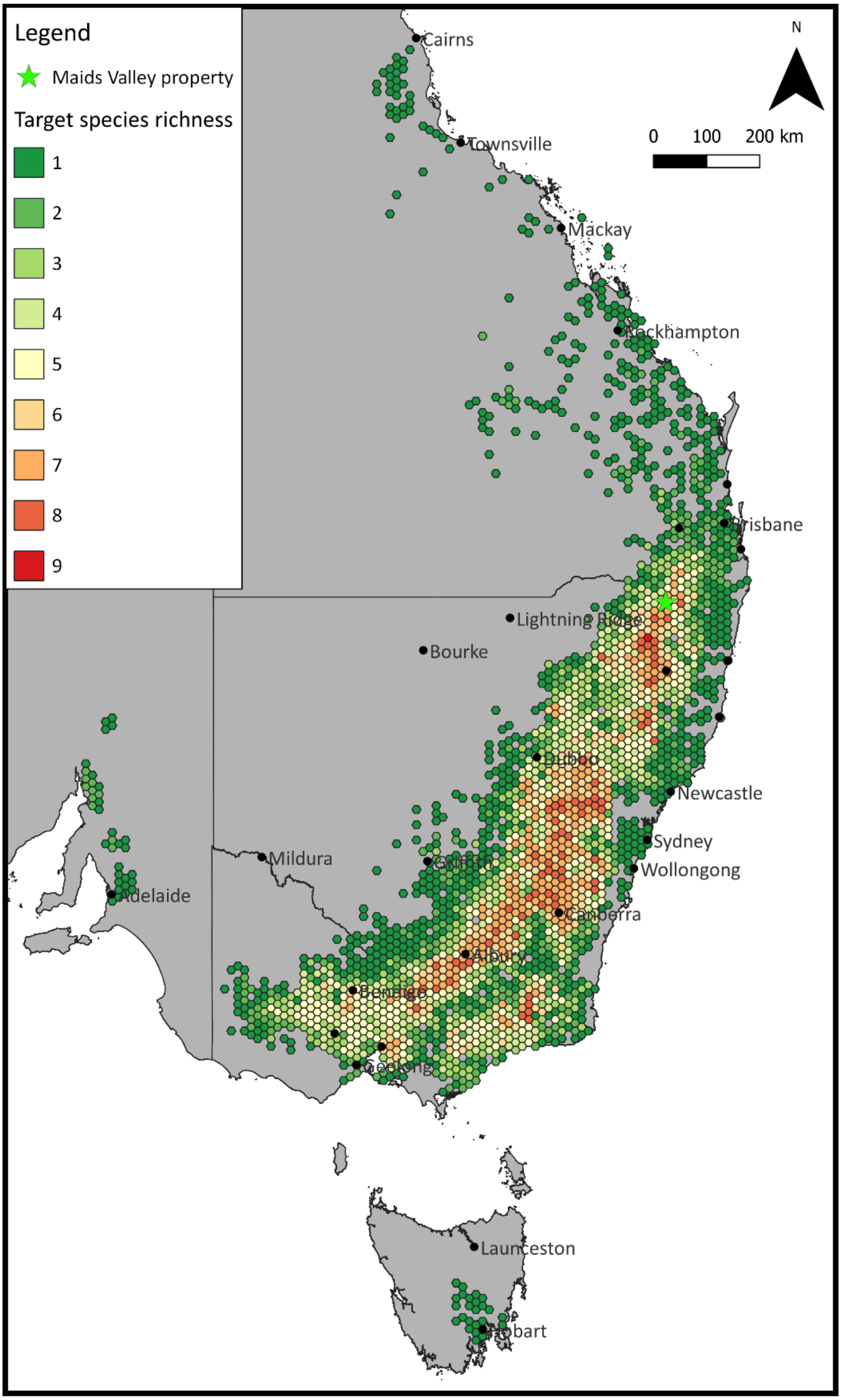


**Figure 4:** Species density map of the 11 target species across a 20 km hexagonal grid in eastern Australia based upon herbarium and survey records. The target species primarily co-occur in a band stretching from central Victoria to the NSW-QLD border which corresponds to the area in which Box-Gum Grassy Woodland (BGGW) occurs. Note that the Maids Valley site is located very near the northern extent of this BGGW band as well as the northern extreme of many targets species distributions, with only two target species having extended distributions in Queensland.

## 2.2 Climate matching and identification of climate-ready provenances

To locate areas where climate-ready provenances can be sourced, the approach taken on the Restore and Renew webtool (Rossetto et al. 2019) was employed. The centroid of the Maids Valley site was calculated and used to find the mean annual precipitation and mean annual temperature of the property using CHELSA (Karger *et al.* 2017) historical data. A buffer of +/- 300 mm and +/- 1.5ºc was then applied to these average rainfall and temperature figures to calculate the envelope of climate conditions that matched those experienced at the site. This envelope was then projected across the current conditions of eastern Australia to identify areas with current climate conditions that matched the Maids Valley site.

This process was repeated to identify areas where climate-ready provenances can be sourced for this site based upon two climate change scenarios: shared socioeconomic pathways 370 and 585. For each of these scenarios, the projected climate of the Maids Valley site between for the years 2070 to 2100 was extracted from 5 CHELSA V2.1 (Karger *et al.* 2017; Karger *et al.* 2020) datasets developed using different global circulation models (gfdl-esm4, ukesm1-0-ll, mri-esm2-0, mpi-esm1-2-hr, and ipsl-cm6a-lr). The resultant envelope for each model was then projected onto current climate conditions to identify areas where the current climate matched the future climate of the Maids Valley site. For both scenarios the resultant areas were then summarised across models so that only areas where current climate conditions matched the future climate of Maids Valley site under all five models were considered suitable for sourcing climate-ready provenances.

Both the current and future climate matched areas were then mapped and compared to the distribution of the eleven target species to locate potential sites that could be used to source climate-ready seed for the restoration of the Maids Valley site. The proportion of total seed lines to be sourced from climate-ready provenances is largely dependent on the goals of the restoration planting, with 20% being proportion used by ReCER in situations where climate readiness is not highly prioritised and 50% being used when climate readiness is a high priority. However, in situations where climate-ready provenances are limited, such as for many of the target species of this project which may be planted at the Maids Valley site, it may be the case that sourcing diverse populations that may not be climate-ready to increase the adaptive potential of the planting becomes more important for climate change adaptation.

## 2.3 Population genetic analyses and guidelines

### 2.3.1 Sampling and genetic data generation

Sample collection and data generation followed the standard Restore and Renew protocols the ReCER (Rossetto *et al.* 2019) has established for restoration genomics studies. Within the context of this pilot project, sample collection was limited to the NSW portion of the target species distribution due to time and permitting constraints. The aim of field collecting was to ensure a sampling site occurred within 50 km of all known populations of each target species, as this is the standard distance over which genetic findings are extrapolated. As a highly cleared and threatened vegetation type, it was not possible to achieve this for the BGGW in some regions where there is limited intact native vegetation in conservation managed land, such as the Liverpool Plains. At all sampling sites, six leaf samples were collected for DNA extraction and sequencing, along with at least a single voucher specimen to allow for latter identification as needed. To allow for the confirmation of taxonomic and distributional boundaries, closely related and putatively hybridising species were collected where they co-occurred with the target species. Overall, 3374 leaf samples (each representing an individual tree) from 669 sites were included in analyses for this project, including 1812 samples for the 11 target species and an additional 1562 samples for closely related and putatively hybridising species to use as outgroups.

Leaf material from all samples were sent to Diversity Array Technologies Pty Ltd (Canberra) for DNA extraction, sequencing, and dataset construction. DArTseq is a reduced representation sequencing technique resulting in tens of thousands of single nucleotide polymorphisms (SNPs) that were used for population genetic analyses.

### 2.3.2 Determining taxonomic and distributional boundaries

The first step taken to develop genetically informed seed sourcing guidelines for the target BGGW eucalypt species was to test taxonomic and distributional boundaries. This was done using datasets that contained the target species and their close relatives that have either been previously sequenced by ReCER or sampled and sequenced during this project. These datasets were analysed using phylogenetic networks (using *Splitstree* v4), principal components analysis (PCoA) and the calculation of pairwise $F_{st}$ values between sites. Any individual samples or sites of the target species which clustered with an outgroup were taken to represent misidentification and removed from the dataset before species specific restoration genetic analyses. Clustering intermediate between the target species and an outgroup were taken to represent hybrids and were also removed from the datasets before further analyses. Voucher specimens were used to confirm genetic identifications using morphology where possible.

In some cases, all collections in part of a species distribution were found to be more closely related to outgroup samples than the target species and therefore likely to represent a misunderstanding of a species taxonomy or distribution. In these cases, the set of sites that represent the target species were identified and used as an operational taxonomic unit (OTU) for the purposes of this report. Work to resolve the taxonomic issues is outside the scope of this project, but the findings contained within this report lay a strong foundation for this work to be undertaken and the guidance provided here is not dependant on the completion of that taxonomic work. Our findings regarding the taxonomic and distributional boundaries of all target species are provided in sections 3.2.1 and 4.

### 2.3.3 Population genetic analyses

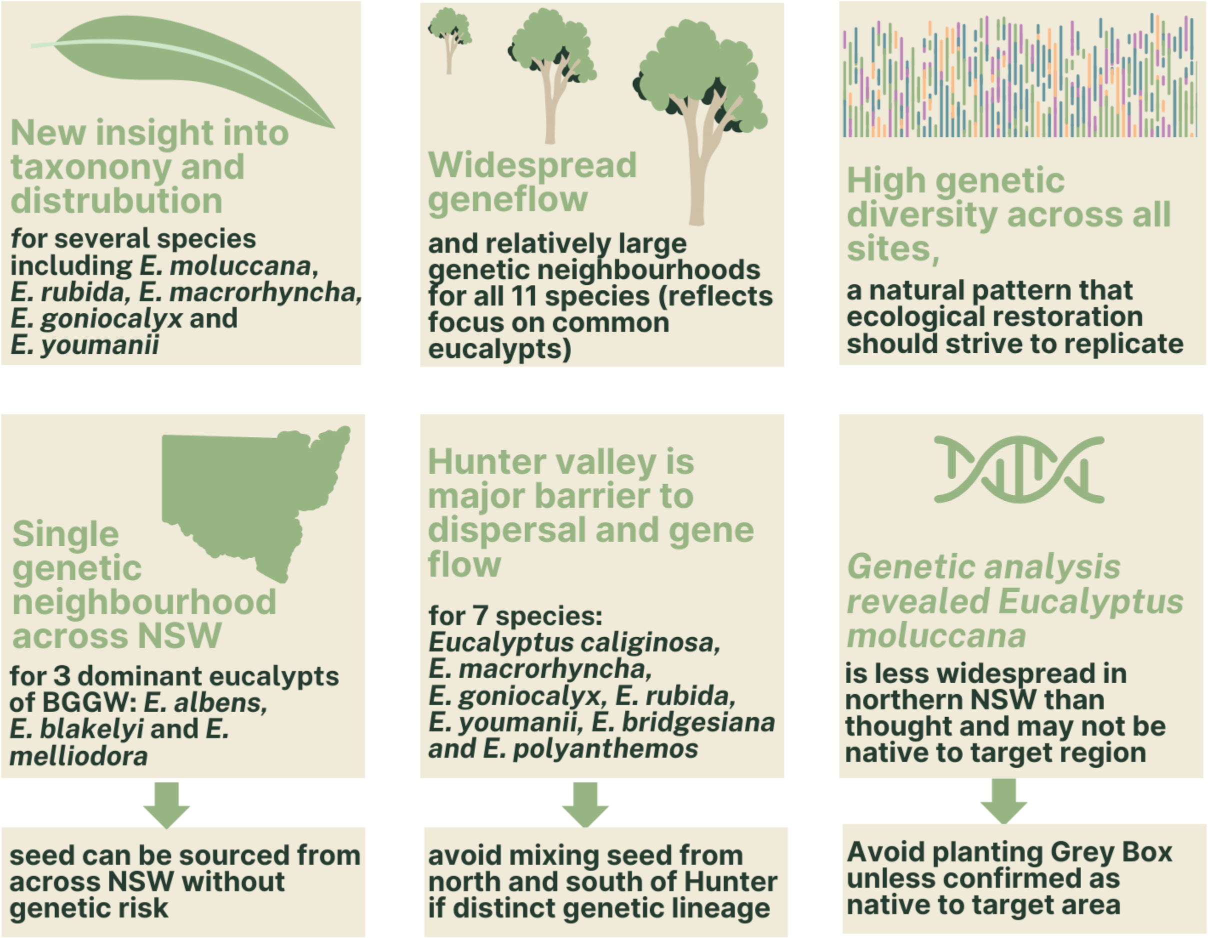
For each species, once the distributional and taxonomic boundaries were established for the target species/OTU, the filtered dataset was used to investigate within species genetic structuring and diversity patterns. First, rates of clonal reproduction were investigated, however as the *Eucalyptus* species targeted in this study have a tree rather than a mallee (spreading, multi-stemmed habit that can lead to clonal vegetative spread) growth form, very few clonal genets were observed across all species. These clonal genets may reflect sampling of the same individual multiple times in the field due to error in identifying individuals, so have little influence on the development of seed sourcing guidance. Within species genetic structuring was then investigated using phylogenetic networks, PCoA's, $F_{st}$ values and admixture analysis (utilising *sNMF*). Genetic neighbourhoods were identified for species where there was congruent clustering across analytical methods that exceeded what is expected due to isolation-by-distance. Levels of genetic diversity and inbreeding were estimated using standard population genetic parameters for all sites with 5 non-hybrid samples of the target species. We provide our findings on the reproductive biology and patterns of genetic structuring and diversity for all target species in Section 4.

### 2.3.4 Development of seed sourcing guidelines

To investigate the level of seed collecting effort required to capture a minimum of 90% of common genetic diversity of target species within restoration plantings, a minimum site approach was taken. Under this approach seed is collected from 5 mother trees at each source location, aiming to ensure maternal trees are at least 100 m apart to minimise the chance of collecting from related trees (Barbour *et al.* 2005; Bezemer *et al.* 2016). While this approach results in a higher total number of maternal lines being needed than if each site is treated as a single line, it decreases the overall seed collecting effort needed to meet genetic diversity capture targets due to a substantial reduction in the number of source locations that must be identified and visited.

Required seed sourcing effort was determined using a random sampling technique of genotyped samples over 1000 iterations for each combination of site and sample number, with the choice of site numbers being the lowest that always resulted in the capture of >90% of all alleles present in the species genetic neighbourhood being targeted for seed sourcing, thus allowing for flexibility in the site choice optimisation (Dimon et al., in prep). The threshold of 90% of common alleles is a standard target used by ReCER based upon United Nations' Convention on Biological Diversity target (United Nations Convention on Biological Diversity, 2021). This approach is easily replicable for different regions and suites of taxa where genetic data is available or can be generated, with the possibility of being automated and used to optimise specific restoration or seed collection projects. Targeted guidance on what a good seed sourcing strategy for a restoration site within the target region is provided based upon the species-specific population genetic findings and seed source optimisation analyses for each species.

# 3. Findings and implications



**New insight into taxonony and distrubution**

**for several species including *E. moluccana*, *E. rubida*, *E. macrorhyncha*, *E. goniocalyx* and *E. youmanii***

**Single genetic neighbourhood across NSW**

**for 3 dominant eucalypts of BGGW: *E. albens*, *E. blakelyi* and *E. melliodora***

**seed can be sourced from across NSW without genetic risk**

**Widespread geneflow**

**and relatively large genetic neighbourhoods for all 11 species (reflects focus on common eucalypts)**

**Hunter valley is major barrier to dispersal and gene flow**

**for 7 species: *Eucalyptus caliginosa*, *E. macrorhyncha*, *E. goniocalyx*, *E. rubida*, *E. youmanii*, *E. bridgesiana* and *E. polyanthemos***

**avoid mixing seed from north and south of Hunter if distinct genetic lineage**

**High genetic diversity across all sites,**

**a natural pattern that ecological restoration should strive to replicate**

***Genetic analysis revealed Eucalyptus moluccana***

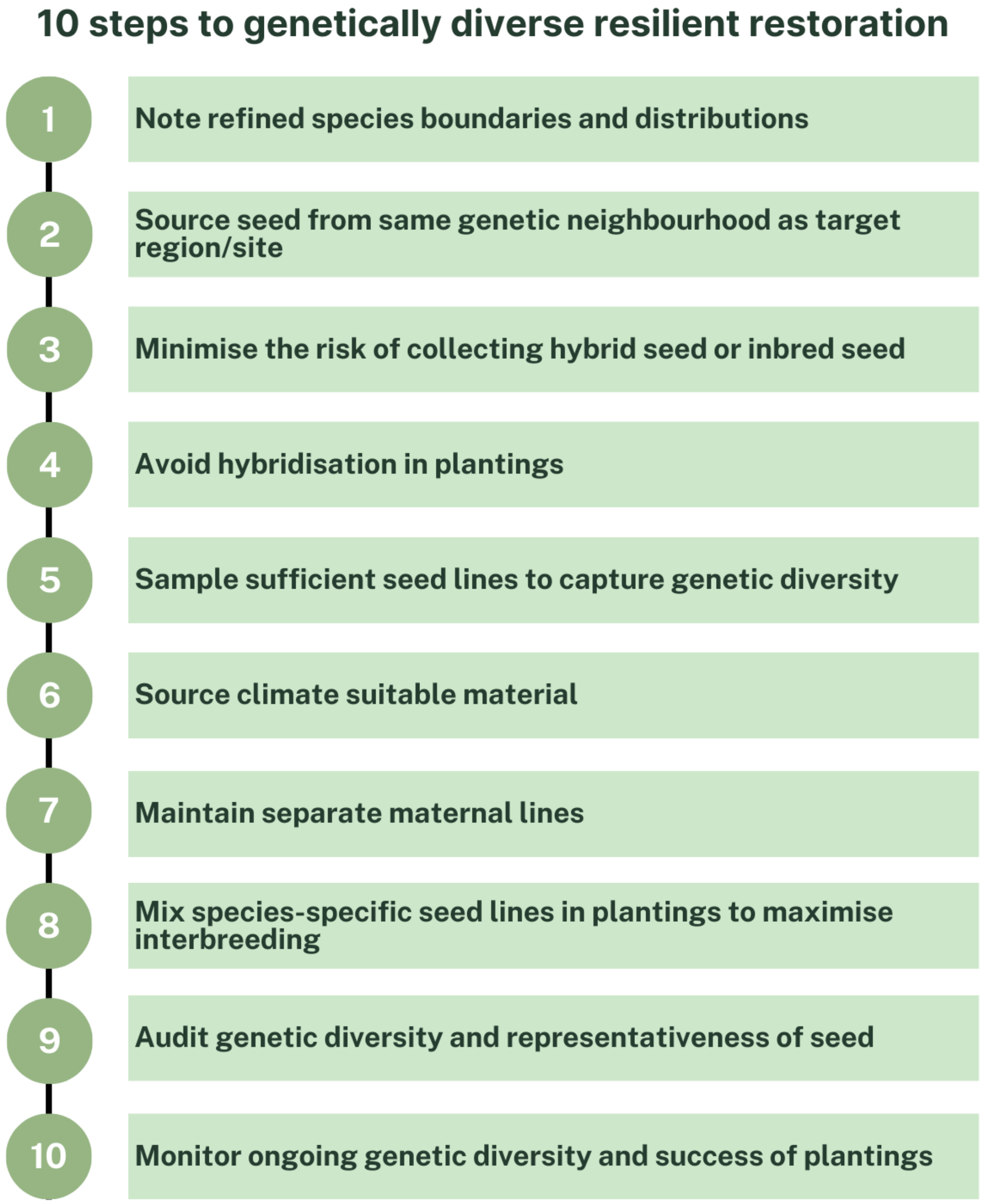
**is less widespread in northern NSW than thought and may not be native to target region**

**Avoid planting Grey Box unless confirmed as native to target area**

**Figure 5:** Snapshot of key genetic findings and implications for restoration of the 11 target *Eucalyptus* species at the Maid Valley site.

## 3.1 An overview of how to use findings to improve restoration

The findings of this restoration genetic research can improve the quality and long-term success of ecological restoration of Box-Gum Grassy Woodland and provides a clear plan for sourcing genetically diverse climate-ready seed for Eucalyptus plantings at the Maids Valley site. Figure 5 provides a snapshot of key findings and their implications, including that new taxonomic and distributional insight means that not all target taxa may be suitable for planting at the Maids Valley site and that plantings should replicate the high genetic diversity of natural populations. Other implications relate to the geographic location of seed source sites, including that it is appropriate to source seed for the three dominant eucalypts of Box-Gum Grassy Woodland (*Eucalyptus albens, E. blakelyi* and *E. melliodora*) from across NSW and into southern Queensland. This insight increases seed availability and will facilitate securing genetically diverse seed from a broad geographic area. However, research outcomes can be used in many more ways beyond these simple key findings. To ensure full appreciation of the actionable outcomes of this research, we summarise key recommendations below and in Figure 6. Each recommendation is discussed in more detail throughout the remainder of Section 3 and in the species-specific section 4.



10 steps to genetically diverse resilient restoration

1. Note refined species boundaries and distributions
2. Source seed from same genetic neighbourhood as target region/site
3. Minimise the risk of collecting hybrid seed or inbred seed
4. Avoid hybridisation in plantings
5. Sample sufficient seed lines to capture genetic diversity
6. Source climate suitable material
7. Maintain separate maternal lines
8. Mix species-specific seed lines in plantings to maximise interbreeding
9. Audit genetic diversity and representativeness of seed
10. Monitor ongoing genetic diversity and success of plantings

**Figure 6:** Summary of ten key steps for genetically diverse climate-ready restoration of Box-Gum Grassy Woodland.

### 3.1.1 Key recommendations for genetically diverse climate-ready restoration of Box-Gum Grassy Woodland.

- **Note refined species boundaries and distributions:** Genetic data provided valuable insight into taxonomic boundaries, with implications for where to source seed and what species to target. (e.g. *Eucalyptus rubida*, *E. macrorhyncha*, *E. youmanii* and *E. moluccana*).
- **Source seed from same genetic neighbourhood as target region/site.** Mapping of genetic neighbourhoods for each target taxa across NSW improves on 'local provenance' generalisations that likely underestimated an evolutionary appropriate seed collection area. By understanding natural patterns of gene flow, the uncertainties attached to moving seed and genotypes across the landscape are removed, increasing confidence in what provenances are suitable for use in individual restoration plantings and broadening the seed available for use.
- **Minimise the risk of collecting hybrid or inbred seed.** Genetic data identified pairs of species that naturally hybridise in the wild. Avoid collecting seed where these species pairs co-occur. It is also important to avoid sourcing seed from small or planted populations as they are likely to have low levels of genetic diversity and/or inbred seed.
- **Avoid hybridisation in plantings:** Pairs of species that are identified as commonly hybridising in natural populations are also likely to hybridise when planted together. For example, hybridisation was most common between *E. blakelyi* and other Red Gum species, and between Stringybark species in this project, both groups in which multiple species would

be appropriate to include in plantings at Maids Valley. Care should be taken to minimise the planting of these hybridising species pairs in close vicinity to avoid recruitment of hybrids in future generations.

- **Sample sufficient seed lines to capture genetic diversity:** By understanding levels of genetic diversity in natural populations, it becomes possible to undertake optimisation approaches to determine what seed sourcing effort is required to meet genetic diversity targets within plantings. While this is very similar between the target species investigated in this project, due to them all being *Eucalyptus* species, previous work has shown that it can vary considerably more between species of other taxonomic groups (e.g. between species of *Acacia* or *Dodonaea*).
- **Source climate suitable material:** After using genetic data to determine a species distribution and genetic structuring, it is then possible to identify what climate ready provenances are most suitable for use in individual restoration plantings. For some species (e.g. *E. caliginosa*, *E. bridgesiana* and *E. youmanii*) this is complicated by Maids Valley being at the north-western edge of their range.
- **Maintain separate maternal lines:** Tracking maternal lines and keeping them separate throughout the entire processes of seed collection to restoration planting enables manipulation of the proportion of seed from each mother so that genetic diversity can be maximised, and relatedness minimised. Maintaining separate maternal lines also considerably reduces the number of collection sites needed to capture adequate genetic diversity.
- **Mix species-specific seed lines in plantings to maximise interbreeding:** By using genetic data to optimise the seed used in restoration plantings, it is then possible to design a planting scheme that maximises the retention of genetic diversity and mixing of seed lines. Maternal lines should be interplanted to allow for maximum interbreeding of maternal lines.
- **Audit genetic diversity and representativeness of seed used in plantings:** With a baseline of genetic patterns in natural population established, it is possible to undertake strategic genetic testing of seed sourced for restoration plantings to test for genetic diversity and health.
- **Monitor ongoing genetic diversity and success of plantings:** When it is understood what the genetic diversity of natural populations is and what seed lines are used in a restoration planting, it becomes possible to monitor the genetic diversity and reproductive success of a planting as it becomes established and self-sustaining.

## 3.2 Taxonomic and distributional uncertainty

The genetic analyses proved a powerful tool for highlighting and resolving taxonomic and distributional uncertainty for several target woodland species, including *Eucalyptus moluccana, E. rubida, E. macrorhyncha*, *E. goniocalyx*, and *E. youmanii*. The details and implications for seed sourcing of clarified species boundaries and distribution is discussed in more detail for each target species in Section 4. In brief we find that generally, taxonomic issues and uncertainty exist where species distributions are not continuous, and several range restricted and geographically isolated taxa should be synonymised with a common and abundant relative, a situation which is easily accommodated in our population genetic analyses and seed sourcing guidance by including these narrow range taxa in the analyses of the common target species. In situations where highly genetically divergent populations of a target species were identified as putatively misidentified or unrecognised taxa, these sites were excluded from the population genetic analyses and seed sourcing guidance pending further study to confirm their taxonomic identity.

For three of the target species (*E. goniocalyx*, *E. macrorhyncha* and *E. moluccana*), we found all populations within large regions of their reputed distributions have been misassigned to the target species. Therefore, we limit our population genetic analyses and seed sourcing guidelines to only the portions of these species distributions where samples form a single genetic lineage. One notable outcome of this is that *E. moluccana* should be removed from the definition of the BGGW in the Nandewar bioregion as the trees identified as this species in the region are not closely related to true *E. moluccana* from coastal NSW. In the case of *E. youmanii*, which we found to be more widespread than stated by previous authors, we include all sites assigned to the species genetically in our population genetic analyses and seed sourcing guidance.

## 3.3 Landscape patterns of diversity in common box-gum grassy woodlands eucalypts

### 3.3.1 Widespread geneflow with a common divergence for tablelands species

Overall, we found widespread geneflow and high levels of outcrossing for all 11 target species, a result that reflects our focus on common eucalypt species, which are known to share very similar population genetic patterns and reproductive biology. Such congruence between restoration genetics findings for individual species' is not found in many other taxonomic groups. Two different genetic and evolutionary patterns were observed across the 11 target BGGW species. For the three abundant species that define the BGGW (*E. albens*, *E. blakelyi* and *E. melliodora*) we found no genetic structuring, consistently high levels of genetic diversity and extensive geneflow across the species' entire NSW distribution. These species occur on flatter land with moderate to high fertility soils and across a very broad climatic range, an environmental niche which gives rise to a contiguous band of occurrence from Victoria to southern Queensland. Seven of the remaining eight species show evidence for the Hunter Valley being a major biogeographical barrier to their dispersal and gene flow. *Eucalyptus caliginosa*, *E. macrorhyncha* (as defined by genetic data in this study), *E. goniocalyx* (as defined by genetic data in this study), *E. rubida* (as defined by genetic data in this study) and *E. youmanii* only occur either to the north or south of the Hunter Valley but show extensive and uninterrupted geneflow across their distributions. Likewise, *E. bridgesiana* occurs on both sides of the Hunter Valley, but the populations on either side are genetically divergent from one another suggesting geneflow is limited across the Hunter Valley gap. While it occurs only to the south of the Hunter Valley, *E. polyanthemos* showed genetic divergence between its two subspecies that occur in NSW.

These results suggest that over evolutionary timescales, habitat connectivity has been high for species that occur in the full range of environmental conditions BGGW experience, but that species with more specialised or narrower environmental niches may not have been able to cross the Hunter Valley gap in the recent evolutionary past. This means that there are different considerations regarding seed movement for these two groups of species. For the common species with broad environmental ranges evolutionarily informed seed movement in NSW can be largely unrestrained. For species which have more habitat specificity and are more marginal members of the BGGW communities, consideration needs to be given to whether the species naturally crosses the Hunter gap and if not, whether the populations on either side, should they exist, are highly genetically divergent, which comes with considerations of outbreeding depressions and localised environmental adaptation.

### 3.3.2 Similar patterns of genetic diversity across species

For the eleven target eucalypt species investigated in this study, we find that patterns of genetic diversity are consistent. Most sampled sites for all species showed high levels of allelic diversity and heterozygosity which were even across the species distribution. Only a small number of sites that represent small, isolated pockets of the species distributions (*E. melliodora* in the Bega Valley, *E. moluccana* from the Nowra district, and *E. caliginosa* from Maryland N.P.) showed notably low genetic diversity, likely the result of genetic bottlenecking. This suggests there are evolutionary factors that mean high levels of standing genetic diversity within populations are beneficial for population survival and reproduction, a finding important for ecological restoration of these species which should try to recreate these natural patterns. We also find the number of seed sources to capture the target level of genetic diversity was consistent across all species, ranging from four to six sites when collecting five maternal lines from each site.

### 3.3.3 Location of climate-ready provenances

Figure 7 shows the areas which match either the current or future climate of the Maids Valley site. Under current conditions, the site is located towards the north-west of a band of similar climate that supports the Box-Gum Grassy Woodlands of NSW and there are limited areas to the north that are climate matched currently. The Queensland-New South Wales border inland of the Main Range roughly corresponds to an area of high turnover of eucalypt flora (Figure 4), possible due to the climatic shift observed in the region. This turnover of eucalypt flora is reflected in the distributions of many of the eleven target *Eucalyptus* species, in that several species that occur naturally in the Maids Valley area do not occur far into Queensland, primarily being limited to the Granite Belt region (*E. albens*, *E. blakelyi*, *E. bridgesiana*, *E. caliginosa*, *E. youmanii*). Only *E. melliodora* and *E. moluccana* are common north of the Granite Belt in Queensland.

As can be seen in Figure 7 however, the projected climate of the Maids Valley site in 2070 under both ssp370 and ssp585 scenarios is matched to the current climate to the north and west of the site. Very little difference is evident in the climate matched areas for these two scenarios, both of which represent severe climate change scenarios beyond the global targets currently in place, thus representing worse case scenarios.

There are areas of climate ready provenances for both *E. melliodora* and *E. moluccana* available in Queensland, although as these populations were not sampled in this project, we cannot comment on their genetic diversity and divergence from the NSW populations around the Maids Valley site. Also, as discussed in Section 4.4, *E. moluccana* may not actually be native to the Maids Valley area. While there are very limited opportunities to locate climate ready provenance from north of the Maids Valley site for the remaining target species that are native to the Maids Valley area, there are small areas to the west within NSW that may be suitable for some of these species. Namely, *E. albens* and *E. blakelyi* occur in climate matched areas such as the Pilliga, and around Bullala and Dthinna Dthinnawan national parks. However, *E. bridgesiana, E. caliginosa* and *E. youmanii* do not occur in any areas which are climate matched for the future climate of the Maids Valley site. For these species, the best approach will be to ensure genetic diversity in plantings is high to increase the adaptive potential of the planted populations, thus maximising the chance it can adapt to the future climate.

We recommend all projects that aim to restore Box-Gum Grassy Woodland consult the Restore and Renew Webtool climate matching tool (restore-and-renew.org.au/sitematch) to find climate matched areas for their restoration site.

## 3.4 Important considerations when undertaking genetically informed restoration.

### 3.4.1 Tracking of maternal lines

It is strongly recommended that to maximise genetic diversity in restoration sites or seed production areas (SPAs), all maternal lines are tracked and kept separate throughout the entire processes of seed collection to restoration planting. Mixing seed from multiple mothers in a single collection removes the ability to control the proportion of seed from each mother and consequently removes any ability to intentionally maximise genetic diversity, minimise relatedness, track unintentional loss of genetic diversity or remove unwanted individuals. Maintaining separate maternal lines also considerably reduces the number of collection sites needed to capture adequate genetic diversity, thus decreasing the required seed collecting effort and cost (van der Merwe *et al.* 2023).

In restoration plantings and SPA’s, maternal lines should be interplanted to allow for maximum interbreeding of maternal lines, but if practicable the identity of each planted individual should still be recorded. This recommendation applies to both single maternal line per site and multiple maternal lines per site strategies. In the latter case, both the source site and mother plant should be tracked.

### 3.4.2 Avoid sourcing from small, planted or hybridising populations

While not something we can directly test with the data underlying this report, previous work has shown that seed coming from small, fragmented *Eucalyptus* populations is often highly inbred, low in genetic diversity due to a lack of pollen donors, and often contains a large proportion of hybrid seed (Broadhurst 2013; Broadhurst *et al.* 2015; Jordan *et al.* 2016). Therefore, to maximise the chances that high quality seed (genetically diverse and not highly inbred) that will perform well in restoration plantings is collected, large stands of the target species should be preferentially chosen for seed collection over lone trees or small stands.

While seed supplies are often limited for ecological restoration, it is best to avoid sourcing seed from plantings, unless designed as seed production areas (SPAs). Even when sourcing seed from established SPAs, it is recommended that seed is tested so that mass plantings of related individuals are avoided. Planted populations experience genetic diversity filtering and represent a limited number of families, therefore seed of SPAs that are not designed to specifically maximise the mixing of families and maternal lines is likely to have a much greater relatedness between seed lines than from natural populations. It is possible to design planting schemes for restoration sites that double as seed production areas, however this requires much greater care be taken with the design and monitoring of plantings and the resulting seed.

Eucalypts have high rates of hybrid compatibility, and hybrids and hybrid zones have been widely observed in natural populations. However, these hybrids tend to occur at the environmental boundaries of species distributions, being comparatively rare in the core habitat of species. This suggests that non-hybrid individuals have competitive advantage over hybrid individuals under most circumstances, and it is therefore important to minimise the use of hybrid seed in restoration plantings unless they are within a known hybrid zone for the target species. However, hybrid seed and seedlings are hard to identify and remove from collections in the absence of genotyping, therefore the best strategy to limit the use of hybrid seed in plantings is to avoid sites where species known to hybridise with the target species also occur. Species known to hybridise are identified in Section 4.

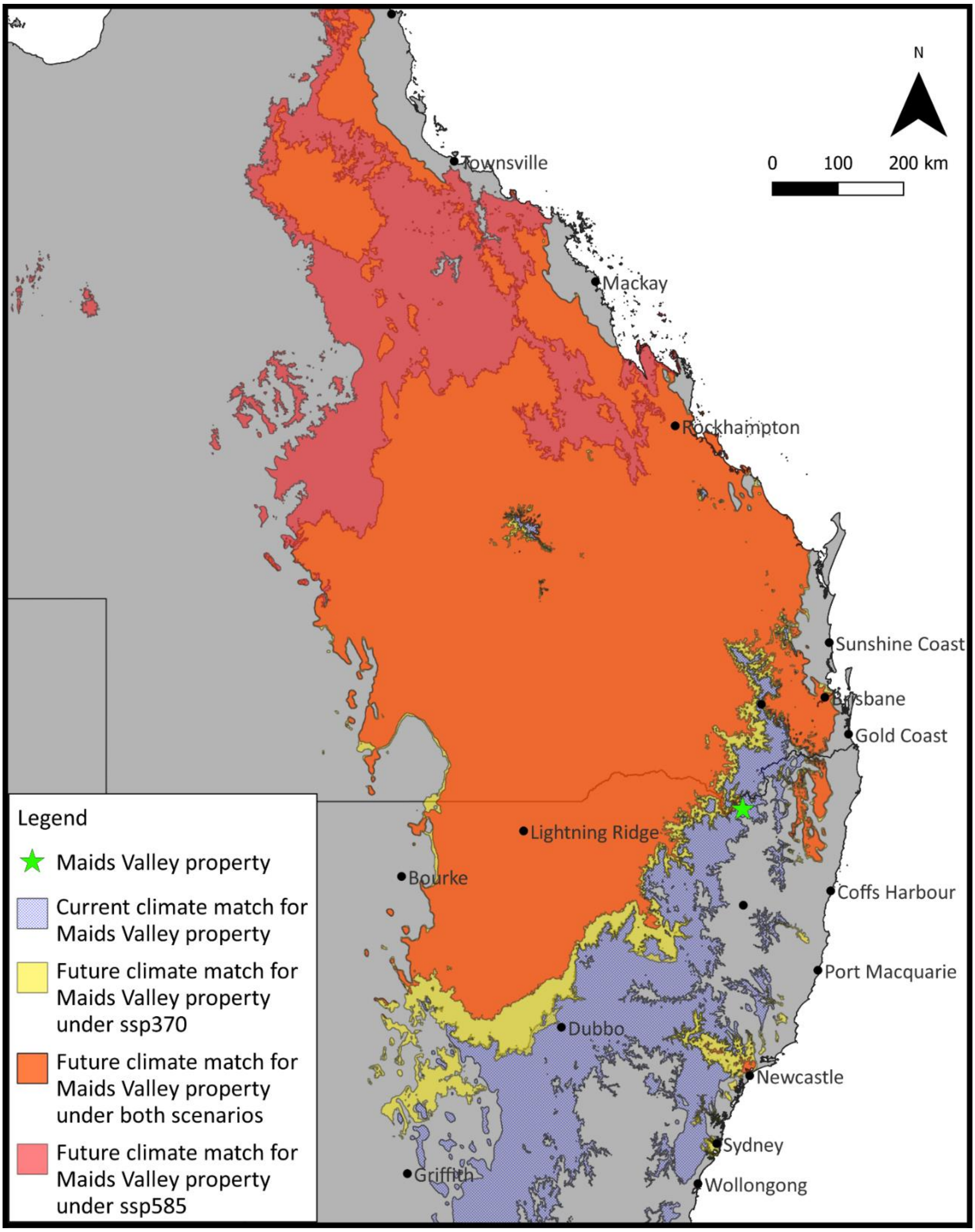


**Figure 7:** Climate matched regions for the predicted climate of the target restoration region in the years 2070-2100. Blue-hashed areas are a match for the current climate of the Maids Valley site (green star), yellow areas are climate matched under the ssp370 climate change projections, red areas are climate matched under ssp585 climate projections, and orange areas are climate-matched under both scenarios. Note that these climate matched areas are largely outside the current distribution of the target species investigated in the project, limiting the availability of climate ready seed for these species.

### 3.4.3 Distribution of seed source sites and mother plant location within sites

The aim of collecting multiple maternal lines is to both maximise the capture of genetic diversity and to ensure limited familiar relationships between individuals in plantings. To do this it is important to consider both the spacing of source sites across the landscape and the location of mother trees at a site. Given the high genetic diversity shown by *Eucalyptus* species at individual sites, the even spread of that diversity across the landscape and what is known regarding the genus's reproductive biology (Griffin *et al.* 2019; Nickolas *et al.* 2019), several conclusions can be reached:

- The number of maternal lines sourced is far more impactful on the genetic diversity represented in seed collections than the exact locations of source sites or the specific mother trees from which seed is collected.

- Sites separated by 20 km are unlikely to be experiencing regular gene flow and thus this is a suitable distance to space source sites across the landscape.
- Given pollen movement distances (Barbour et al. 2005; Bezemer et al. 2016), mother trees within a site should be at least 100 m apart, with 200 m optimal, so that different pollen donors are represented.
- If collecting seed from mother trees closer than 100 m because no other choices are available, seed from mother trees within 50 m of one another should not be treated as separate maternal lines due to the high chance of familial relationships between the parental trees.

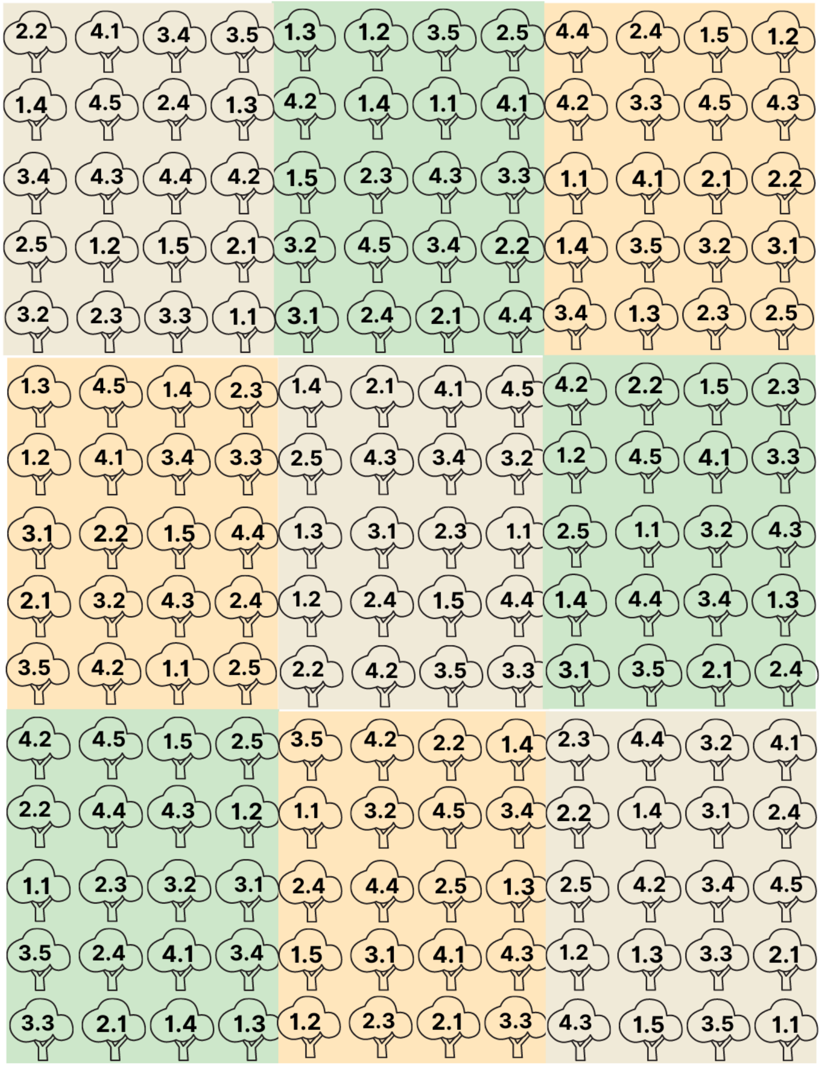
### 3.4.4 Monitoring for success of restoration planting

An important aspect of genetically informed restoration is the monitoring of ongoing growth, survival, and reproductive success of plantings. The ability to achieve this is greatly increased when maternal lines are tracked and the identity of planted individuals is recorded, as this allows the success of maternal lines and source sites to be determined from simple surveys of survival and growth of individuals within plantings. We recommend that such surveys are undertaken at intervals dependant on the life history of the species, as fast-growing, short-lived species will grow and reach reproductive capacity over shorter timelines, and therefore correctional activities will need to be implemented sooner, than for long-lived tree species such as the eucalypts that are the focus of this report. For eucalypts, we suggest more intensive monitoring (e.g. undertaking growth and survival surveys annually) during the establishment phase (first 3-5 years post planting), followed by less frequent surveys once the population is established.

Additionally, the recruitment of new individuals into the restored area should be surveyed once the planting reaches reproductive age to measure the trajectory of the population overtime. Genetic survey of seed or recruited individuals from restored plantings can be used to confirm that the initial genetically informed seed sourcing strategy has resulted in a population that is producing sufficiently genetically diverse offspring to ensure its long-term survival, growth and adaptation to local conditions. If all seed and offspring are being produced by maternal lines that are particularly vigorous and fecund or different maternal lines are not interbreeding, problems such as a loss of genetic diversity, inbreeding depression or poor adaptation to changing environmental conditions may result. Such processes may not be readily apparent based upon the success of planted individuals or the number of newly recruited individuals alone, however, genetic surveys are a tool that allows such issues to be identified early and corrective actions undertaken to allow for a self-sustaining and healthy restored population to be achieved.

### 3.4.5 Planting of maternal lines within sites

While planting strategies are highly variable and heavily informed by the practicalities of the location, scale and target vegetation of restoration projects, some generalised guidance for genetically informed plantings are as follows. To maximise the chance of establishing genetically healthy and self-sustaining restored populations of the target *Eucalyptus* species, we recommend a planting block system is used. Each block should consist of a single species for ease of planting and to maximise the chance of pollination without hybridisation. The block size is dependent on the number of maternal lines and their representation within each block, as all lines should be represented in all blocks, and individual numbers within each block should be even across maternal lines. This maximises the chance that there will be interbreeding between the full suite of maternal lines and all genetic diversity will be represented in future generations of the population. However, the number of representatives of each maternal line can be varied between blocks based upon on-ground factors and planting techniques. Finally, the planting of representatives of maternal lines within each block should be randomised or undertaken using a scheme that minimises the relatedness of neighbouring individuals. Figure 8 below is an example of a randomised block planting design for three species with one representative from each of 20 maternal lines within each block.



| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.2 | 4.1 | 3.4 | 3.5 | 1.3 | 1.2 | 3.5 | 2.5 | 4.4 | 2.4 | 1.5 | 1.2 |
| 1.4 | 4.5 | 2.4 | 1.3 | 4.2 | 1.4 | 1.1 | 4.1 | 4.2 | 3.3 | 4.5 | 4.3 |
| 3.4 | 4.3 | 4.4 | 4.2 | 1.5 | 2.3 | 4.3 | 3.3 | 1.1 | 4.1 | 2.1 | 2.2 |
| 2.5 | 1.2 | 1.5 | 2.1 | 3.2 | 4.5 | 3.4 | 2.2 | 1.4 | 3.5 | 3.2 | 3.1 |
| 3.2 | 2.3 | 3.3 | 1.1 | 3.1 | 2.4 | 2.1 | 4.4 | 3.4 | 1.3 | 2.3 | 2.5 |
| 1.3 | 4.5 | 1.4 | 2.3 | 1.4 | 2.1 | 4.1 | 4.5 | 4.2 | 2.2 | 1.5 | 2.3 |
| 1.2 | 4.1 | 3.4 | 3.3 | 2.5 | 4.3 | 3.4 | 3.2 | 1.2 | 4.5 | 4.1 | 3.3 |
| 3.1 | 2.2 | 1.5 | 4.4 | 1.3 | 3.1 | 2.3 | 1.1 | 2.5 | 1.1 | 3.2 | 4.3 |
| 2.1 | 3.2 | 4.3 | 2.4 | 1.2 | 2.4 | 1.5 | 4.4 | 1.4 | 4.4 | 3.4 | 1.3 |
| 3.5 | 4.2 | 1.1 | 2.5 | 2.2 | 4.2 | 3.5 | 3.3 | 3.1 | 3.5 | 2.1 | 2.4 |
| 4.2 | 4.5 | 1.5 | 2.5 | 3.5 | 4.2 | 2.2 | 1.4 | 2.3 | 4.4 | 3.2 | 4.1 |
| 2.2 | 4.4 | 4.3 | 1.2 | 1.1 | 3.2 | 4.5 | 3.4 | 2.2 | 1.4 | 3.1 | 2.4 |
| 1.1 | 2.3 | 3.2 | 3.1 | 2.4 | 4.4 | 2.5 | 1.3 | 2.5 | 4.2 | 3.4 | 4.5 |
| 3.5 | 2.4 | 4.1 | 3.4 | 1.5 | 3.1 | 4.1 | 4.3 | 1.2 | 1.3 | 3.3 | 2.1 |
| 3.3 | 2.1 | 1.4 | 1.3 | 1.2 | 2.3 | 2.1 | 3.3 | 4.3 | 1.5 | 3.5 | 1.1 |

**Figure 8:** Example of a regular block planting scheme for a three species planting with seed collected using a four site by 5 maternal lines per site scheme. The colour represents the species planted and the numeric code corresponds to the maternal line the seedling originates from. The number of representatives from each maternal lines within blocks can be varied but should always be even across maternal lines to maximise genetic diversity and minimise relatedness between individuals within blocks. The planting of maternal lines within blocks can be randomised or regular in some way that limits the relatedness of neighbouring individuals, but individuals from a single maternal line should not be planted in a subblock together.

# 4. Species specific patterns of diversity and guidelines for use in restoration

## 4.1 How to interpret species-specific figures and tables

In this section we describe species specific patterns of diversity and provide seed collection guidelines for 11 species. Figure captions are self-explanatory; however, we provide a brief introduction below.

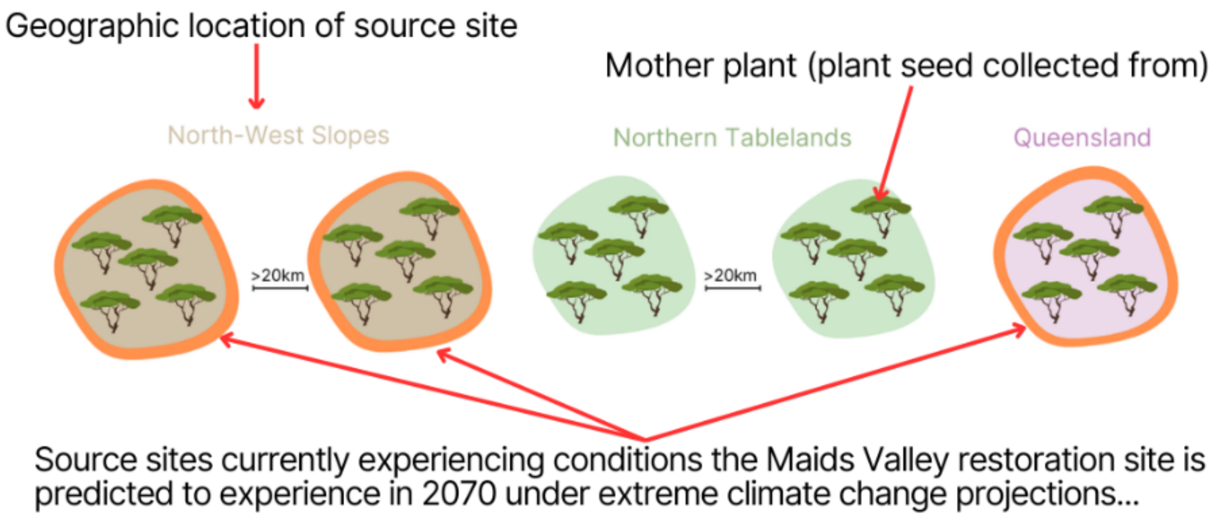
Worth noting, is that given the widespread geneflow and high levels of outcrossing apparent for all target species, genetic neighbourhoods and therefore optimal seed collection areas cover a relatively broad geographic area. This result reflects the focus on eucalypts and leads to limited variation in guidance for seed collection between species, which focusses mostly on ensuring the correct taxon is identified, maximising genetic diversity and minimising the relatedness of individuals planted together. Significantly more variation in population genetic patterns and seed collection guidance is expected for non-eucalypt taxa.

### 4.1.1 Genetic neighbourhood maps

Genetic neighbourhoods are mapped across NSW for each species, with the Maids Valley site identified. The known occurrence of each species (herbarium and survey records) is identified by an ‘X’ and the location of sites sampled for genetic analysis are represented by coloured points as per the individual figure caption. Sites within the same genetic neighbourhood are represented by the same colour, whereas sites from a different genetic neighbourhood or genetic lineage are a different colour. Each map also depicts the areas where current climate conditions match modelled climate conditions at the Maids Valley site in the years 2070-2100 under severe (ssp585 – red area) climate change projections (as per Figure 6).

### 4.1.2 Optimised seed source strategy

A schematic representation of an ideal seed collection strategy, where maternal lines are maintained and at least 90% of observed genetic diversity is captured, is depicted for each species. The minimum collection effort required to source genetically diverse seed suitable for use in restoration of the Maids Valley site (or another site within the same genetic neighbourhood) is depicted. Each shape represents a seed collection site, whereby colour is representative of geographic location. Each tree represents a maternal plant. In all cases seed is sourced from at least five maternal plants within each site. When possible, collection strategies incorporating 50% severe climate ready material are depicted. Source sites surrounded by an orange shape are to be located within areas identified as currently experiencing conditions the restoration site will experience in 2070 under severe (ssp585) climate change projections.



## 4.2 *Eucalyptus albens*

*Eucalyptus albens*, White Box, is one of the defining species of the BGGW and is a common woodland tree from north-western Victoria through the inland slopes and tablelands of NSW and into south-east Queensland, with an isolated population in the southern Flinders Ranges of South Australia (Figure 9). It is characterised by the box type bark of its trunk and large branches, elongated, often cylindrical, buds and fruit, and broad, dull coloured juvenile leaves. Our sampling and recommendations for use of this species in restoration activities is limited to the NSW portion of its distribution, which is contiguous apart from populations in the southern parts of Kosciuszko National Park and in the Southern Highlands between the Wollondilly River and Bullio. The latter of these isolated populations was unsampled in this project and therefore we do not make comment on it. This species has been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects across the species NSW distribution.

### 4.2.1 Species limits and hybridisation

- The existing taxonomic and geographic circumscription of this species, widely accepted amongst botanical authorities, is supported by the genetic data generated in this project.
- We find that this species hybridises primarily with its close relatives, the three species of Grey Box (*E. microcarpa*, *E. moluccana* and *E. woollsiana*).
- Populations ascribed to *E. moluccana* in the North-West Slopes and western edge of the Northern Tablelands regions in northern NSW are genetically intermediate between *E. albens* and *E. microcarpa*, with no genetic relationship to true *E. moluccana*. This suggests hybridisation between *E. albens* and *E. microcarpa* is particularly common in these regions. (See figure 10, section 4.5).
- Despite what has been suggested by previous authors (Hill 1991a), we find no evidence of intergrading of *E. albens* and *E. moluccana* in the Hunter Valley, with sampled sites from the Muswellbrook area being pure *E. albens* and those from the Broke area and Belford NP being pure *E. moluccana*. However, it remains possible there is a narrower intergradation zone between these areas which we have not sampled in this project.
- One *E. albens* x *moluccana* hybrid was observed in the genetic data, originating from Kwiambal NP near the northern end of the North-West Slopes.

### 4.2.2 Genetic structuring and neighbourhoods

- *Eucalyptus albens* showed no genetic structuring across its distribution in NSW (Figure 9), with the geographically isolated population in Kosciuszko National Park showing no signal of genetic isolation from the other sampled sites. This corresponds to the findings of previous genetic studies on the species (Prober and Brown 1994; Murray *et al.* 2019).
- There was very limited genetic divergence between sampled sites across NSW, with a weak isolation-by-distance signal being observed.
- For these reasons, we recognised only a single genetic neighbourhood for this species, including all populations in NSW.

### 4.2.3 Reproductive biology

- Three putatively clonal genets were observed for *E. albens*, each containing two samples from a single site. As this species possesses a lignotuber and may be multi-stemmed, this may be the result from accidental sampling of different parts of the same individual.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.2.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. albens*.
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

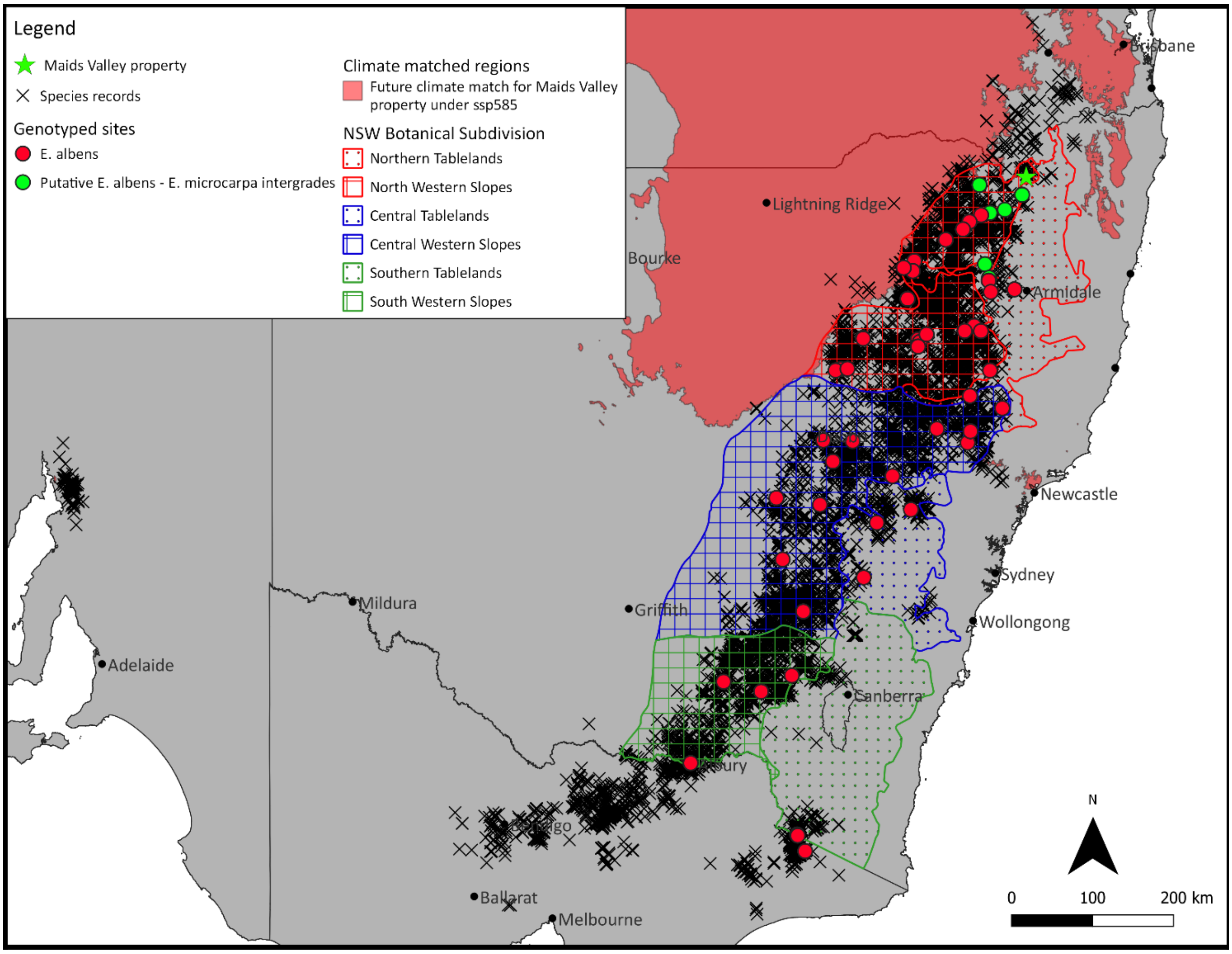


**Figure 9:** Sites sampled for genetic analysis of *Eucalyptus albens* indicating genetic neighbourhoods, with red circles being those belonging to the only neighbourhood observed across the species distribution within NSW and green being sites that were identified as *E. moluccana* in the field but that were closer to, and intermediate between, *E. albens* and *E. microcarpa* genetically. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

### 4.2.5 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- Seed can be sourced from across the species distribution within NSW for use in restoration plantings within the state.
- **Minimum seed sourcing effort**: At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed:
- *Eucalyptus microcarpa* (Grey Box, Inland Box)
- *Eucalyptus moluccana* (Grey Box)
- *Eucalyptus woollsiana* (Narrow-leaved Grey Box)

#### Maids Valley site recommendations

- Optimal seed sourcing for *Eucalyptus albens* when undertaking ecological restoration at the Maids Valley site (Figure 10) would:
- Use five maternal lines collected from each of five seed source sites (25 maternal lines total) at least 20 km apart within the North-West Slopes and Northern Tablelands regions of NSW if sourcing from Queensland is not possible.

- If sourcing from Queensland is possible, one to two seed source sites can be located within that state for *E. albens*. Although we have not genotyped these populations to check their genetic distinctness from NSW populations, this is expected to be low given the distribution wide patterns observed for this species.
- Three of the five seed source sites (again 20 km apart from all other sites) would be in future climate matched regions at the western edge of the species distribution to provide climate change ready provenances.

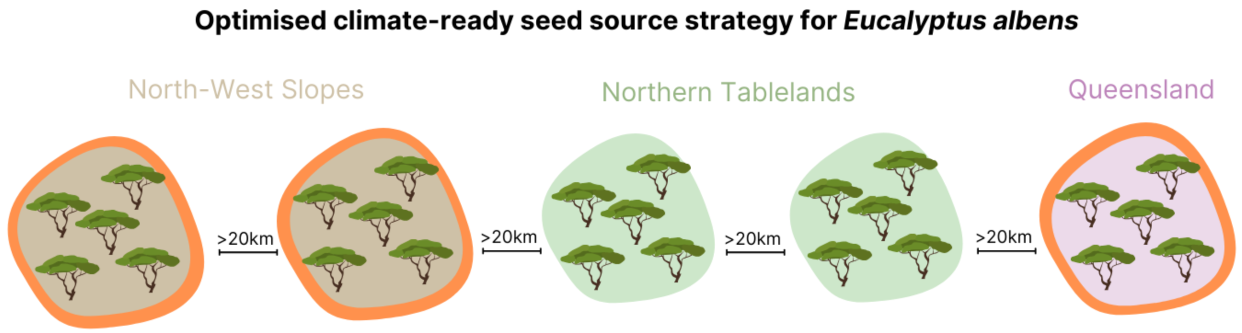


**Figure 10:** Schematic representation of seed collection effort required for *Eucalyptus albens* to source genetically diverse seed (90% of total genetic diversity) suitable for use in restoration of the Maids Valley site, including incorporating severe climate readiness. Each circle represents a collection site and each tree represents a maternal line. Orange circles indicate future climate matched sites (severe scenario). Avoid sites where *Eucalyptus microcarpa, E. moluccana and Eucalyptus woollsiana* co-occur.

## 4.3 *Eucalyptus blakelyi*

*Eucalyptus blakelyi*, Blakely's Red Gum, is one of the defining species of the BGGW and is a common woodland and open forest species of the inland slopes and tablelands of south-eastern Australia, from the Granite Belt of south-east Queensland to the Victorian Midlands. It is characterised by its smooth bark, elongated bud caps (opercula), broad juvenile leaves on square stems, and black, toothed seeds. Our sampling and recommendations for use of this species in restoration activities is limited to the NSW portion of its distribution (Figure 11). The only outlying population of the species in NSW is in Cocoparra National Park and the immediate surrounding areas in the South-West Plains region. This population was not sampled during this project, as it was of low relevance to BGGW restoration activities in the target region. This species has been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects across the species NSW distribution.

### 4.3.1 Species limits and hybridisation

- This species is part of complex of closely related and intergrading species that co-occur across the inland slopes of NSW, differing in the soils and positions in the landscape they occupy. The species which have overlapping distributions and intergrade and/or hybridise include:
  - *Eucalyptus blakelyi:* Abundant on moderate to high fertility soils, generally on flats or gentle hills.
  - *Eucalyptus chloroclada* (Baradine Red Gum): Occurs on deeper sands in the western half of the North-West Slopes region of NSW north into Queensland.
  - *Eucalyptus dealbata* (Tumble-down Red Gum): Occurs on skeletal soils, often those derived from granite or porphyry, primarily in the north and central slopes and tablelands of NSW north into Queensland.
  - *Eucalyptus dwyeri* (Dwyer's Red Gum): Occurs in shallow soils on sandstone ridges in the western half of the Central and North-West slopes and the upper Hunter Valley regions of NSW.
  - *Eucalyptus nandewarica* (Mallee Red Gum): A narrow range endemic restricted to the lower slopes of the Nandewar Range.
- Our genetic data support these taxa as being distinct despite their broad sympatry, frequent intergradation and difficulties differentiating them based upon morphology. We find a strong correlation between soil type and genetic group, suggesting these species are strongly adapted to the soils in which they occur, which may be the driven of their genetic divergence.
  - We found suspected *E. blakelyi* x *dwyeri* hybrids at two sites in the South-West Slopes region.
- In addition to the complex of inland woodland species, *E. tereticornis* (Forest Red Gum), a common and widespread species of woodlands and forests in coastal eastern Australia, is also closely related to *E. blakelyi*. It has long been known that these two species intergrade in areas where their distributions meet, such as the Hunter Valley (Hill 1991b). Our genetic data supports this, with a broad zone of intergradation across the Northern Tablelands, western Hunter Valley and Sydney Basin, and Southern Highlands identified in our genetic data (Figure 12).
  - This intergradation zone has important implications for the restoration of Red Gum populations in these regions, as the populations intermediate between *E. blakelyi* and *E. tereticornis* may have a different intermediate environmental niche than either of the true species.
- Many hybrids with *E. camaldulensis* (River Red Gum) were recorded based upon the genetic data, however, there appears to be a bias towards these hybrids being identified as *E. camaldulensis* rather than *E. blakelyi* based upon morphology.

### 4.3.2 Genetic structuring and neighbourhoods

- When hybrid and introgressed populations were excluded, *E. blakelyi* showed no genetic structuring across its distribution in NSW.
- There was very limited genetic divergence between sampled sites across NSW, with a weak isolation-by-distance signal being observed.
- For these reasons, we recognised only a single genetic neighbourhood for this species, including all populations in NSW.

### 4.3.3 Reproductive biology

- No clonal samples were recorded across the samples of *E. blakelyi*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

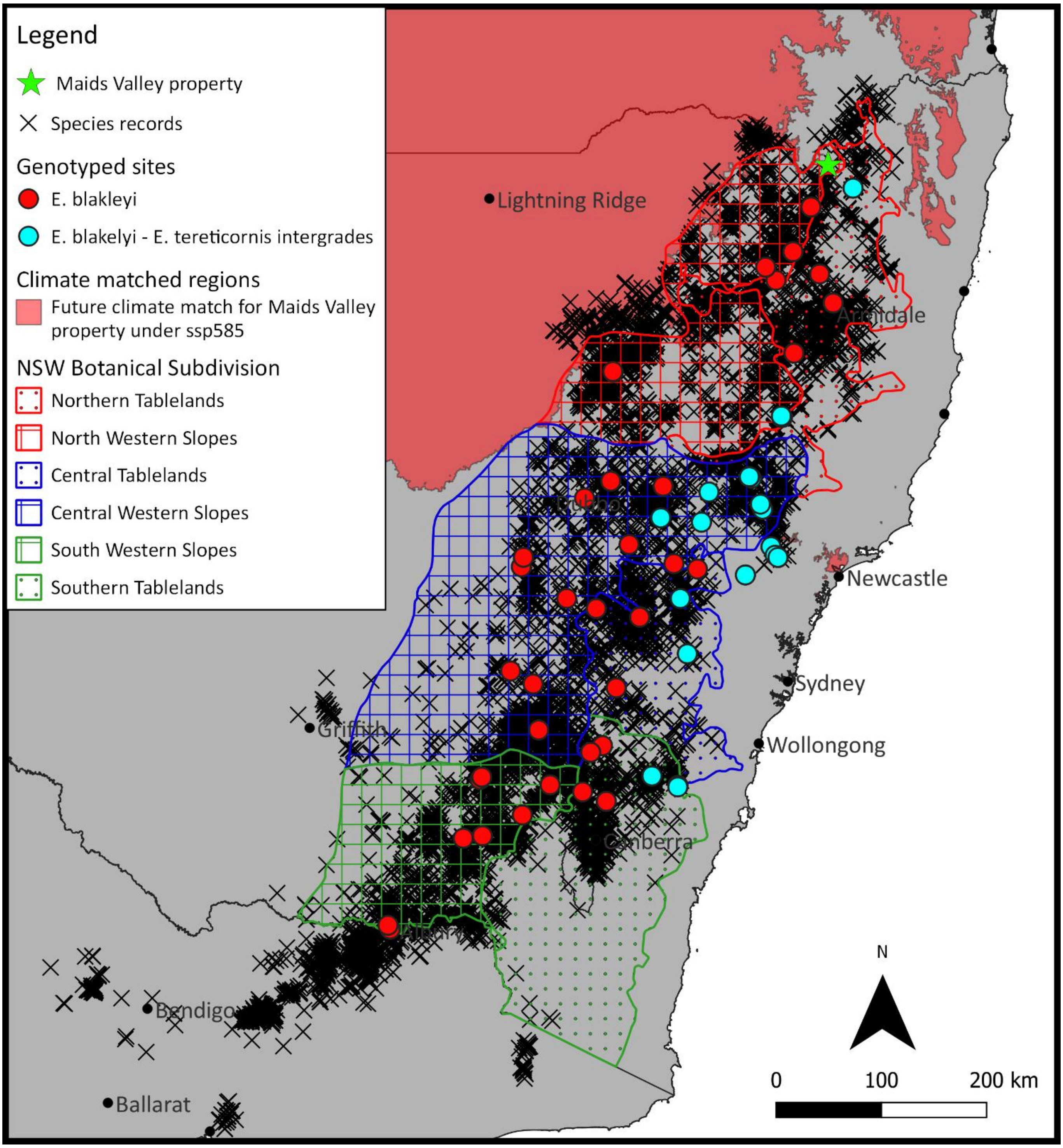


**Figure 11:** Sites sampled for genetic analysis of *Eucalyptus blakelyi* indicating genetic neighbourhoods, with red circles being those belonging to the only neighbourhood observed across the species distribution within NSW. Also shown on the map are sites where samples were identified as genetically intermediate between *E. blakelyi* and *E. tereticornis* (teal circles). Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

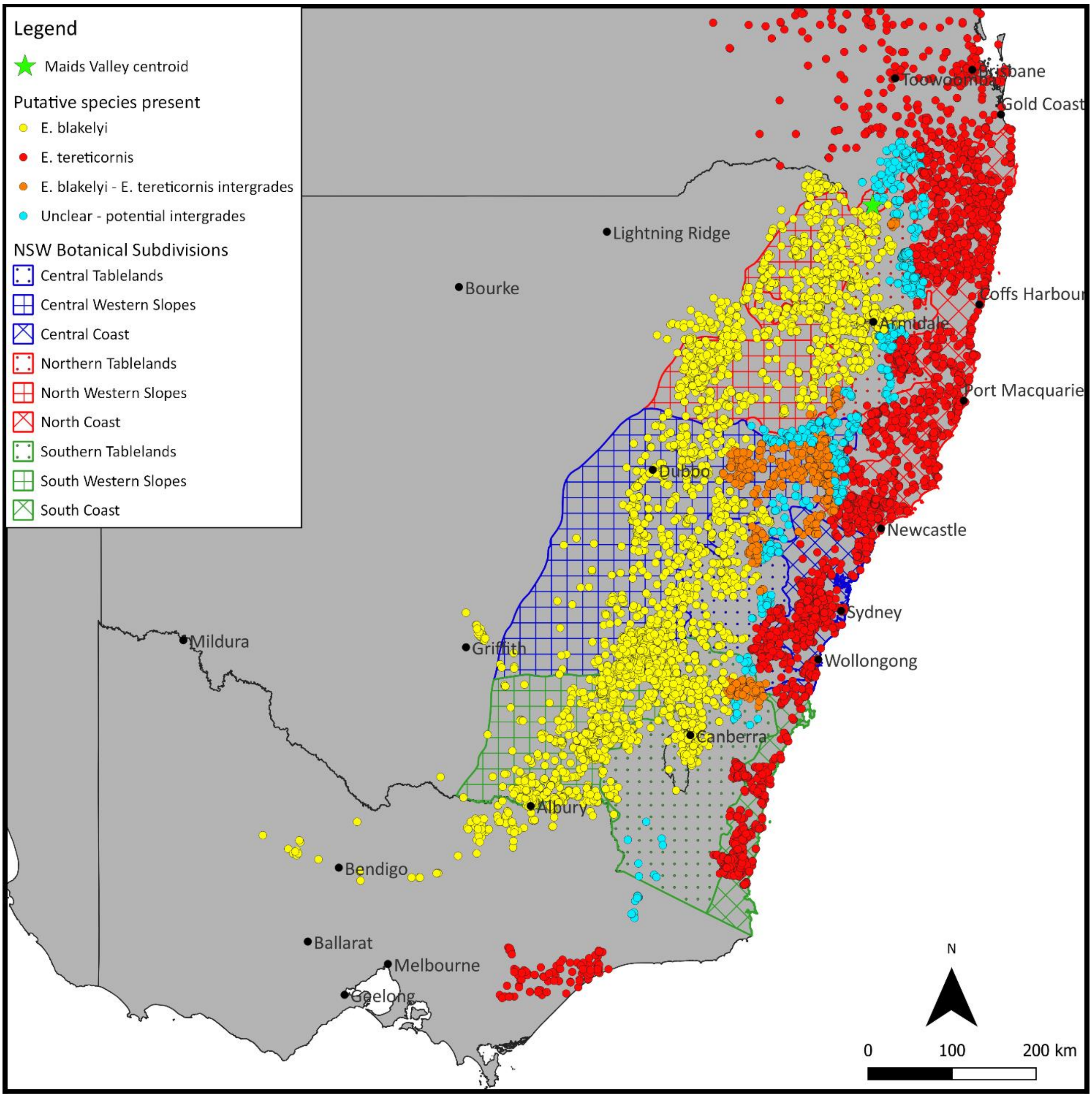


**Figure 12:** Map illustrating our understanding of the relative distributions of *E. blakelyi, E. tereticornis* and intergrades of these two taxa based upon our genetic findings. Yellow points are Atlas of Living Australia records we consider to represent *E. blakelyi*, red points are those we consider to represent *E. tereticornis*, and orange points are the areas we are confident intergrades occur. Teal points represent records in areas we are unable to clarify which species is present, with the potential these areas are also populated by intergrades. Note that Maids Valley is a location right of the edge of the zone where both species may be present.

### 4.3.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. blakelyi.*
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

### 4.3.5 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- Seed can be sourced from across the species distribution within NSW for use in restoration plantings within the state.
- **Minimum seed sourcing effort**: At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.

- Given the division of the landscape by the inland red gum species, seed source sites that are core habitat for *E. blakelyi*, namely soils of at least moderate fertility in flats or depressions, should be chosen to help minimise the chance of hybrid or mis-identified seed being used in restoration.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed:
    - Eucalyptus camaldulensis (River Red Gum)
    - Eucalyptus chloroclada (Baradine Red Gum)
    - *Eucalyptus dealbata* (Tumble-down Red Gum)
    - *Eucalyptus dwyeri* (Dwyer's Red Gum)
- Seedling leaf morphology is moderately diagnostic in the Red Gums, and therefore can be used to assist in screens for hybrid individuals. Seedling differentiation per EUCLID (Brooker *et al.* 2015) is as follows:
    - *Eucalyptus blakelyi:* stems rarely glaucous; leaves ovate to broadly so, 4.8–13 cm long, 2.5–7 cm wide, green, blue-green or rarely glaucous.
    - *Eucalyptus camaldulensis:* stems non-glaucous; leaves narrowly lanceolate, 7.5–16 cm long, 1.3–4.2 cm wide, dull green.
    - *Eucalyptus chloroclada:* stems non-glaucous; leaves ovate, 4–8 cm long, 2–5 cm wide, usually green.
    - *Eucalyptus dealbata:* stems usually glaucous; leaves ovate, deltoid or orbicular, 5–11.5 cm long, 3.3–6.2 cm wide, usually glaucous.
    - *Eucalyptus dwyeri:* stems non-glaucous; leaves narrowly lanceolate to lanceolate, 7–12 cm long, 1–2.5 cm wide, dull green.
- As the intergradation zone between *E. blakelyi* and *E. tereticornis* is very large, all populations within this zone are intermediate between these species and the two species have very similar ecological niches, use of intergrades in restoration plantings on the eastern edge of *E. blakelyi's* distribution reflects natural patterns and should not be avoided.

### Maids Valley site recommendations

- Optimal seed sourcing for *Eucalyptus blakelyi* when undertaking ecological restoration at the Maids Valley site (Figure 13) would:
    - Use five maternal lines collected from each of five seed source sites (25 maternal lines total) at least 20 km apart within the North-West Slopes and Northern Tablelands regions of NSW.
    - Sourcing seed from Queensland is not plausible for *E. blakelyi* as it has an extremely limited distribution in that state.
    - Three of the five seed source sites (again 20 km apart from all other sites) would be in future climate matched regions at the western edge of the species distribution to provide climate change ready provenances.

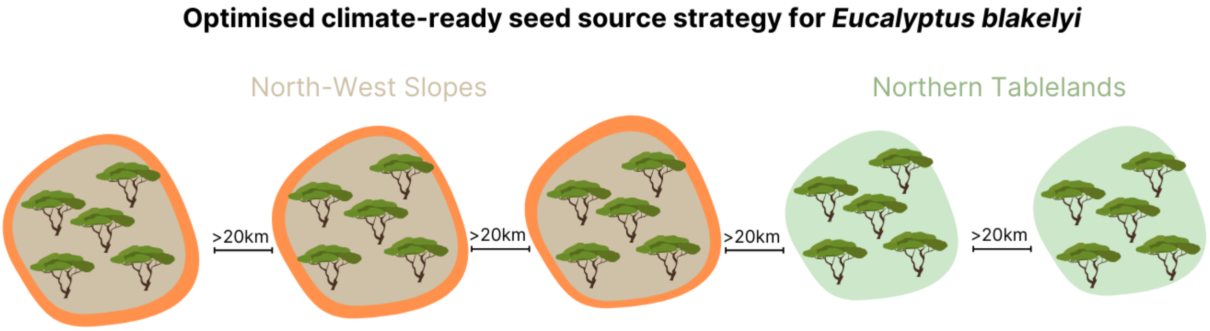


**Figure 13:** Schematic representation of seed collection effort required for *Eucalyptus blakelyi* to source genetically diverse seed (90% of total genetic diversity) suitable for use in restoration of the Maids Valley site, including incorporating severe climate readiness. Each circle represents a collection site and each tree represents a maternal line. Orange circles indicate future climate matched sites (extreme scenario). Avoid sites where *Eucalyptus camaldulensis*, *E. chloroclada*, *E dealbata* and *E. dwyeri* co-occur.

## 4.4 *Eucalyptus melliodora*

*Eucalyptus melliodora*, Yellow Box, is one of the defining species of the BGGW and is a common and abundant species in woodlands of south-eastern Australia. There are scattered populations in Queensland as far north as Carnarvon and Kroombit Tops national parks, with the more contiguous core of the species distribution running from the Darling Downs of Queensland through the inland slopes, tablelands and highlands of NSW and Victoria as far south-west as the Wimmera. Geographically isolated populations of *E. melliodora* occur in the far South Coast and in Kosciuszko National Park, both of which were sampled during this project (Figure 14). A species with highly variable morphology, *E. melliodora* is best recognised by its flaky to box-type bark, the presence of staminodes (non-fertile stamen) in its flowers, and fruit in clusters of seven with a conspicuous staminal ring that is not shed after flowering. This species has been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects across its NSW distribution.

### 4.4.1 Species limits and hybridisation

- The existing taxonomic and geographic circumscription of this species, widely accepted amongst botanical authorities, is supported by the genetic data generated in this project.
- We find that the closest relative of *E. melliodora* is *E. bosistoana*, which has an overlapping distribution in the Southern Highlands and South Coast of NSW. However, no hybrids between these species were observed in the genetic data.
- *Eucalyptus melliodora* is primarily known to hybridise with two ironbark species in NSW, *E. crebra* (Narrow-leaved Ironbark) and *E. sideroxylon* (Mugga Ironbark), and we found genetic evidence for hybrids with the former at multiple sites where they co-occurred. For this reason, sites where either ironbark species is present should be avoided when sourcing *E. melliodora* seed for use in restoration to limit the amount of hybrid material used.

### 4.4.2 Genetic structuring and neighbourhoods

- *Eucalyptus melliodora* showed no genetic structuring across its distribution in NSW (Figure 15), with the geographically isolated populations in Kosciuszko National Park and on the South Coast showing no signal of genetic isolation from the other sampled sites. This corresponds to the findings of previous genetic studies on the species (Supple *et al.* 2018).
- There was very limited genetic divergence between sampled sites across NSW, with a weak isolation-by-distance signal observed.
- For these reasons, we recognised only a single genetic neighbourhood for this species, including all populations in NSW.

### 4.4.3 Reproductive biology

- One putatively clonal genet was observed for *E. melliodora*, containing two samples from a single site. As this species possesses a lignotuber and may be multi-stemmed, this may be the result from accidental sampling of different parts of the same individual.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.4.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. melliodora.*
- This diversity was evenly spread across the landscape, however, our single sampled site on the South Coast of NSW showed low genetic diversity. This suggests that while this population is not genetically divergent from others of the species, it may have experienced a genetic bottleneck due to its small size leading to low genetic diversity. For this reason, ecological restoration of the species in the region should give extra consideration regarding levels of genetic diversity in restored populations.

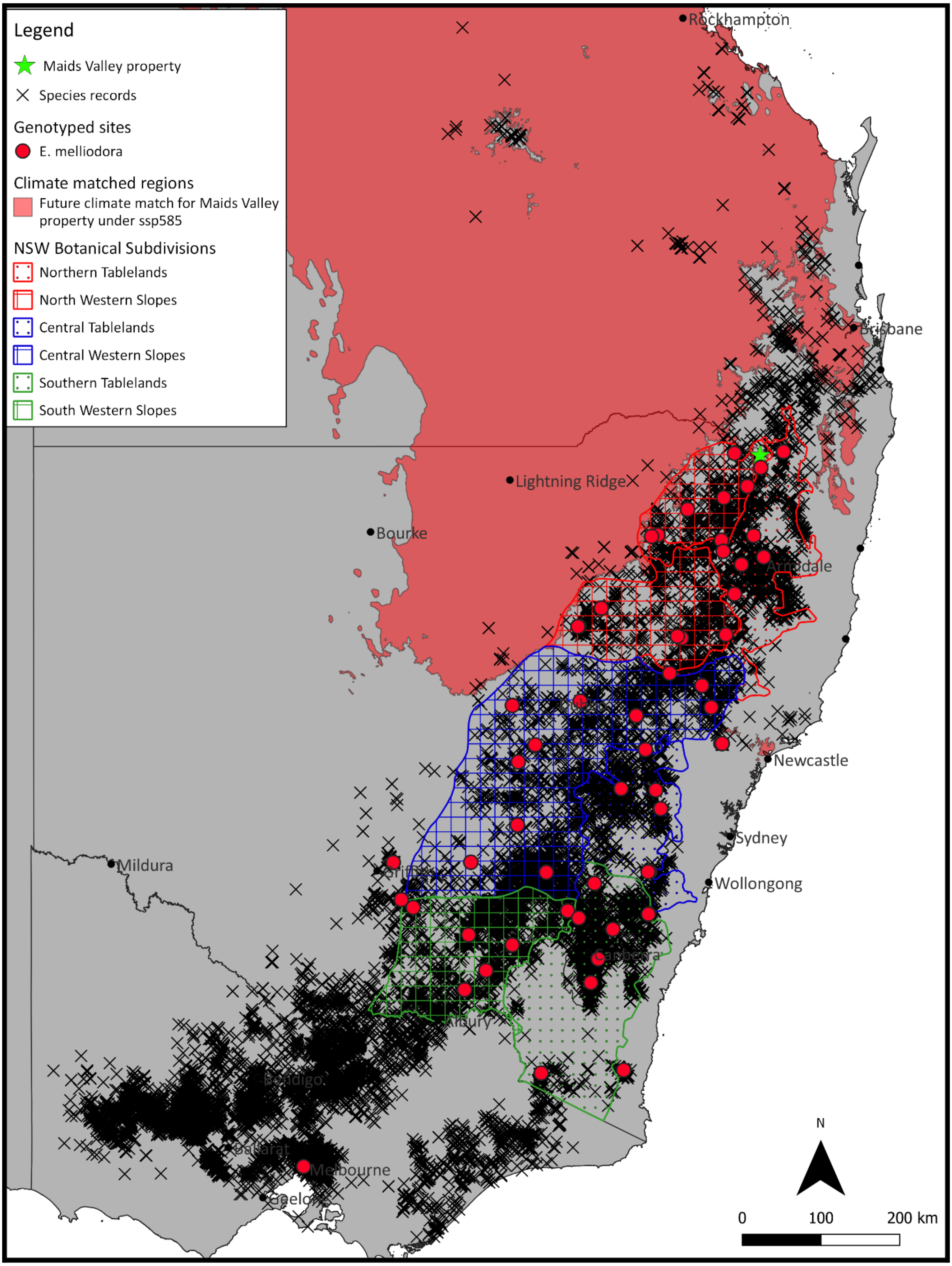


**Figure 14:** Sites sampled for genetic analysis of *Eucalyptus melliodora* indicating genetic neighbourhoods, with red circles being those belonging to the only neighbourhood observed across the species distribution within NSW. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

### 4.4.5 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- Seed can be sourced from across the species distribution within NSW for use in restoration plantings within the state.
- **Minimum seed sourcing effort**: At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed:
  - *Eucalyptus crebra* (Narrow-leaved Ironbark)
  - Eucalyptus sideroxylon (Mugga Ironbark)

#### Maids Valley site recommendations

- Optimal seed sourcing for *Eucalyptus melliodora* when undertaking ecological restoration at the Maids Valley site (Figure 15) would:
  - Use five maternal lines collected from each of five seed source sites (25 maternal lines total) at least 20 km apart within the North-West Slopes and Northern Tablelands regions of NSW.
  - If sourcing from Queensland is possible, two or three seed source sites can be located within that state for *E. melliodora*. Although we have not genotyped these populations to check their genetic distinctness from NSW populations, this is expected to be low given the distribution wide patterns observed for this species.
  - Three of the five seed source sites (again 20 km apart from all other sites) would be in future climate matched regions at the western edge of the species distribution to provide climate change ready provenances.

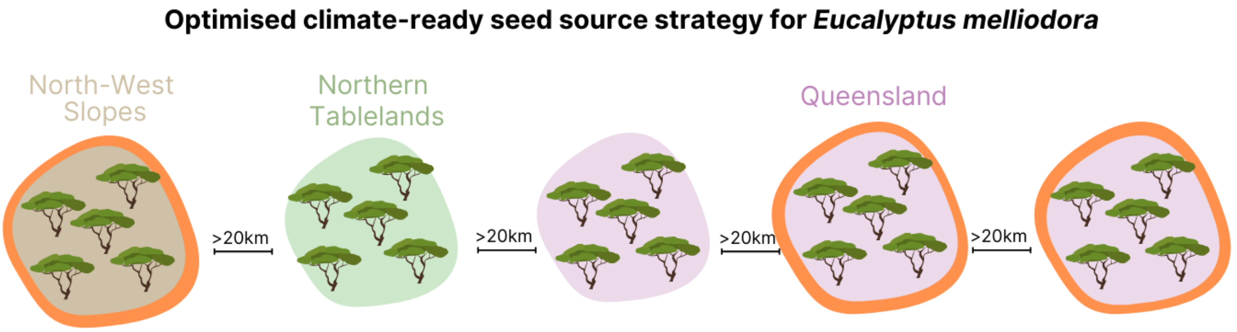


**Figure 15:** A schematic representation of seed collection effort required for *Eucalyptus melliodora* to source genetically diverse seed (90% of total genetic diversity) suitable for use in restoration of the Maids Valley site, including incorporation of severe climate readiness. Each circle represents a collection site and each tree represents a maternal line. Orange circles indicate future climate matched sites (extreme scenario). Note that other options with less sites from Qld, and consequently more sites from North-West Slopes, also meet recommendations. Avoid sites where *Eucalyptus crebra*, and *E. sideroxylon* co-occur.

## 4.5 *Eucalyptus moluccana*

Under the definition of the BGGW used in its conservation listing, *E. moluccana,* Grey Box, is considered a defining species only in the Nandewar bioregion of northern NSW. The species is considered to occur in coastal valley woodlands from Nowra in NSW to Atherton in Queensland, with inland populations in NSW in the Northern Tablelands and North-West Slopes regions. As explained below, genetic data shows the species is significantly less widespread in northern NSW than previously understood (Figures 16 and 17) and its role as a defining species of the BGGW needs to be reconsidered. This species can be recognised by its box type bark on the trunk, wide, glossy green adult leaves, and barrel shaped fruit.

### 4.5.1 Data generation issues

- DNA extraction from this species proved difficult using the standard laboratory protocols employed by Diversity Array Technologies, and the timeline of this project limited options to resolve this issue.
  - This led to only ~70% of samples being successfully being genotyped, and a high proportion of sampling sites not being represented by 5 or more samples, limiting what analyses could be employed.
  - Genetic boundaries and structuring, and levels of genetic diversity are established using a reduced number of sampling sites, and seed sourcing guidelines developed.
  - This species could not be made available using the standard model employed on the Restore and Renew webtool.

### 4.5.2 Species limits and hybridisation

- We find that *E. moluccana* is the most genetically distinct species amongst the Grey Box species, with *E. albens*, *E. microcarpa* and *E. woollsiana* being more closely related to one another than to *E. moluccana*.
- Genetic data shows this species' distribution has been overestimated by surveys misidentifying *E. albens* and *E. microcarpa*, or intergrades thereof, as *E. moluccana*, particularly in northern NSW.
  - It is limited in how far west in occurs in northern NSW to the very eastern edge of the Northern Tablelands.
  - All records further west may represent *E. albens – E. microcarpa* intergrades that have a morphology very similar to *E. moluccana*.
  - The species appears to occur further west in south-east Queensland and in the very edge of northern NSW between Tenterfield and Bonshaw, however more targeted work is needed to establish its exact distributional boundaries.
- No hybrid zone between *E. albens* and *E. moluccana* was observed in the Hunter Valley despite previous observations based upon morphology.
- A single hybrid between *E. moluccana* and *E. albens* was recorded at Kwiambal NP.
- A genetically divergent site at Dangars Falls in the New England Tablelands may represent an area of hybridisation, although a secondary parent could not be identified.

### 4.5.3 Genetic structuring and neighbourhoods

- Once misidentified sites were removed, *E. moluccana* showed no genetic structuring across its distribution in NSW.
- There was very limited genetic divergence between sampled sites across NSW, with a weak isolation-by-distance signal being observed.
- For these reasons, we recognised only a single genetic neighbourhood for this species, including all populations in NSW referrable to this species using genetic data.

### 4.5.4 Reproductive biology

- No clonal samples were recorded across the samples of *E. moluccana*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.5.5 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. moluccana.*
- This diversity was evenly spread across the landscape, although the most southerly population sampled from Falls Creek, south of Nowra, showed evidence of genetic bottlenecking, a reduction in genetic diversity due to a small population size.

### 4.5.6 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- Seed can be sourced from across the species distribution within NSW for use in restoration plantings within the state.
- **Minimum seed sourcing effort:** At least 5 maternal lines from 4 distinct sites (20 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed:
  - Eucalyptus albens (White Box)
  - *Eucalyptus microcarpa* (Grey Box, Inland Box)
  - *Eucalyptus woollsiana* (Narrow-leaved Grey Box)

#### Maids Valley site recommendations

- Given the findings outlined above showing many populations attributed to *E. moluccana* in Northern NSW do not genetically align with this species, further field surveys and/or genetic testing may be needed to determine if *E. moluccana* is native to the Maids Valley area.
- If *E. moluccana* is to be planted at the Maids Valley site, optimal seed sourcing for the species when undertaking ecological restoration (Figure 18) would:
  - Use five maternal lines collected from each of four seed source sites (20 maternal lines total) at least 20 km apart within the North- Coast and Northern Tablelands regions of NSW.
  - If sourcing from Queensland is possible, two seed source sites can be located within that state for *E. moluccana*. Although we have not genotyped these populations to check their genetic distinctness from NSW populations, this is expected to be low given the distribution wide patterns observed for this species.
  - As there are no populations genetically assignable to *E. moluccana* in climate matched regions of NSW, two of the four seed source sites (again 20 km apart from all other sites) would be in future climate matched regions in Queensland if it is possible to source seed interstate.

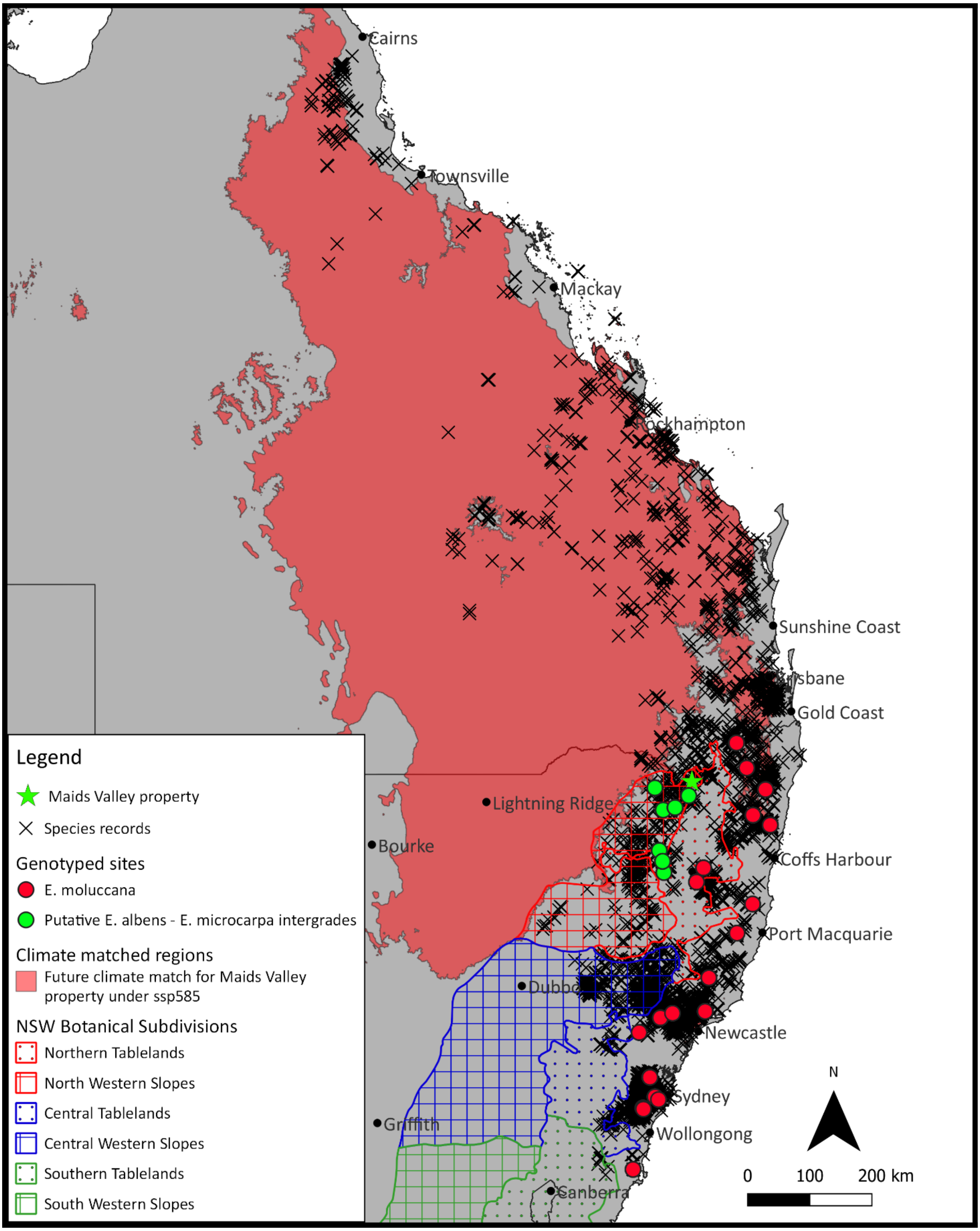


**Figure 16:** Sites sampled for genetic analysis of *E. moluccana* indicating genetic neighbourhoods, with red circles being those belonging to the only neighbourhood observed across the species distribution within NSW. Green circles represent sites where samples were collected as *E. moluccana* but were genetically intermediate between *E. albens* and *E. microcarpa*. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

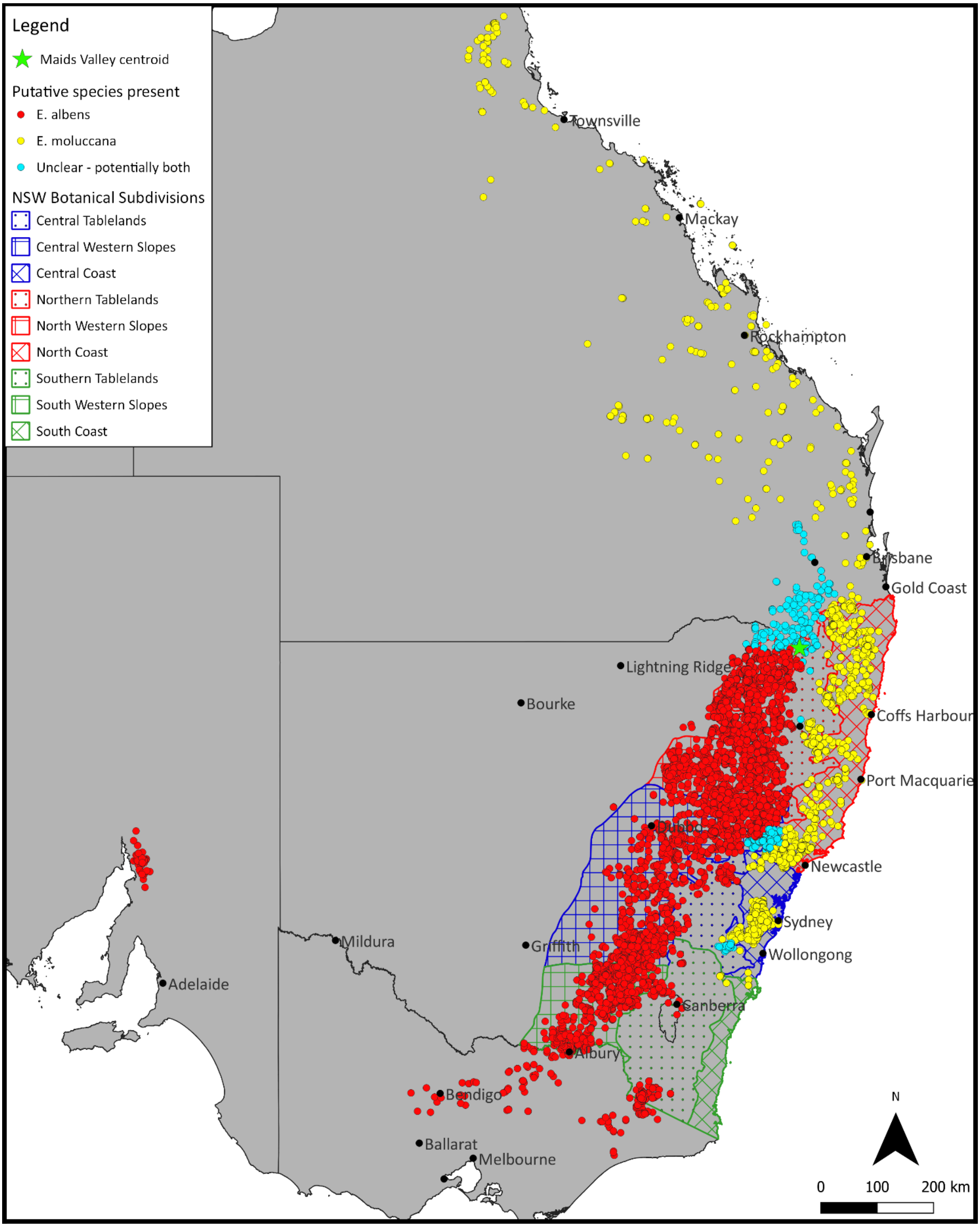


**Figure 17:** Map illustrating our understanding of the relative distributions of *E. albens* and E. *moluccana* based upon our genetic findings. Yellow points are Atlas of Living Australia records we consider to represent *E. moluccana*, red points are those we consider to represent *E. albens* or close relatives thereof, and teal points represent records in areas we are unable to clarify which species is present, with the potential both are present and hybridising. Note that Maids Valley is a location right of the edge of the zone where both species may be present.

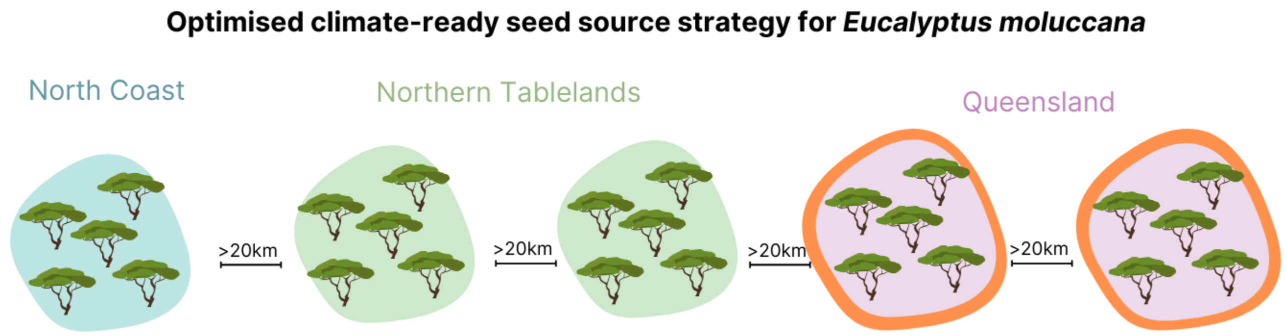


**Figure 18:** If *Eucalyptus moluccana* is confirmed native to the Maids Valley area, a schematic representation of seed collection effort required to source genetically diverse seed (90% of total genetic diversity) suitable for use in restoration, incorporating severe climate readiness. Each circle represents a collection site and each tree represents a maternal line. Orange circles indicate future climate matched sites (extreme scenario). Note that other options with less sites from Queensland, and consequently more sites from North-West Slopes, also meet recommendations. Avoid sites where *Eucalyptus albens*, *E. microcarpa* and *E. woollsiana* co-occur.

## 4.6 *Eucalyptus bridgesiana*

*Eucalyptus bridgesiana*, Apple Box, is a species associated with the BGGW, being widespread in grassy woodlands on slopes with shallower soils from Gippsland in eastern Victoria to the Granite Belt in south-east Queensland. Being associated with slopes and upland areas, *E. bridgesiana* is absent from the Hunter Valley and Liverpool Plain, which form a gap in its distribution. An isolated population is known from the Warrumbungle Range of central NSW, that was not sampled during this project. The species can be identified by its rough, tessellated bark, green crown leaves, fruit that exceed 5 mm in length and subopposite juvenile leaves. This species has been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects across its NSW distribution.

### 4.6.1 Species limits and hybridisation

- The existing taxonomic and geographic circumscription of this species, widely accepted amongst botanical authorities, is supported by the genetic data generated in this project.
- *Eucalyptus* sp. Howes Swamp Creek from Wollemi N.P. has recently been resolved as a *E. bridgesiana* x *viminalis* hybrid swarm (Bell *et al.* 2022), showing these species can infrequently hybridise.
- One hybrid individual with *E. goniocalyx* (Long-leaved Box, Bundy) was observed in the genetic data, although overall, there is no evidence this species frequently hybridises with any other taxon.
- Two sampled sites misidentified as *E. bridgesiana* were reidentified as *E. nova-anglica* (New England Peppermint*)* based on genetic data. This species, while not closely related to, nor hybridising with *E. bridgesiana*, occurs in similar woodland habitat in the Northern Tablelands and can be difficult to differentiate from *E. bridgesiana* based upon morphology, especially if juvenile leaves (opposite in *E. nova-anglica* vs alternate to sub-opposite in *E. bridgesiana*) are not present.

### 4.6.2 Genetic structuring and neighbourhoods

- *Eucalyptus bridgesiana* showed two genetic neighbourhoods in NSW, separated by the Hunter Valley (Figure 19):
  - A southern genetic neighbourhood from the Gulgong-Wollar region in the Central Tablelands south to the Victorian border, including populations both east and west of the Australian Alps.
  - A northern genetic neighbourhood from the Liverpool Range north through the Northern Tablelands and into the Granite Belt of South-east Queensland.
- As the outlying population in the Warumbungles was not sampled in this project, it cannot be assigned to either genetic neighbourhood.

### 4.6.3 Reproductive biology

- No clonal samples were recorded across the samples of *E. bridgesiana*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.6.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. bridgesiana.*
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

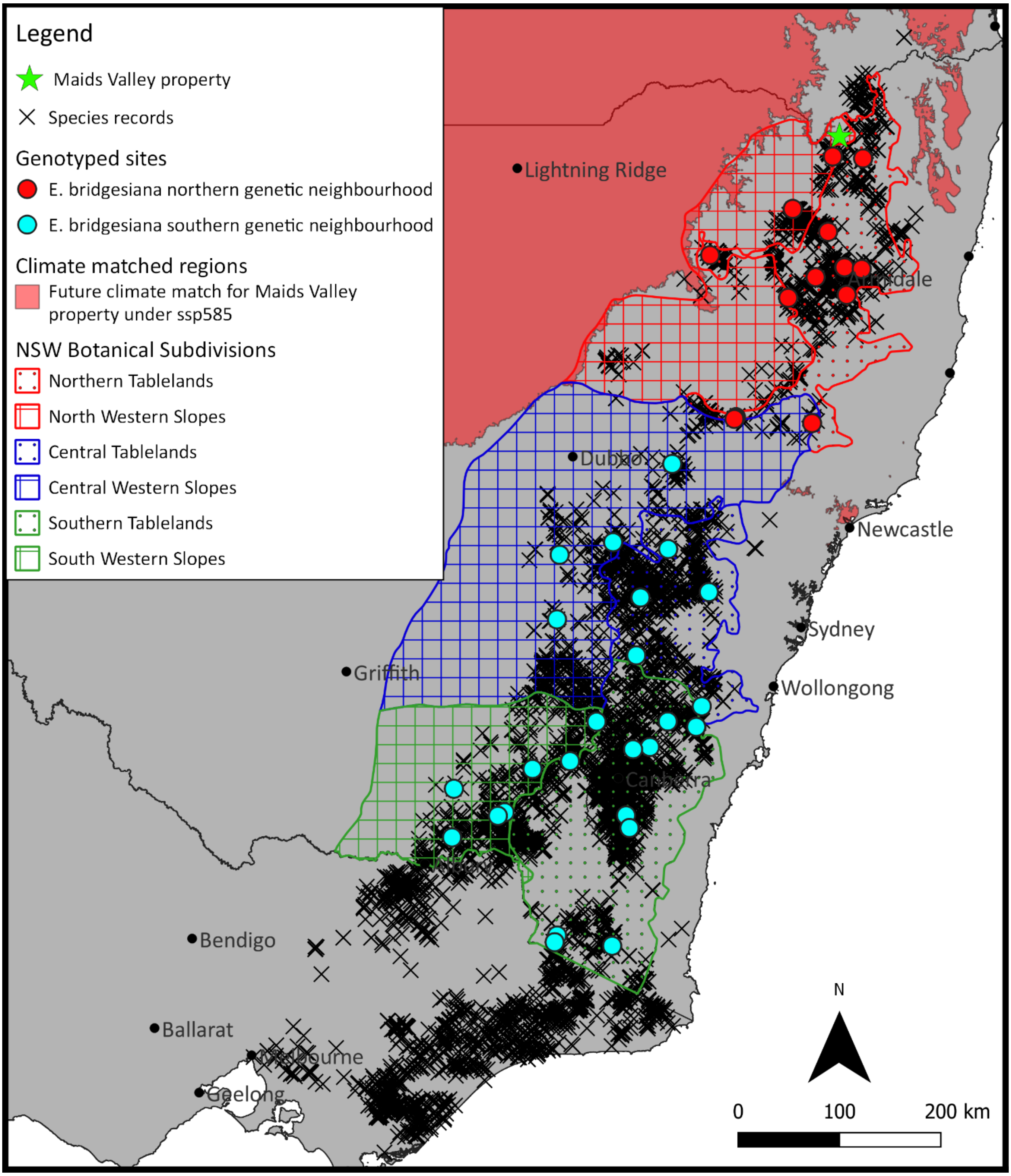


**Figure 19:** Sites sampled for genetic analysis of *Eucalyptus bridgesiana* indicating genetic neighbourhoods. Teal circles are those belonging to the southern neighbourhood and red circles those belonging to the northern neighbourhood, which are separated by the Hunter Valley. Due to a lack of sampling, the isolated population in the Warrumbungles cannot be assigned to either neighbourhood. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

## 4.6.5 Seed sourcing for ecological restoration

### Generalised species wide recommendations

- Seed can be sourced from across the species distribution within NSW for use in restoration plantings within the state.
- Minimum seed sourcing effort:
  - **Northern genetic neighbourhood**: At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
  - **Southern genetic neighbourhood**: At least 5 maternal lines from 6 distinct sites (30 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Despite occasional hybrids being observed, no other species are known to commonly hybridise with E. bridgesiana and therefore there are no co-occurring species that should impact site choice.
- In the Northern Tablelands care needs to be taken to avoid the morphologically similar *E. nova-anglica* when collecting seed. These species can be separated in the seedling stage by the position of leaves on the stems:
  - *Eucalyptus bridgesiana*: alternate to sub-opposite seedling leaves.
  - *Eucalyptus nova-anglica*: strictly opposite seedling leaves.

### Maids Valley site recommendations

- Optimal seed sourcing for *Eucalyptus bridgesiana* when undertaking ecological restoration at the Maids Valley site (Figure 20) would:
  - Use five maternal lines collected from each of five seed source sites (25 maternal lines total) at least 20 km apart within the Northern Tablelands region of NSW.
  - Sourcing seed from Queensland is not plausible for *E. bridgesiana* as it has an extremely limited distribution in that state.
  - Utilising the above seed sourcing scheme to capture high genetic diversity and adaptive potential will be very important for *E. bridgesiana* site as no climate-matched provenances exist for the Maids Valley site for this species.

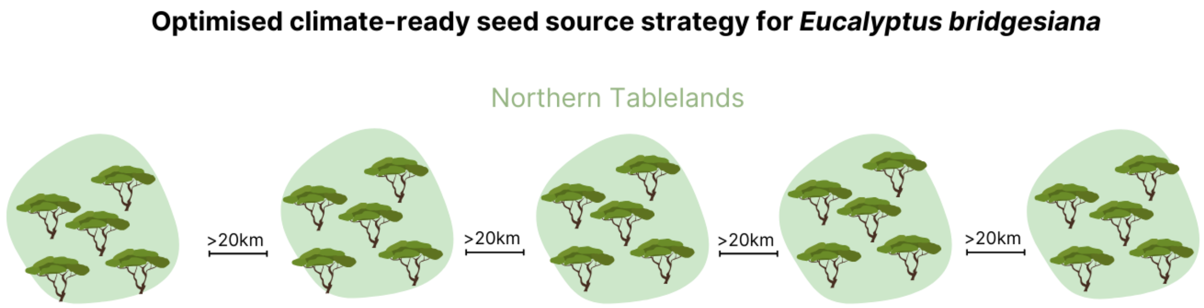


**Figure 20:** A schematic representation of seed collection effort required for *Eucalyptus bridgesiana* to source genetically diverse seed (90% of total genetic diversity) suitable for use in restoration of the Maids Valley site. Each circle represents a collection site and each tree represents a maternal line. No climate-matched provenances are available. Avoid the morphologically similar *E. nova-anglica.*

## 4.7 *Eucalyptus caliginosa*

*Eucalyptus caliginosa*, New England Stringybark, is a common and locally dominant member of open woodland communities in the Northern Tablelands of NSW and Granite Belt of Queensland, often found growing in association with BGGW. The species distribution is largely contiguous, with small outlying populations to its south on the Liverpool Range (Figure 21). It is recognisable by its fibrous rough bark to the small branches, broad adult leaves with numerous small island oil glands, pedicellate, hemispherical fruit, and juvenile leaves which are scabrid on the midrib, margin, and petiole only. This species has been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects across its NSW distribution.

### 4.7.1 Species limits and hybridisation

- *Eucalyptus caliginosa* is a species of stringybark, a group of *Eucalyptus* species that are difficult to differentiate based upon morphology, especially if all parts of the plant (leaves, buds, fruits and seedlings) are not available.
- The existing taxonomic and geographic circumscription of this species is broadly supported by the genetic data generated in this project.
- *Eucalyptus eugenioides*, Thin-leaved Stringybark, is closely related to *E. calignosa*, and differentiating these species can be difficult, however *E. eugenioides* occurs further east than *E. caliginosa* on the Great Dividing Range in northern NSW.
    - We find that the distribution of this species extends further south than previously suggested by some authorities, with populations sometimes attributed to *E. eugenioides* on the Liverpool Range found to be better placed within *E. caliginosa* based on our genetic data (Figure 22).
    - The stabilised hybrid *E.* x *conjuncta*, known only from the vicinity of Murrundi in the Upper Hunter, is also found to likely be an *E. caliginosa* x *E. sparsifolia* rather than *E. eugenioides* x *E. sparsifolia* hybrid, as previously suggested.
- Hybrid individuals between *E. caliginosa* and both *E. williamsiana* and *E. laevopinea* were recorded in the Northern Tablelands region.

### 4.7.2 Genetic structuring and neighbourhoods

- *Eucalyptus caliginosa* showed no genetic structuring across its distribution in NSW (Figure 20), with the geographically isolated populations on the Liverpool Range showing no signal of genetic isolation from the other sampled sites.
- There was very limited genetic divergence between sampled sites across NSW, with a weak isolation-by-distance signal being observed.
- For these reasons, we recognised only a single genetic neighbourhood for this species, including all populations in NSW.

### 4.7.3 Reproductive biology

- No clonal samples were recorded across the samples of *E. caliginosa*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.7.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. caliginosa.*
- This diversity was evenly spread across the landscape, apart from the most northly sampling site from near Maryland National Park which showed notably low genetic diversity and evidence for inbreeding.

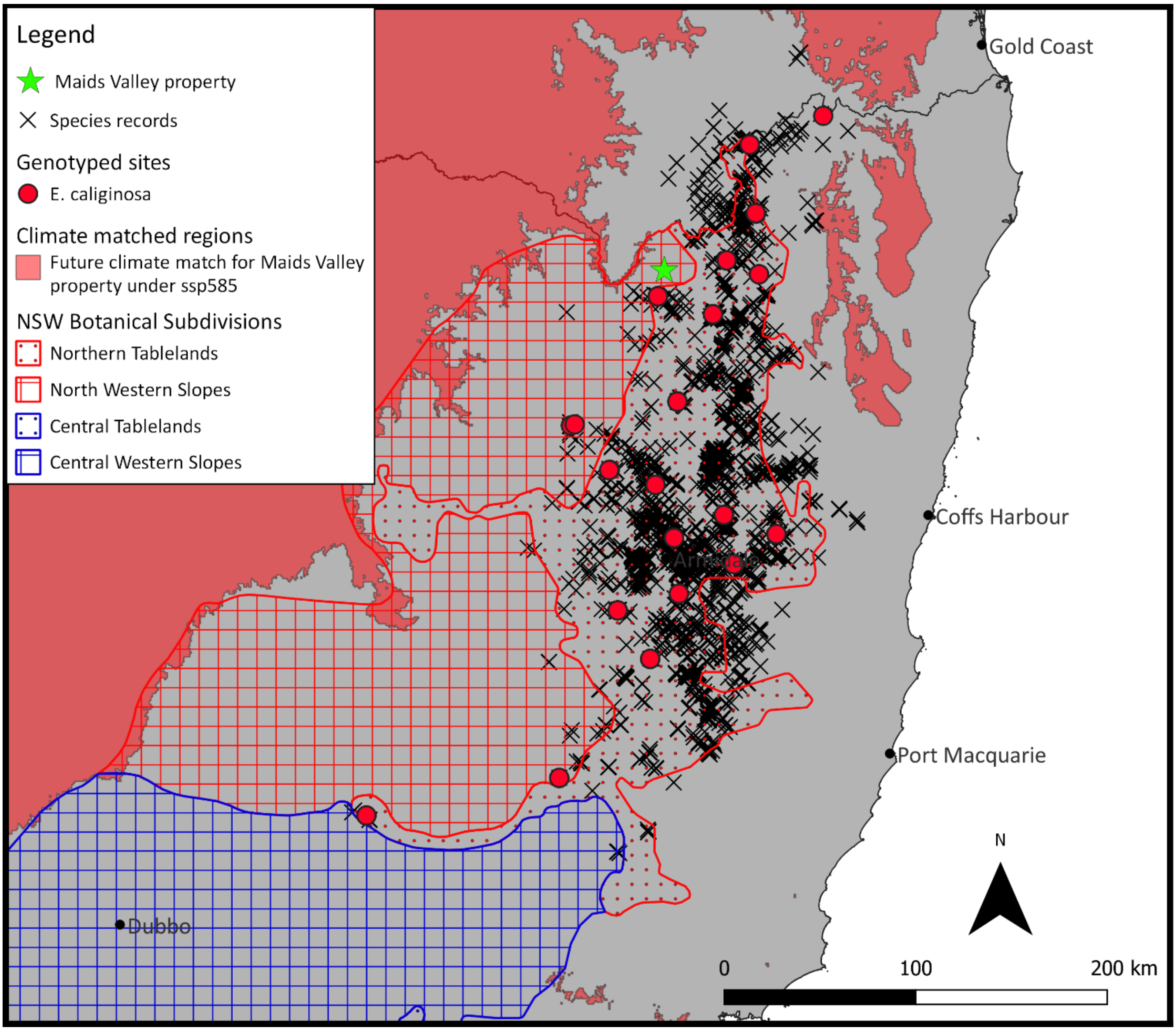


**Figure 21:** Sites sampled for genetic analysis of Eucalyptus caliginosa indicating genetic neighbourhoods, with red circles being those belonging to the only neighbourhood observed across the species distribution within NSW. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

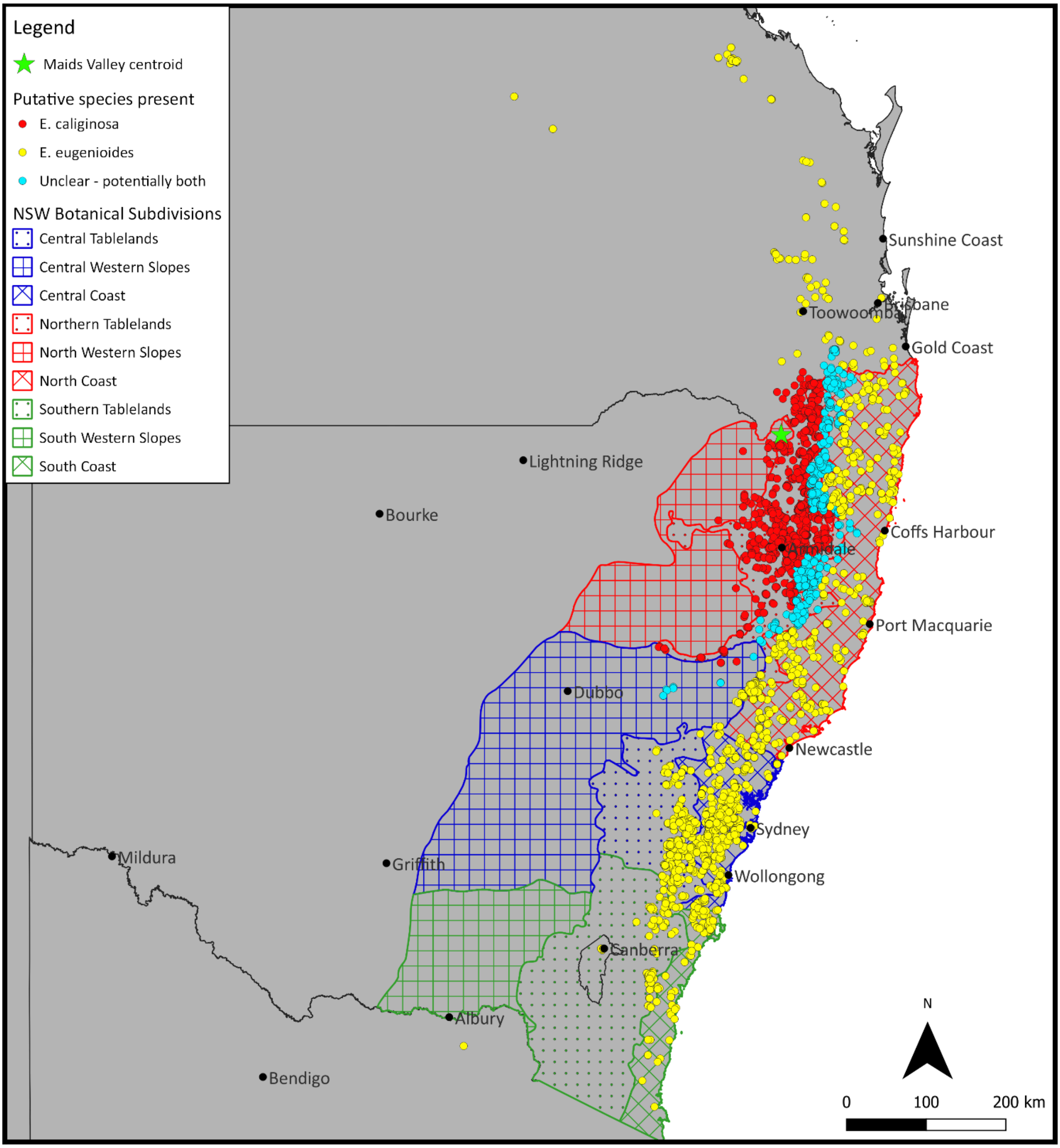


Figure 22: Map illustrating our understanding of the relative distributions of *E. caliginosa* and *E. eugenioides* based upon our genetic findings. Red points are Atlas of Living Australia records we consider to represent *E. caliginosa,* red points are those we consider to represent *E. eugenioides*, and teal points represent records in areas we are unable to clarify which species is present, with the potential both are present and hybridising.

## 4.7.5 Seed sourcing for ecological restoration

### Generalised species wide recommendations

- Seed can be sourced from across the species distribution within NSW for use in restoration plantings within the state.
- **Minimum seed sourcing effort:** At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Care should be taken when seed is collected to make sure the correct stringybark species is being collected. Seedling morphology is diagnostic between the stringybarks, and therefore can be used to assist in screens for misidentified or hybrid seed. Seedling differentiation between the key species per EUCLID (Brooker *et al.* 2015) is as follows:
  - *Eucalyptus caliginosa*: stems densely hairy, seedling leaves initially sessile and opposite, quickly becoming petiolate and alternate by node 8, leaves ovate (to 8 cm long and 4.5 cm wide) and discolourous and only ever hairy on margins, petiolate and lower midrib.
  - *Eucalyptus eugenioides*: stems densely hairy, seedling leaves always petiolate, transitioning from opposite to alternate by node 10, leaves ovate to lanceolate (to 10 cm long and 4.5 cm wide) and discolourous with sparse hairs on both surfaces and margins.
  - *Eucalyptus laevopinea*: stems initially densely hairy becoming smooth by node 8, seedling leaves sessile and opposite to node 6, then alternate and petiolate, leaves broadly lanceolate (to 13 cm long and 5.5 cm wide) and initially discolourous and hairy on margins and midrib before quickly transitioning to concolorous and hairless by node 6.
  - *Eucalyptus williamsiana*: stems initially densely hairy becoming smooth, seedling leaves subsessile to shortly pedicellate and opposite to node 5, then alternate, leaves ovate (to 11 cm long and 6 cm wide) and initially discolourous and hairy before quickly transitioning to concolorous and hairless.
  - *Eucalyptus youmanii*: stems densely hairy, seedling leaves opposite to node 7, then alternate, always petiolate, leaves ovate to lanceolate (to 11cm long and 5 cm wide) and discolourous with hairs on surface, margins, and midrib.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed: *Eucalyptus eugenioides*, *E. laevopinea*, *E. williamsiana* and *E. youmanii.*

### Maids Valley site recommendations

- Optimal seed sourcing for *Eucalyptus caliginosa* when undertaking ecological restoration at the Maids Valley site (Figure 23) would:
  - Use five maternal lines collected from each of five seed source sites (25 maternal lines total) at least 20 km apart within the Northern Tablelands region of NSW.
  - Sourcing seed from Queensland is not plausible for *E. caliginosa* as it has an extremely limited distribution in that state.
  - Utilising the above seed sourcing scheme to capture high genetic diversity and adaptive potential will be very important for *E. caliginosa* site as no climate-matched provenances exist for the Maids Valley site for this species.

**Optimised climate-ready seed source strategy for *Eucalyptus caliginosa***

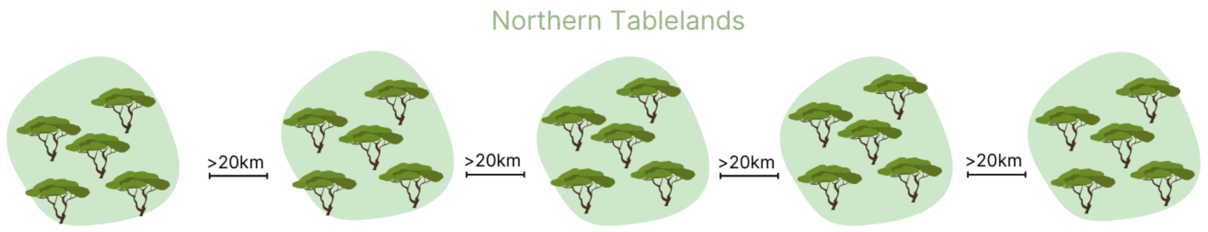


**Figure 23:** A schematic representation of seed collection effort required for *Eucalyptus caliginosa* to source genetically diverse seed (90% of total genetic diversity) suitable for use in restoration of the Maids Valley site, including incorporation of severe climate readiness. Each circle represents a collection site and each tree represents a maternal line. No climate-matched provenances are available. Avoid sites where *Eucalyptus eugenioides*, *E. laevopinea*, *E. youmanii* and *E. williamsiana* co-occur.

## 4.8 *Eucalyptus goniocalyx*

*Eucalyptus goniocalyx*, Long-leaved Box/Bundy, is a species associated with the BGGW, being common in open woodlands on drier slopes with shallow soils. Three subspecies are recognised, however only *E. goniocalyx* subsp. *goniocalyx* is present in New South Wales, from Goulburn River National Park south on the inland slopes and tablelands into Victoria (Figure 24). Outlying isolated populations are present on the Liverpool Range, Warrumbungle Range, and to the south-west of the Australian Alps in Kosciuszko National Park. This species can be identified by its loose, fibrous rough bark on the trunk and largest branches, long, glossy green adult leaves, large, sessile fruit, and its orbicular, opposite juvenile leaves. Populations of this species south of the Hunter Valley have been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects within the area.

### Species limits and hybridisation

- *Eucalyptus goniocalyx* is member of *Eucalyptus* subseries *Clivosae*, a group in which species circumscriptions and boundaries are controversial among botanical authorities.
- *Eucalyptus nortonii*, Mealy Bundy, is primarily distinguished from *E. goniocalyx* by the production of wax leading to glaucous buds and fruit and dull rather than glossy leaves, however, both species occur in similar habitat and have near complete distributional overlap. As *E. nortonii* was not extensively sampled during this project, we cannot comment on the evolutionary distinctiveness nor relative distributions of these two species here but believe this is something that warrants further investigation.
- Populations in the Warrumbungle and Nandewar ranges previously considered to represent *E. goniocalyx* were described as *E. volcanica* by Johnson and Hill (1990).
  - We find that the type population for this taxon in the Kaputar Range is genetically distinct from both *E. goniocalyx* and the Warrumbungle range *E. volcanica* population (Figure 25).
  - The Warrumbungle Range *E. volcanica* population is genetically closer to *E. goniocalyx* than the Kaputar Range *E. volcanica* population.
- In addition, we find that the populations identified and sampled as *E. goniocalyx* on the Liverpool Range are more genetically divergent to populations of the species to the south of the Range, than all NSW populations south of the Liverpool Range are to one another.
  - These populations likely represent *E. nortonii* rather than *E. goniocalyx*, but we can not be certain of this given our sampling.
- Overall, these findings show further taxonomic study is needed to fully understand the species and distributional boundaries of taxa in this group.
- We limit our restoration genomics guidance to populations of *E. goniocalyx* subsp. *goniocalyx* from south of the Hunter Valley, as movement of material between the highly genetically divergent populations in the Liverpool, Warrumbungle and Nandewar ranges is not recommended regardless of taxonomy.
- A single *E. goniocalyx* x *E. bridgesiana* was recorded in our genetic dataset.

### 4.8.1 Genetic structuring and neighbourhoods

- When only populations of *E. goniocalyx* subsp. *goniocalyx* from south of the Hunter Valley are considered, there is no genetic structuring (Figure 24).
- There was very limited genetic divergence between sampled sites across southern NSW, with a weak isolation-by-distance signal being observed.
- For these reasons, we recognised a single genetic neighbourhood for this species south of the Hunter Valley.

### 4.8.2 Reproductive biology

- No clonal samples were recorded across the samples of *E. goniocalyx*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.8.3 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. goniocalyx*.
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

### 4.8.4 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- Seed can be sourced from across the species distribution south of the Hunter Valley for use in restoration plantings south of the Hunter Valley.
- North of the Hunter Valley, seed should only be moved within but not between the Warrumbungle, Liverpool and Kaputar ranges until species boundaries have been resolved.
- **Minimum seed sourcing effort:** At least 5 maternal lines from 6 distinct sites (30 total maternal lines) should be used to ensure capture of 90% of common genetic diversity south of the Hunter Valley in seed collection.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed:
  - Eucalyptus cypellocarpa
  - Eucalyptus nortonii

#### Maids Valley site recommendations

- As this species is not native to the Northern Tablelands region, the species should not be used in ecological restoration within that region.

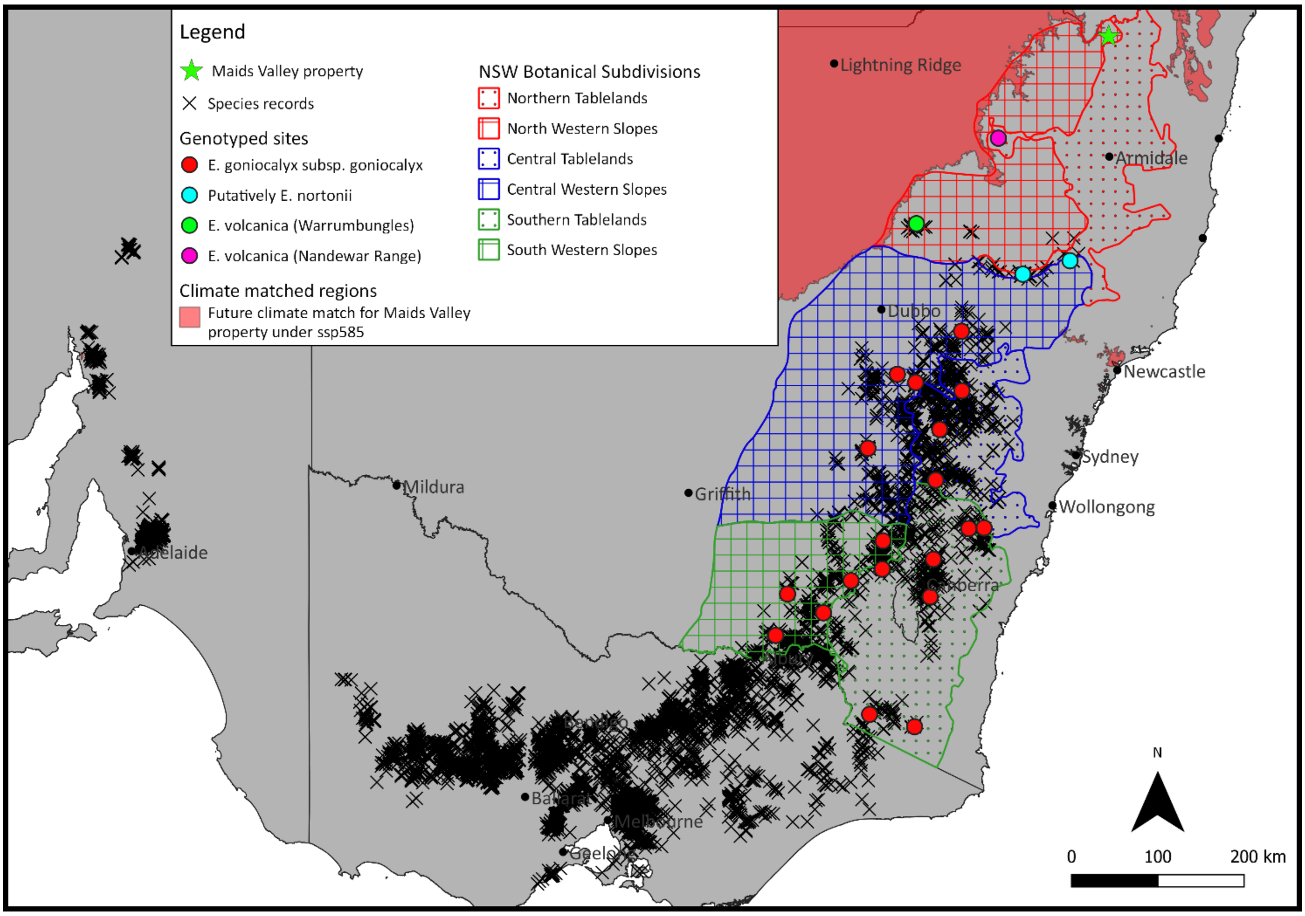


**Figure 24:** Sites sampled for genetic analysis of *Eucalyptus goniocalyx* indicating genetic lineages, with red circles being those belonging to the main *E. goniocalyx* subsp. *goniocalyx* lineage, teal circles being the Liverpool Range putative *E. nortonii* genetic lineage, green circles being the Warrumbungle *E. volcanica* lineage, and pink circles being the Kaputar Range *E. volcanica* lineage. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

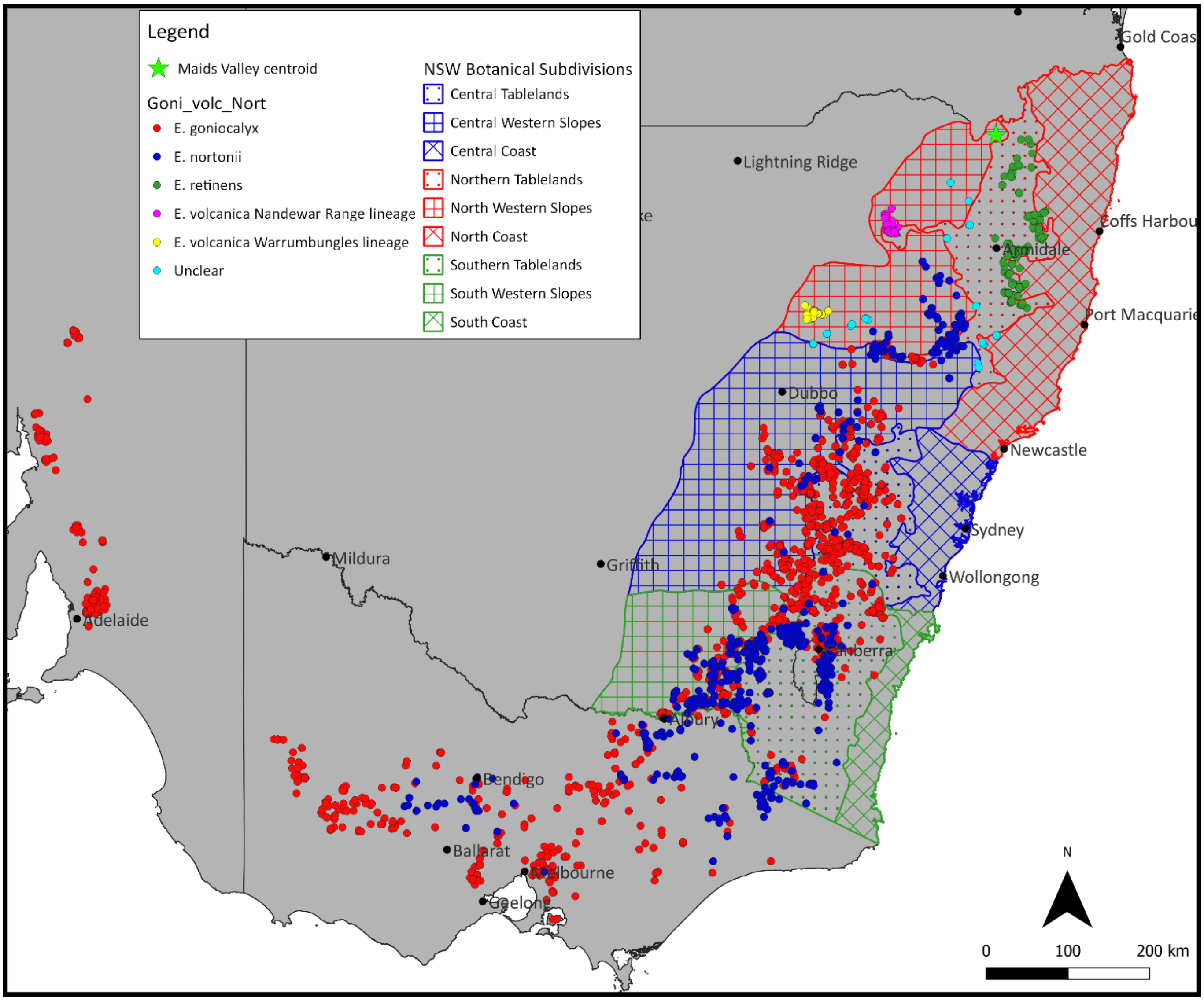


**Figure 25:** Map illustrating our understanding of the relative distributions of *Eucalyptus goniocalyx* and close relatives based upon our genetic findings. Red points are Atlas of Living Australia records we consider to represent *E. goniocalyx*, blue points are those we consider to represent *E. nortonii*. Note the largely overlapping distribution of these two species that are distinguished by a single morphological trait (the production of wax) and we therefore suggest the relative distributions of each species warrants further investigation. Also shown are the distributions of *E. retinens green points)*, *E. volcanica* (pink points) and the populations in the Warrumbungles previously referred to *E. volcanica* that are genetically closer to *E. goniocalyx* (yellow points). Teal points represent records in areas we are unable to clarify what species is present.

## 4.9 *Eucalyptus macrorhyncha*

*Eucalyptus macrorhyncha*, Red Stringybark, is a common and widespread member of woodland on hills and slopes with dry, poor soils. Its distribution stretches from the Northern Tablelands of NSW south through the inland slopes and tablelands of NSW and Victoria (Figure 26), with outlying populations in Cocoparra National Park in south-west NSW and the Clare Valley of South Australia. Morphologically aberrant populations in the Capertee-Rylestone-Lidsdale area of central NSW are variably considered a distinct subspecies (*E. macrorhyncha* subsp. *cannonii*) or a closely related, but distinct species (*E. cannonii*) depending on the taxonomic authority followed. These populations are listed as a vulnerable taxon under the *E. cannonii* name under NSW state legislation (Office of Environment and Heritage 2019), however were taken off the federal legislation in 2013 (Threatened Species Scientific Committee 2013).  This species can be identified by its stringy bark on the trunk and branches, green to blue-green, concolorous adult leaves, large, pedicellate buds that are beaked and diamond-shaped, and its large, pedicellate fruit that are almost spherical. Populations of *E. macrorhyncha* subsp. *macrorhyncha* from south of the Hunter Valley have been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.9.1 Species limits and hybridisation

- *Eucalyptus macrorhyncha* is a species of Stringybark, a group of *Eucalyptus* species that are difficult to differentiate based upon morphology, especially if all parts of the plant (leaves, buds, fruits and seedlings) are not available.
- We find the classification of the morphologically aberrant Capertee-Rylestone-Lidsdale populations as *E. macrorhyncha* subsp. *cannonii* is supported by the genetic data rather than as the separate species *E. cannonii*.
  - *Eucalyptus* macrorhyncha subsp. *macrorhyncha* is believed to also occurs in this area, with the two subspecies differentiated by the parts of the slopes they occupy. *Eucalyptus macrorhyncha* subsp. *cannonii* is restricted to the upper slopes and plateaus, with *E. macrorhyncha* subsp. *macrorhyncha* present on the lower slopes.
- All samples collect as E. *macrorhyncha* subsp. *macrorhyncha* north of the Hunter were moderately to highly divergent from samples of the taxon south of the Hunter.
  - Samples from one site near Nundle in the Northern Tablelands were subsequently identified as being *E. laevopinea*
  - All other sites in the Northern Tablelands were genetically indistinguishable from *E. youmanii*, showing that *E. macrorhycnha* is absent from this region entirely.
- Samples from the one sampled site in the Warrumbungle Range were genetically divergent from all of *E. macrorhyncha* subsp. *macrorhyncha, E. youmanii* and *E. laevopinea,* suggesting they may represent an unrecognised taxon, putatively most closely related to *E. youmanii.*
- We limit our information for ecological restoration of *E. macrorhyncha* to only populations south of the Hunter Valley.
  - Populations north of the Hunter are addressed under a broadly defined *E. youmanii* (Section 4.12).
- Hybrids between E. *macrorhyncha* subsp. *macrorhyncha* and both *E. sparsifolia* and *E. eugenioides* were recorded in our genetic dataset.

### 4.9.2 Genetic structuring and neighbourhoods

- The two subspecies of *E. macrorhyncha*, subspecies *macrorhyncha* and subspecies *cannonii*, were genetically divergent, despite a lack of geographic separation.
- There was very limited genetic divergence between sampled sites of *E. macrorhyncha* subsp. *macrorhyncha* south of the Hunter, with a weak isolation-by-distance signal being observed.
- For these reasons, we recognised that the two subspecies need to be managed separately, despite not representing discreet genetic neighbourhoods.

### 4.9.3 Reproductive biology

- No clonal samples were recorded across the samples of *E. macrorhyncha*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.9.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. macrorhyncha.*
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

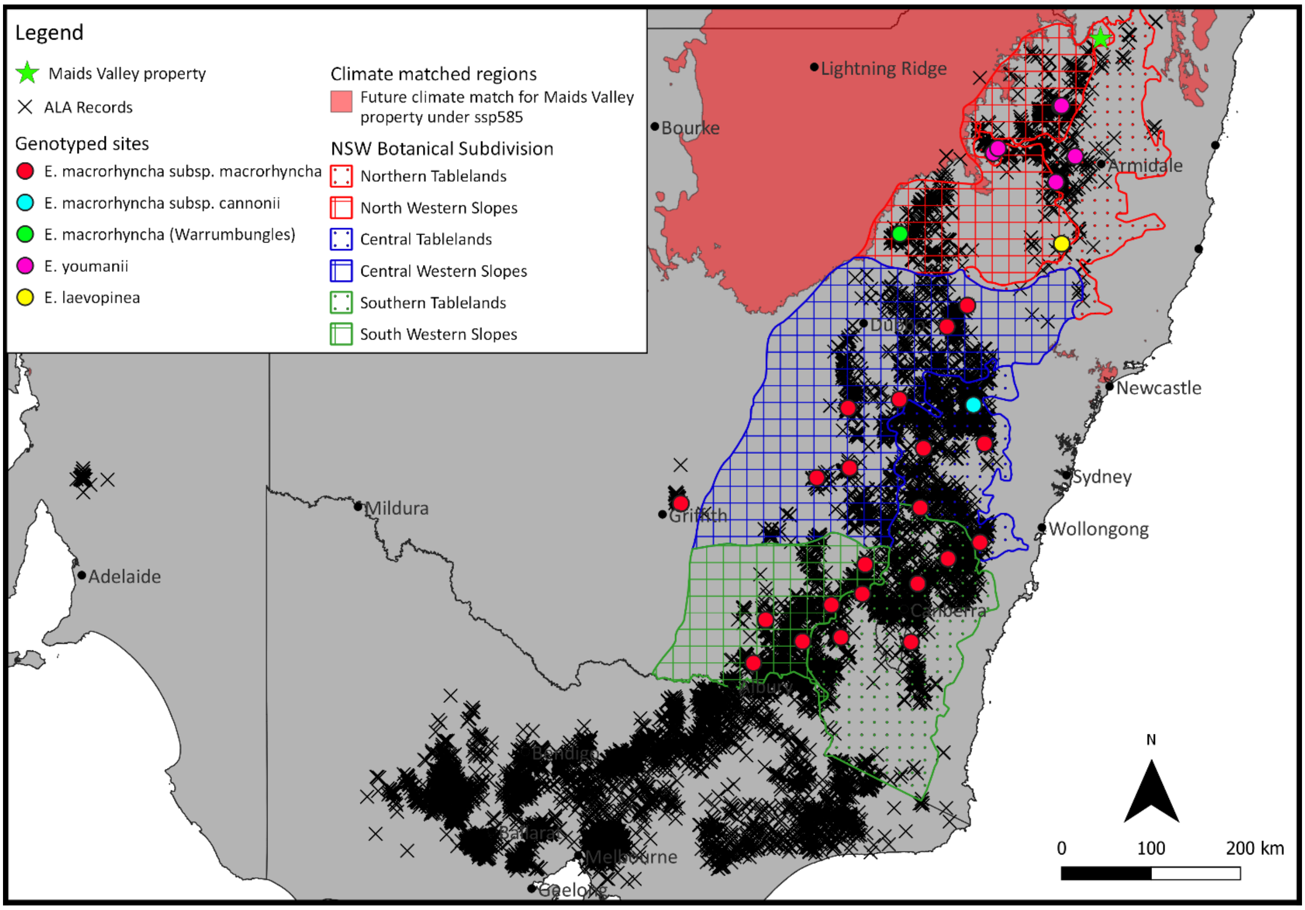


**Figure 26:** Sites sampled for genetic analysis of *Eucalyptus macrorhyncha* indicating genetic lineages, with red circles being those belonging to the main *E. macrorhyncha* subsp. *macrorhyncha* lineage, teal circles being *E. macrorhyncha* subsp. *cannonii*, and green circles being the divergent Warrumbungle Range *E. macrorhyncha* lineage. Pink and yellow circles indicate sites where samples were identified as *E. macrorhyncha* subsp. *macrorhyncha* in the field but that were genetically identified as *E. youmanii* or *E. laevopinea* respectively. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

### 4.9.5 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- *Eucalyptus macrorhyncha* subsp. *macrorhyncha* s.s. seed can be sourced from across the subspecies distribution south of the Hunter Valley for use in restoration plantings south of the Hunter Valley.
- *Eucalyptus macrorhyncha subsp. cannonii* is under conservation management and therefore conservation genomics rather than restoration genomic approaches should be used to inform seed sourcing for this subspecies.
- *Eucalyptus macrorhyncha* s.l. seed should only be moved within the Warrumbungle Range and Pilliga areas until the taxonomic identity of these populations has been resolved.
- Minimum seed sourcing effort:
  - ***Eucalyptus macrorhyncha* subsp. *macrorhyncha:*** At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed:
  - Eucalyptus sparsifolia
  - Eucalyptus eugenioides
  - Eucalyptus laevopinea

#### Maids Valley site recommendations

- As our genetic data shows this species is not native to the Northern Tablelands region (as discussed above), the species should not be used in ecological restoration within that region.

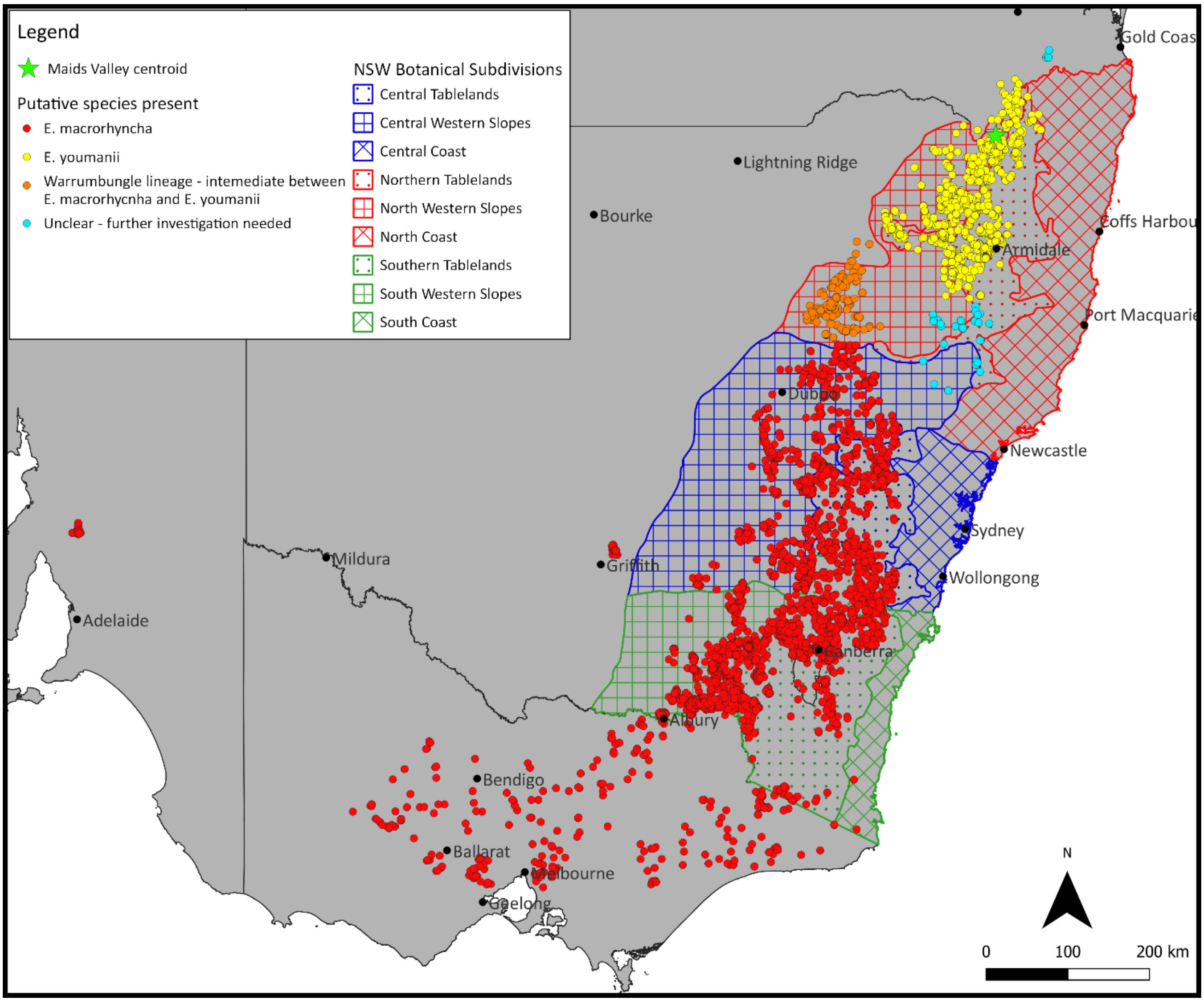


**Figure 27:** Map illustrating our understanding of the relative distributions of *Eucalyptus macrorhyncha* and *E. youmanii* based upon our genetic findings. Yellow points are Atlas of Living Australia records we consider to represent *E. youmanii*, red points are those we consider to represent *E. macrorhyncha*. Orange points represent populations in the Pilliga and Warrumbungles that are hypothesised to be a distinct lineage intermediate between *E. macrorhyncha* and *E. youmanii*, with teal points represent records in areas we are unable to clarify what species is present.

## 4.10 *Eucalyptus polyanthemos*

*Eucalyptus polyanthemos*, Red Box, is a species associated with the BGGW in central and southern NSW and Victoria. The species distribution ranges from Gulgong in the central west of NSW to Western Victoria, including through Gippsland and into the south-east corner of NSW. Three subspecies are recognised, two of which are native to NSW, *E. polyanthemos* subsp. *polyanthemos* which is characterised by having predominately smooth bark and *E. polyanthemos* subsp. *vestita* with rough box-type bark to the small branches. The distribution of these two subspecies in NSW are not well characterised, with subspecies *polyanthemos* being the more northly of the two, with the southern edge of its distribution being somewhere between Tumut and the Victorian border. Subspecies *vestita* is widespread in Victoria and extends north into NSW to approximate Tumut, as well as into the south-east corner of the state. The species can be identified based upon its dull, broad, ovate to orbicular crown leaves, terminal inflorescences of obconical, thin-walled fruit and broad ovate to orbicular, blue grey to glaucous juvenile leaves. This species has been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects across its NSW distribution.

### 4.10.1Species limits and hybridisation

- The existing taxonomic and geographic circumscription of this species, widely accepted amongst botanical authorities, is supported by the genetic data generated in this project.
- We find the two subspecies present in NSW represent distinct genetic lineages, with samples from Livingstone NP and Downfall Nature Reserve south, along with those in the south-east corner being *E. polyanthmos* susbsp. *vestita*. All samples from site form Tumut and Ellerslie Nature Reserve and north being *E. polyanthemos* subsp. *polyanthemos* genetically.
  - Without more granular sampling outside the scope of this project, we cannot determine the precise distribution boundaries of these subspecies.
- No hybrids were observed in the genetic dataset for this species.

### 4.10.2 Genetic structuring and neighbourhoods

- *Eucalyptus polyanthemos* showed two genetic neighbourhoods in NSW, corresponding to the two recognised subspecies:
  - A northern genetic neighbourhood equivalent to *E. polyanthemos* subsp. *polyanthemos* from the northern half of the South-West Slopes region to the northern extent of the species distribution in the Central-West of NSW.
  - A southern genetic neighbourhood equivalent to *E. polyanthemos* subsp. *vestita* in the southern half of the South-West slopes region and the south-east corner.

### 4.10.3 Reproductive biology

- No clonal samples were recorded across the samples of *E. polyanthemos*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.10.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. polyanthemos.*
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

### 4.10.5 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- Seed sourcing should be undertaken independently for the two subspecies present in NSW, but seed can be freely moved within the distributions of both subspecies within NSW.
- Minimum seed sourcing effort:
  - ***Eucalyptus polyanthemos* subsp. *polyanthemos***: At least 5 maternal lines from 4 distinct sites (20 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
  - ***Eucalyptus polyanthemos* subsp. *vestita***: At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- No other species are known to commonly hybridise with *E. polyanthemos* and therefore there are no co-occurring species that should impact site choice.

## Maids Valley site recommendations

- As this species is not native to the Northern Tablelands region, the species should not be used in ecological restoration within that region.

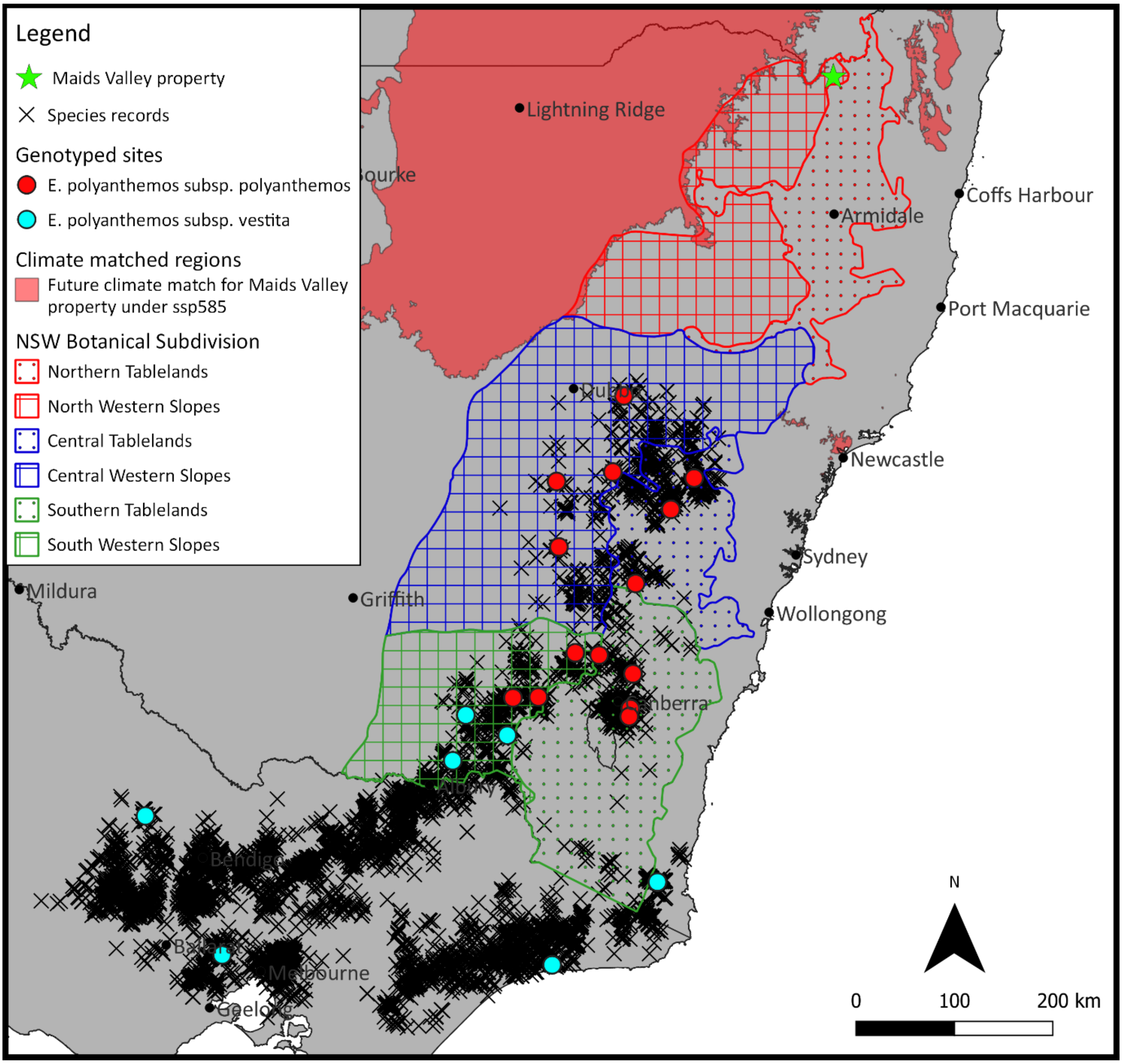


**Figure 28:** Sites sampled for genetic analysis of *Eucalyptus polyanthemos* indicating genetic neighbourhoods, which correspond to the accepted subspecies. Red circles are those belonging to the more northly distributed *E. polyanthemos* subsp. *polyanthemos* and teal circles are those belonging to the more southerly distributed *E. polyanthemos* subsp. *vestita*. Note that there is no clear geographic barrier that separates the two species distribution despite the observed genetic divergence. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

## 4.11 *Eucalyptus rubida*

*Eucalyptus rubida*, Candlebark, is a species associate with the BGGW primarily in the Central and Southern Tablelands of NSW and Victoria. The subspecies *E. rubida* subsp. *rubida* is considered to occur from the western edge of Wollemi NP south, including all populations in southern NSW, Victorian and Tasmania (Figure 29). The small populations in the Northern Tablelands of NSW are classified as *E. rubida* subsp. *barbigerorum,* a listed vulnerable taxon under both federal (Department of the Environment, Water, Heritage and the Arts 2008b) and state (Office of Environment and Heritage 2022) legislation. In addition, a population of trees on Mount Canobolas near the central-west NSW town of Orange, variously considered a subspecies of *E. rubida* (subsp. *canobolensis*) or the distinct species *E. canobolensis*, is listed as endangered under both federal (Department of the Environment, Water, Heritage and the Arts 2008a) and state (Office of Environment and Heritage 2023) legislation. *Eucalyptus rubida* is recognised by its smooth barked trunk (basal stocking of rough bark in subsp. *barbigerorum*) often with horizontal insect scars, greyish green adult leaves, cup-shaped fruit in groups of 3 (occasionally 7), and sessile, opposite, and very glaucous juvenile leaves. Due to a lack of genetic differentiation between sites, this species has not yet been added to the Restore and Renew webtool.

### 4.11.1 Species limits and hybridisation

- *Eucalyptus rubida* is closely related to, and difficult to distinguish morphologically from, *E. dalrympleana* (Mountain Gum). Our genetic data supports these taxa as distinct and strong habitat differentiation exist between them.
  - *Eucalyptus rubida* is a tree of poorer form that occurs on less fertile, shallow soils.
  - *Eucalyptus dalrympleana* is a tree of better form that occupies moderate to high fertility soils.
- We find that the current taxonomic circumscription of *E. rubida* and relatives in NSW is not supported by evolutionary genetic analyses (Figure 30).
  - Our genetic data suggests *E. canobolensis* should be reduced to *E. rubida* subsp. *canobolensis,* as minimal genetic divergence exists between it and *E. rubida* subsp. *rubida*.
  - We also find that *E. rubida* subsp*. barbigerorum* is genetically indistinguishable from the co-occurring *E. dalrympleana* subsp. *heptantha* and should be synonymised with it. Therefore, we find *E. rubida* does not occur in the Northern Tablelands of NSW.
- Putative hybrids between *E. rubida* subsp. *rubida* and *E. mannifera* subsp. *mannifera* (Brittle Gum) were recorded at one sampling site.

### 4.11.2 Genetic structuring and neighbourhoods

- The two subspecies of *E. rubida* we recognise in this report, subspecies *rubida* and subspecies *canobolensis*, were not genetically divergent.
- There was very limited genetic divergence between sampled sites of *E. rubida* subsp. *rubida*, with no signal of isolation-by-distance being observed.
- For these reasons, we recognised that the two subspecies need to be managed separately, despite not representing discreet genetic neighbourhoods.

### 4.11.3 Reproductive biology

- No clonal samples were recorded across the samples of *E. rubida*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.11.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. rubida.*
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

### 4.11.5 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- *Eucalyptus rubida* subsp. *rubida* seed can be sourced from across the subspecies distribution within NSW for use in restoration plantings within the state.
- *Eucalyptus rubida* subsp. *canobolensis* is under conservation management and therefore conservation genomics rather than restoration genomic approaches should be used to inform seed sourcing for this subspecies.

- Minimum seed sourcing effort:
    - ***Eucalyptus rubida*** **subsp.** ***rubida:*** At least 5 maternal lines from 5 distinct sites (25 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed:
    - Eucalyptus dalrympleana (Mountain Gum)
    - Eucalyptus mannifera (Brittle Gum)

### Maids Valley site recommendations

- As our genetic data shows this species is not native to the Northern Tablelands region (as discussed above), the species should not be used in ecological restoration within that region.

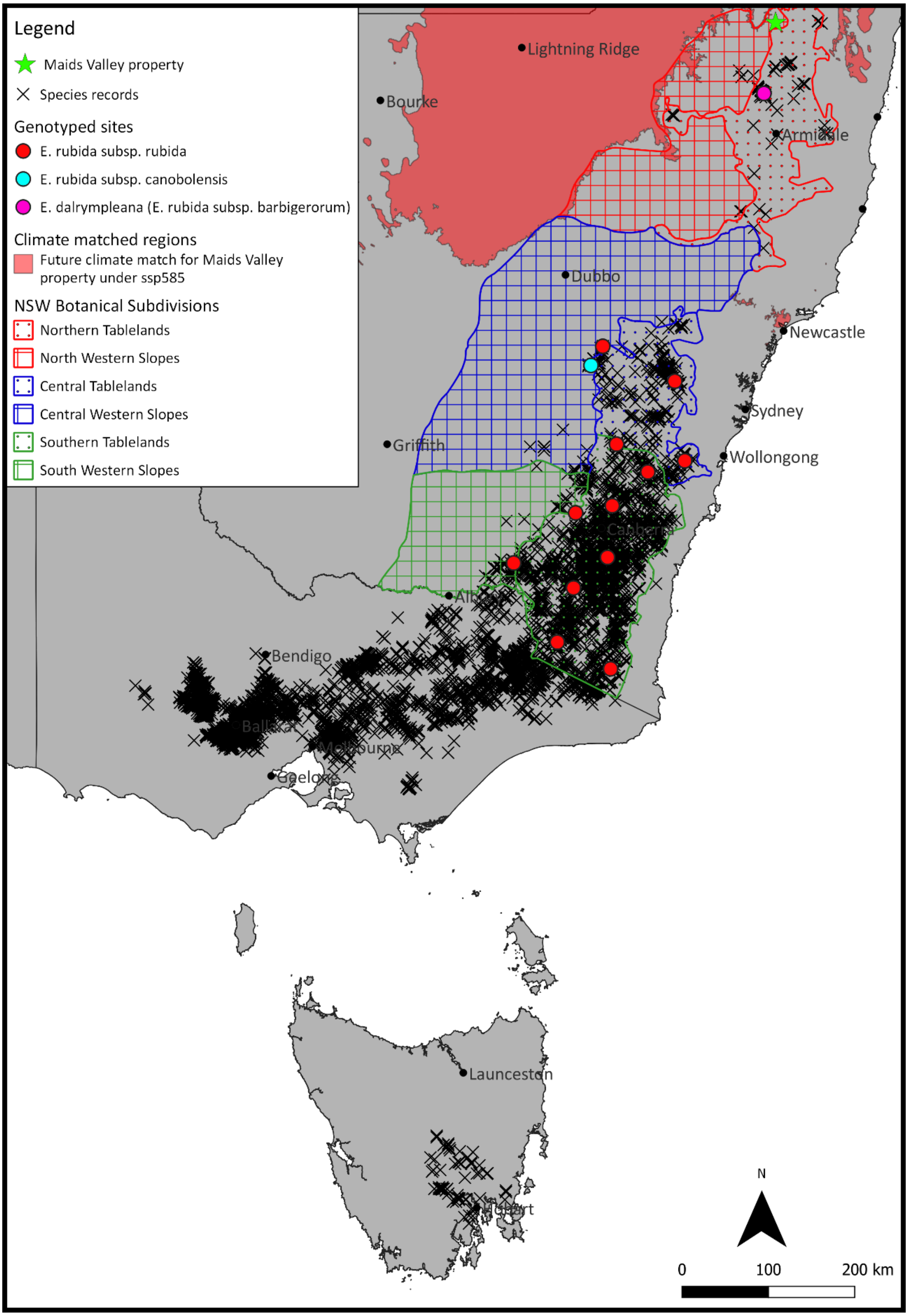


**Figure 29:** Sites sampled for genetic analysis of *Eucalyptus rubida* indicating genetic neighbourhoods. Red circles are those belonging to *E. rubida* subsp. *rubida*, teal circles are those belonging to *E. rubida* subsp. *canobolensis*, and pink circles those belonging to *E. rubida* subsp. *barbigerorum* which is genetically indistinguishable from *E. dalrympleana* subsp. *heptantha* and therefore not a valid taxon. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

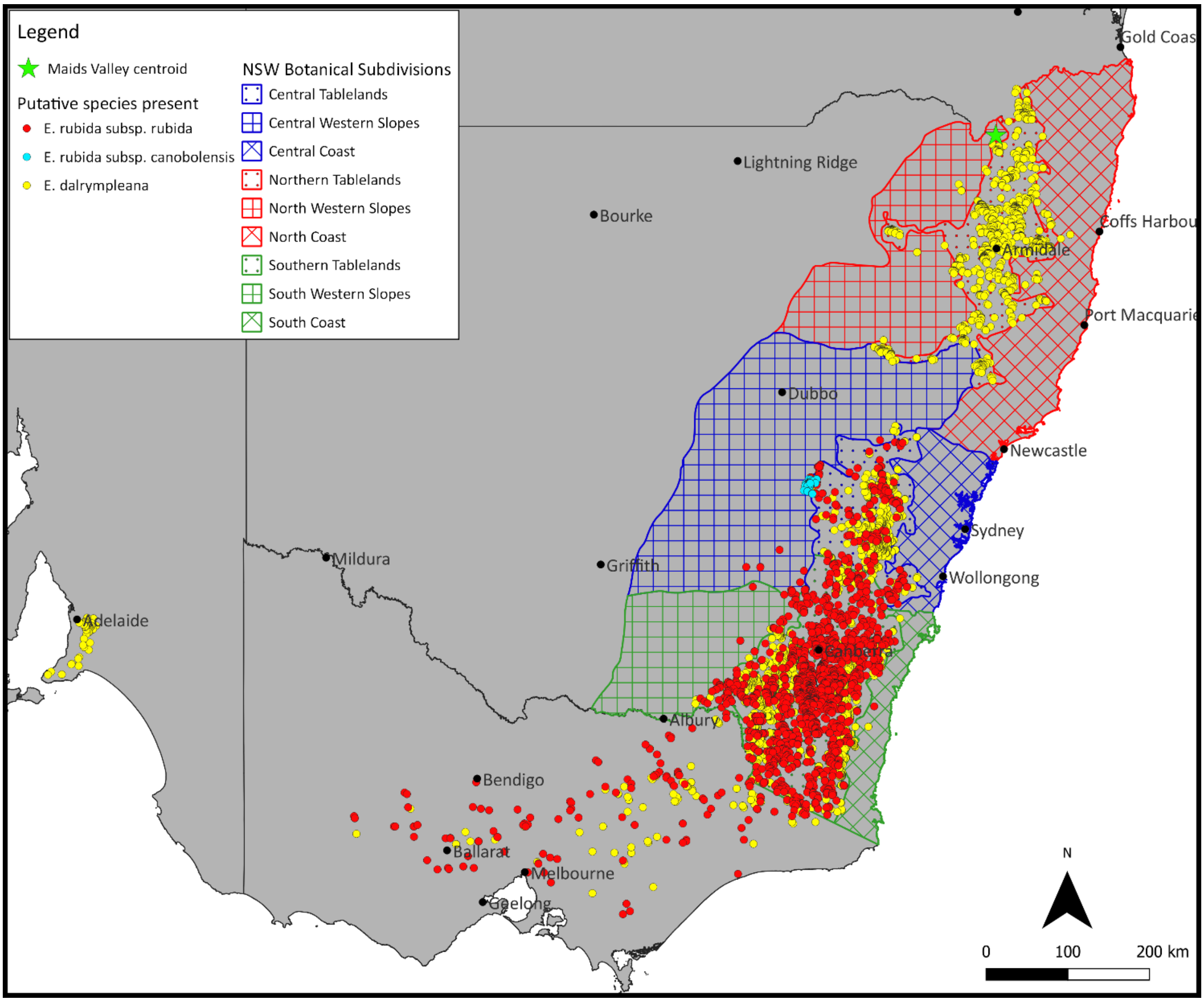


**Figure 30:** Map illustrating our understanding of the relative distributions of *E. rubida* and *E. dalrympleana* based upon our genetic findings. Red points are Atlas of Living Australia records we consider to represent *E. rubida subsp. rubida*, teal points representing *E. rubida* subsp. *canobolensis* and yellow points are those we consider to represent *E. dalrympleana*.

## 4.12 *Eucalyptus youmanii*

*Eucalyptus youmanii*, Youman's Stringybark, is a woodland tree species associated with the BGGW in the Northern Tablelands of NSW and Granite Belt of QLD. Populations of this species occur on low to moderate fertility soils between Nundle SF in the south, Cathedral Rock NP in the east, Dalveen in the north and the Kaputar Range in the west. We follow the taxonomy employed by the EUCLID authors (Brooker *et al.* 2015) for this taxon and closely related taxa, namely that *E. subtilior* (considered distinct by NSW authorities (Hill 1991c)) and *E. stannicola* (both from the Northern Tablelands region) are synonyms of *E. youmanii*, and that *E. prominula* (a species endemic to the Yengo region of the Sydney Basin) is a distinct species despite it being listed as a synonym of *E. youmanii* on the Australian Plant Census (Council of Heads of Australasian Herbaria 2016). *Eucalyptus youmanii* can be recognised by its stringy bark to the small branches, green to grey-green lanceolate adult leaves, subtly to distinctly longitudinal ridged buds borne on pedicels in groups of 7, large (1.7-1.3 cm wide) hemispherical fruit, and petiolate juvenile leaves that are hairy on the margins and midvein. This species has been added to the Restore and Renew webtool, enabling exploration of appropriate areas for seed sourcing for site specific restoration projects across its NSW distribution.

### 4.12.1 Species limits and hybridisation

- *Eucalyptus youmanii* is a species of Stringybark, a group of *Eucalyptus* species that are difficult to differentiate based upon morphology, especially if all parts of the plant (leaves, buds, fruits, and seedlings) are not available.
- We find that our genetic data supports the position we took that *E. subtilor* and *E. stannicola* are conspecific with *E. youmanii.*
- We did not sample the Sydney Basin endemic *E. prominula,* which occurs outside of the geographic scope of this project, so cannot comment on its relationship to *E. youmanii*.
- All samples collect as *E. macrorhyncha* subsp. *macrorhyncha* (Red Stringybark) north of the Hunter were genetically indistinguishable from *E. youmanii*, suggesting the understanding of these two species distributions may need revision (See figure 17 under the *E. macrorhyncha* section).
  - Such revisions would likely involve widening *E. youmanii's* distribution, increasing its abundance and ecological importance in the Northern Tablelands of NSW.
  - We include our collections of E. *macrorhyncha* subsp. *macrorhyncha* from the Northern Tablelands in our information for ecological restoration of *E. youmanii.*
  - No hybrids between this species and other Stringybarks were recorded in our genetic dataset.

### 4.12.2 Genetic structuring and neighbourhoods

- *Eucalyptus youmanii* showed no genetic structuring across its distribution (Figure 31).
- There was very limited genetic divergence between sampled sites across NSW, with a weak isolation-by-distance signal being observed.
- For these reasons, we recognised only a single genetic neighbourhood for this species, including all populations.

### 4.12.3 Reproductive biology

- No clonal samples were recorded across the samples of *E. youmanii*.
- Genetic patterns in mature wild populations of this species suggest a mixed to preferentially outcrossing breeding system, with no evidence for inbreeding or selfing in any population.

### 4.12.4 Genetic diversity

- Within site allelic richness and heterozygosity was high for *E. youmanii.*
- This diversity was evenly spread across the landscape.

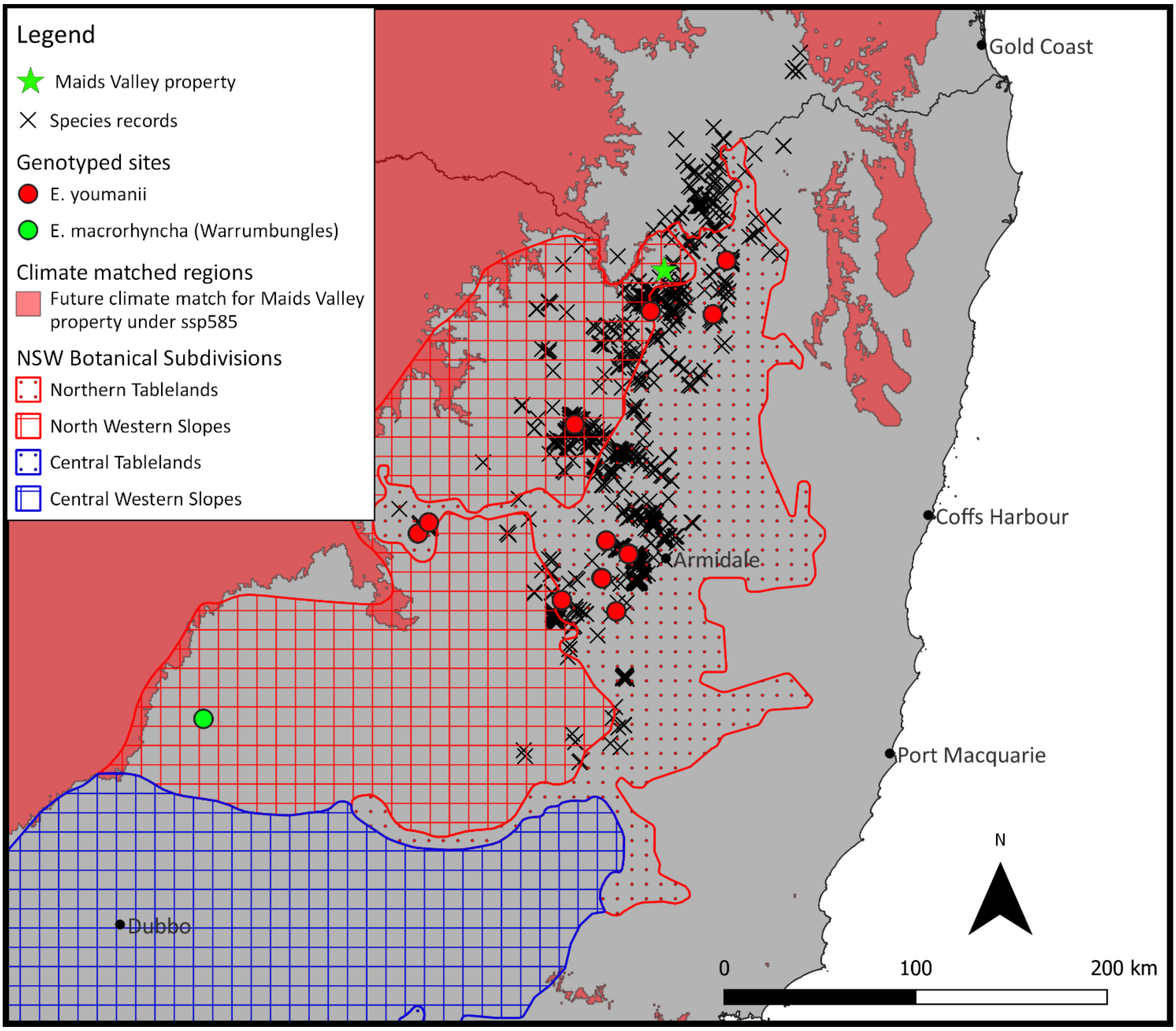


**Figure 31:** Sites sampled for genetic analysis of *Euclyptus youmanii* indicating genetic lineages, with red circles being those belonging to the only genetic lineage that exists across the species distribution. This includes sites in the Northern Tablelands that have previously been ascribed to *E. macrorhyncha*, *E. subtilor* and *E. stannicola*, all of which are not genetically distinguishable from *E. youmanii*. The green circle represents the divergent *E. macrorhyncha* Warrumbungle lineage which was intermediate between *E. youmanii* and *E. macrorhyncha*. Black crosses represent records of the species to indicate the known distribution of the species while the green star represents the Maids Valley property that may be restored. Red overlaid areas are those which match the predicted climates of the Maids Valley site in 2070-2100 ssp585 projections of mean average precipitation and mean average temperature.

### 4.12.5 Seed sourcing for ecological restoration

#### Generalised species wide recommendations

- Seed can be sourced from across the species distribution within NSW for use in restoration plantings within the state.
- **Minimum seed sourcing effort**: At least 5 maternal lines from 3 distinct sites (15 total maternal lines) should be used to ensure capture of 90% of common genetic diversity within NSW in seed collection.
- Sites where this species co-occurs with any of the following closely related species should be avoided to minimise the use of hybrid seed: *Eucalyptus caliginosa* and *E. laevopinea.*
- Care should be taken when seed is collected to make sure the correct stringybark species is being collected. Seedling morphology is diagnostic between the stringybarks, and therefore can be used to assist in screens for misidentified or hybrid seed. Seedling differentiation between the key species per EUCLID (Brooker *et al.* 2015) is as follows:
  - *Eucalyptus caliginosa* (New England Stringybark): stems densely hairy, seedling leaves initially sessile and opposite, quickly becoming petiolate and alternate by node 8, leaves ovate (to 8 cm long and 4.5 cm wide) and discolourous and only ever hairy on margins, petiolate and lower midrib.

  - *Eucalyptus eugenioides* (Thin-leaved Stringybark): stems densely hairy, seedling leaves always petiolate, transitioning from opposite to alternate by node 10, leaves ovate to lanceolate (to 10 cm long and 4.5 cm wide) and discolourous with sparse hairs on both surfaces and margins.
  - *Eucalyptus laevopinea* (Silver Top Stringybark): stems initially densely hairy becoming smooth by node 8, seedling leaves sessile and opposite to node 6, then alternate and petiolate, leaves broadly lanceolate (to 13 cm long and 5.5 cm wide) and initially discolourous and hairy on margins and midrib before quickly transitioning to concolorous and hairless by node 6.
  - *Eucalyptus youmanii*: stems densely hairy, seedling leaves opposite to node 7, then alternate, always petiolate, leaves ovate to lanceolate (to 11cm long and 5 cm wide) and discolourous with hairs on surface, margins, and midrib.

## Maids Valley site recommendations

- Optimal seed sourcing for *Eucalyptus youmanii* when undertaking ecological restoration at the Maids Valley site (Figure 32) would:
  - Use five maternal lines collected from each of three seed source sites (15 maternal lines total) at least 20 km apart within the Northern Tablelands region of NSW.
  - Sourcing seed from Queensland is not plausible for *E. youmanii* as it has an extremely limited distribution in that state.
  - Utilising the above seed sourcing scheme to capture high genetic diversity and adaptive potential will be very important for *E. youmanii* site as no climate-matched provenances exist for the Maids Valley site for this species.

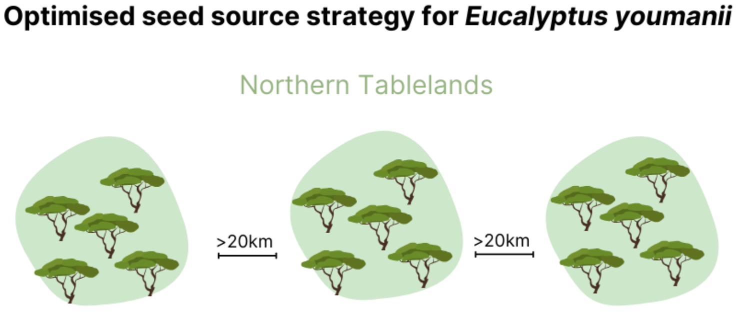


**Figure 32:** FA schematic representation of seed collection effort required for *Eucalyptus youmanii* to source genetically diverse seed (90% of total genetic diversity) suitable for use in restoration of the Maids Valley site. Each circle represents a collection site and each tree represents a maternal line. No climate-matched provenances are available. Care should be taken when seed is collected to make sure the correct stringybark species is being collected.

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