Quaternary Climate Change
&
Podocarpus elatus (Podocarpaceae)

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ABSTRACT

Understanding the effect of Quaternary climate change on the distribution, diversity and divergence of *Podocarpus elatus* (R.Br. ex Endl.) will contribute to the conservation of the east Australian rainforests in light of increasing ecological damage, rapid human population growth and anthropogenic-induced global warming. To know how intraspecific diversification in a wide-ranging long-lived species is driven by climate change will improve our understanding of how climatic-drivers are involved in the evolutionary process.

This thesis examined the genetic consequences of Quaternary climate change in *Podocarpus elatus*, a long-lived rainforest conifer endemic to Australia. Firstly, eight polymorphic nuclear microsatellite and one chloroplast loci were isolated/characterised in *P. elatus*. I demonstrated the microsatellite primers could be applied to other podocarps (i.e. *P. grayi*, *P. lawrencei*, and *P. smithii*.).

The markers were used to investigate the genetic diversity and structure of *P. elatus* throughout the broad-distributional range. Populations throughout the east Australian rainforests were screened and two divergent regions separated by the dry Clarence River valley (New South Wales) were discovered (i.e. Clarence River Corridor). Niche modelling techniques were employed to verify the incidence of climatic/habitat divergence between the two regions.

Phylogeographic analysis and environmental niche modelling were combined to determine: (1) if post-glacial distributional dynamics could be described; (2) if the range-contractions suggested by southern fossil records are uniform across the entire distribution; and (3) if there is agreement between environmental niche modelling and molecular-based regional dynamics.

Niche-modelling indicated that at the Last Glacial Maximum (21 Ka), the habitats suiting the two genetically differentiated regions of *P. elatus* were geographically disjunct. The northern distributional region persisted through the LGM in a small refugial area, which during post-glacial periods has expanded. Conversely the southern range followed the opposite trend and has contracted since the LGM, but overall had greater genetic diversity. Coalescence-based analyses support these differential dynamics across the distribution of the species.

The future climate-induced range shift of the two genetically differentiated regions of *P. elatus* were modelled and compared to coalescence-based inference of regional gene flow, genetic boundaries and expansion/contraction dynamics to provide information with regard to community response to climate cycles.

A total of 405 occurrence records were obtained to model species' distribution (21, 6, and 0 Ka) based on the current environment using MAXENT and forecasting future distribution (2050 A2) using an ensemble of thirteen atmospheric-oceanic global climate models. The analysis suggests the geographic shift in genetic diversity of *P. elatus* according to future climate change scenarios.

Together these data sources provide a means to predict future distribution of genetic diversity, and infer rainforest areas at increased risk of localised extinction. It was found that *P. elatus* is considerably more threatened than shown by its current distribution, and I suggest the use, and extension, of habitat corridors to accommodate future climate-induced range shift of fragmented rainforest habitats along the east coast of Australia.

DECLARATION

I Rohan Mellick certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works (*) contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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STATEMENT OF CONTRIBUTION

Chapter Two

Author contributions: Rohan Mellick collected material, generated the microsatellite library, screened clones, designed primers and wrote the manuscript. Carolyn Connelly supervised the microsatellite library generation. Maurizio Rossetto supervised the writing, screening of clones and primer design. Andrew Lowe, Robert Hill and Maurizio Rossetto funded the project.

Chapter Three

Author contributions: Rohan Mellick, Andrew Lowe and Maurizio Rossetto conceived the ideas. Rohan Mellick collected samples, extracted DNA, completed the analyses, modelled the species' distribution and wrote the manuscript. Andrew Lowe and Maurizio Rossetto supervised the molecular analyses, interpretation of results and writing. Andrew Lowe, Robert Hill and Maurizio Rossetto funded the project.

Chapter Four

Author contributions: Rohan Mellick, Andrew Lowe and Maurizio Rossetto conceived the ideas. Rohan Mellick collected samples, extracted DNA, analysed the data, modelled the species' distribution, generated the coalescent-based model and wrote the manuscript. Chris Allen supervised the processing of environmental layers and completed the bathymetry extrapolation. Andrew Lowe, Robert Hill and Maurizio Rossetto supervised the molecular analyses, interpretation of results and writing. Andrew Lowe, Robert Hill and Maurizio Rossetto funded the project.

Chapter Five

Author contributions: Rohan Mellick, Andrew Lowe and Maurizio Rossetto conceived the ideas. Rohan Mellick analysed the data, generated the coalescent-based model, modelled the species' distribution and wrote the manuscript. Chris Allen supervised the processing of environmental layers, completed the bathymetry extrapolation and helped with Figure 1 generation. Peter D. Wilson supplied future data and projected models onto the future ensemble. Maurizio Rossetto, Robert Hill and Andrew Lowe supervised the writing, analyses and interpretation of results. Andrew Lowe, Robert Hill and Maurizio Rossetto funded the project.

CONTEXTUAL STATEMENT

This thesis has been submitted for assessment as a portfolio of publications (Chapter 2-5). The overall aim is to determine the level of genetic diversity and structure within naturally occurring populations of *Podocarpus elatus*, and establish if this is more strongly influenced by historic or contemporary drivers. I aim to describe via Environmental Niche Modelling (ENM), the past-current-future distributional dynamics of suitable habitat for *P. elatus*. I seek to correlate the observed fossil record and regional genetic boundaries to the inferred distributional dynamics, i.e. is palynology, the ENM-based, and molecular-based regional boundaries and expansion/contraction dynamics in *P. elatus* in agreement?

Furthermore, I attempt to combine knowledge of gene flow dynamics and future ENM of *P. elatus* to be applied to the conservation of these fragmented habitats. The application of molecular and ecological niche modelling techniques could guide the integration of the natural and artificial landscape with the aim of allowing unimpeded range shift in response to rapid climate change.

Chapter One (Literature review): a broad-contextual statement to frame the study for a broader audience in light of current knowledge and understanding.

Chapter Two (Almany, *et al.* 2009): we developed nine species-specific markers to explore the species' genetic diversity and structure.

Chapter Three (Mellick, *et al.* 2011): we determined diversity and structure of *P. elatus* through traditional molecular techniques to understand the effect of recent and historical habitat fragmentation on the observed intraspecific divergence. Two environmental niche models were developed for each genetic region and both were found to be statistically significant. The models suggested climatic-drivers were responsible for the species' intra-specific divergence.

Chapter Four (Mellick, *et al.* 2012): we inferred changes in paleodistribution in response to post-glacial warming by projecting the current model, from Chapter Three, onto the Last Glacial Maximum (21 Ka), Holocene Climatic Optimum (6 Ka) and Pre-Industrial climatic estimates. We also incorporated traditional and coalescent-based molecular techniques to understand the species' demographic patterns.

Chapter Five (Mellick, *et al.* submitted): we inferred future distribution by projecting the current models onto a future climatic ensemble. We used traditional and coalescent-based molecular techniques to understand population divergence and ancestral gene flow (as a proxy to natural gene flow in the absence of anthropogenic effects), for the conservation of *P. elatus* rainforest habitat. We propose conservation strategies in the context of a rapidly changing environment.

Chapter Six: (Conclusion): this includes set sub-sections, summary information and future ideas for the study system. The latter includes conservation strategies, a phylogenetic hypothesis and author's remarks.

Linking past climatic divergence patterns of species, with well-defined environmental realms of tolerance will allow the forecasting of future threats from likely anthropogenic climate change. A changing climate has caused species to shift in range from ancestral to present-day distributions. The corresponding changes in diversity and structure are known to follow climatic patterns. In this thesis an inter-disciplinary methodology is explored in an attempt to correlate intra-specific divergence patterns to climate. Observing divergence patterns in the future from that inferred in the past will validate this methodology and shape future evolutionary and conservation research.

1. Prologue

Broad-leaved podocarps have been recorded in the Gondwanan fossil record for over 144 million years (My) (Hill, 1994; Quilty, 1994). These conifers are a remnant of a world dramatically different from the world we live today. In high latitudes, rainforests were then extremely seasonal and trees evolved to sub-annual periods of light and darkness (McLoughlin, 2001). Broad-evergreen foliage needed to be tough and versatile to endure months of relentless cold and darkness.

The browsers and dispersers that evolved with *Podocarpus* in Australia (~65 million years ago; Ma) are long extinct (Bartholomai and Molnar, 1981; Molnar, 1996), but elements of this ancient environment remain. *Dacrydium* (Podocarpaceae) and associated swamp forest in Australian Wet Tropics is believed to have gone extinct only six thousand years ago (Ka) (Kershaw *et al.*, 2007a).

We observe the evolutionary ecology of these ancient environments through fossil morphology, community association and depositional characters, yet, inference is inevitable. As shown with *Wollemia nobilis*, a recently rediscovered conifer thought to be extinct for 65 My, discrepancies can occur between the fossil and the living specimen with the simplest of traits, let alone distribution (Chambers *et al.*, 1998).

The Southern Hemisphere is dry due to a number of causes, such as northern tectonic drift and the establishment of the Antarctic circumpolar current about 18 Ma (Barker and Thomas, 2004). This has dramatically limited the fossil record in Australia due to the lack of deposition, especially for the Quaternary (1.8 Ma to present). Subsidiary methodology may support the current understanding of historical community structure.

Slow morphological adaptation (evolutionary senescence), restriction to mesic communities, and broad distribution makes *Podocarpus elatus* an invaluable tool in understanding changes in community structure according to climate. Understanding the genetic disjunctions and range shift of *Podocarpus elatus* in response to past-climate change may help in predicting the future habitat suitability of the east Australian rainforests.

2. Background

The Australian vegetation today is the consequence of a dynamic history of climate change, latitudinal change, continental isolation, interaction with an evolving fauna, fire and more recently anthropogenic affects (Hill, 1994). This study is interested in understanding how *Podocarpus elatus* habitat (i.e. drier rainforest) has responded to late Quaternary climate change. Community turnover (including distributional change) between drier rainforest types, containing *Araucaria* and *Podocarpus* species, and wetter types containing *Nothofagus* species along the east coast of Australia have been successional in response to recent glacial cycles (Kershaw *et al.* 1994; Hill, 1994).

The Quaternary (1.8 Ma to present) is a period of particular importance due to extant plants and animals coming to establish broader communities and to reside within their environmental limits of tolerance (environmental niche). Although many have adapted to a changing environment, the fossil record indicates some species, including *Podocarpus*, have had a similar ecology (e.g. co-occurrence with palaeo-counterparts and reliance on mesic habitat) since the Tertiary (65–1.8 Ma), largely unaffected by the changing environment (Brodribb and Hill, 2003) – that is not to say changes in distribution have not facilitated their survival and trait preservation.

Throughout the Quaternary in the Australian Wet Tropics (AWT), rainforest was the dominant vegetation type with large tracts of dry rainforest fringing areas of complex rainforest in wetter areas (Kershaw *et al.*, 2005) similar in type to present-day dry rainforest communities typified by *Araucarian* emergents. Information from the fossil record indicates these dry *Araucarian* forests were the major habitat type of *Podocarpus* species. These transitional communities fringed floristically complex rainforest core areas, and where reduced angiosperm competition allowed for slower growing rainforest gymnosperms to establish, and in some circumstances thrive.

Australian vegetation is very diverse and reflective of the heterogeneous landscape and diversity of ecosystems that occur across our vast continent. This diversity is primarily due to the huge variation in rainfall across the landscape which over time has changed considerably and promoted rapid adaptation and extinction of a variety of plant taxa.

Increasing aridity during the Neogene (33.7–1.8 Ma) into the Quaternary is responsible for the transformation of the Australian landscape from one dominated by broad-leaf rainforest communities to one composed predominantly of open vegetation communities (sclerophyllous-type) with wetter rainforest restricted to present day locally moist areas (Kershaw *et al.*, 1994; Hill, 2004; Martin, 2006; Rossetto, 2006). Detailed information of the Australian climate throughout the Cretaceous and Cenozoic (145.5 Ma to present) has emerged over the last few decades but there is limited data to document short term climatic cycles (i.e. Kershaw *et al.*, 2007a), particularly for the Quaternary from eastern Australia where fossil evidence is sporadic and poorly dated (Quilty, 1994).

The change in distribution of major forest types provides a good assessment of abiotic change (e.g. climate, soil and fire). Biotic conditions (e.g. competition, pathogens, symbionts, and people) also influence the expansion/contraction of plant communities. Because a plant taxon requires a suitable environment for growth, its distribution is mainly controlled by the abiotic environment, with biotic interactions being associated with abiotic limits of tolerance and adaptability.

Past climatic change is inferred from evidence incorporated into deposits and especially from contained fossils (Hill, 1994; Quilty, 1994). If palaeoclimate is reconstructed solely on that indicated by fossil plants, it is susceptible to false interpretation. It is ideal to reach the same conclusion from a number of sources of evidence that are independent. Australia is deprived of palaeoclimatic data sources, especially ice cores that provide unique archives of past climate and environmental changes. Environmental niche modelling, used in this study, is reliant on known global fluctuations of climate, which for the large part has been sourced from Antarctic ice cores.

Australia is a continent where continuous onshore sedimentary records, particularly of those deposits that accumulated in a non-marine environment, are uncommon, and research often depends on the offshore marine sequences, themselves incomplete, around the continent margins, supplemented by other diverse convoluted records (Quilty, 1994). While carbonate deposition has characterised the offshore history during the Cretaceous to Cenozoic, they are gallingly incomplete in so far as records of onshore conditions (Quilty, 1994). The Australian paucity of reliable chronostratigraphic records emphasises the need for exploration of further palaeo-data sources and techniques for inferring Quaternary community structure.

3. The history of Podocarpaceae

Gymnosperms were once dominant in the Australian landscape, now, Podocarpaceae are one of only a few gymnosperm families to still occur in angiosperm dominated tropical rainforests (Hill, 1994). Historically they were a major component of the Gondwanan flora, but a changing physical environment (Createaceous-Paleogene boundary ~ 65 Ma), and rapid diversification of the angiosperms, resulted in the displacement and consequential extinction of many coniferous lineages. In comparison to the flowering plants, which when all taxonomy is finally done may add up to 300 000 species, conifers are a small group of woody seed plants, with only 630 species (Hill, 1994).

Extant Podocarpaceae are only a very small representation of a once highly diverse group (Hill, 1994). The family-wide morphology has stayed conserved through evolutionary time (Biffin *et al.*, 2012), yet recent radiations of the family suggest *Podocarpus* is more dynamic and adaptive than other podocarp genera (Biffin *et al.*, 2011). The community associations of the podocarps have also stayed conserved (e.g. Araucariaceae, Nothofagaceae and Cunoniaceae); even in peripheral ranges such as Central America they continue co-occurring with their palaeo-counterparts (Brodribb and Hill, 2003).

The ecological and environmental requirements of Podocarpaceae are specific, with almost all species restricted to wet montane and rainforest environments (Brodribb and Hill, 2003). Regeneration is usually continual recruitment of shade-tolerant seedlings, or in less shade tolerant species it is reliant on small-scale disturbances or topographic features such as ecotones, rivers and ridgelines to open the canopy (Brodribb and Hill, 2003).

3.1 Taxonomy of Podocarpaceae

Podocarpaceae is comprised mainly of Southern Hemisphere conifers and is a large family of 18 genera and 173 species (Hill, 1994; Quinn *et al.*, 2003). *Podocarpus* is by far the largest genus with 110 species, with the next largest genus being *Dacrydium* with 16. Of the 18 genera of Podocarpaceae only seven are represented in Australia, with most species restricted to northeast Queensland and Tasmania (Hill, 1994). The family was endemic to the ancient super continent of Gondwana and is a classic member of Antarctic flora (Quinn *et al.*, 2003). The main centre of diversity is Australasia, mainly New Caledonia, Tasmania and New Zealand, and to a smaller extent, Malesia and South America.

Fig 1 Variation in leaf morphology of shade-tolerant genera from the family Podocarpaceae (modified from source: Tim Brodribb, University of Tasmania).

Podocarpus has a lancolate leaf with a prominent mid-vein with no secondary venation (Fig 1). *Retrophyllum* has opposing leaflets arranged so the abaxial surface on one side is up and on the other is down (Hill and Pole, 1992). This possibly is a light-harvesting trait left over from its high-latitude origin, where the sun remains low in the sky (Ed Biffin, University of Adelaide, *pers. comm.*).

Podocarpaceae members are evergreen shrubs or trees usually with a straight trunk and mostly horizontal branches. The leaves are usually spirally arranged and are sometimes opposite. The leaves are scale-like, needle-like or flat and leaf-like, and are linear to lanceolate. Members are dioecious or rarely monoecious trees or shrubs with spirally inserted (opposite in *Microcachrys*) oblong to scale-like leaves (functionally replaced by flattened branches in *Phyllocladus*). Pollen cones are catkin-like and have many stamens. Male cones are terminal on axillary shoots including numerous spirally arranged sporophylls every one with two abaxial microsporangia (Mcarthy, 1998; http://www.ibiblio.org/pic/GymnospKey/gymnosperm_key_glossary.html).

Pollen grains are winged (saccate or bisaccate). Female cones are pendant and mature in one year. They are terminal on branches or terminal on short axillary shoots. Scales are persistent or deciduous and including axes are fleshy or dry and not woody at maturity. The seeds are completely covered by a fleshy structure referred to as an epimatium and are wingless (Mcarthy, 1998). Epimatium and integument sometimes connate and form a leathery testa. Germination is phanerocotular with two cotyledons and two parallel vascular bundles (Van Royen, 1979; Pocknall, 1981; Silba, 1986).

3.2 The origin of Podocarpaceae

Podocarpaceae originated in the Southern Hemisphere about 242 million years ago (Ma) and later migrated north of the equator probably during the last 15 million years (My) when the Australian plate contacted the Southeast Asian plate (Quilty 1994; Hill and Brodribb 1999). They also migrated north through Central America to Mexico during the same period. The podocarps appeared in the fossil record at the beginning of the Triassic when the great super continent Pangaea broke up. They grew along side araucarians, ginkophytes, cycads, tree ferns, giant club mosses and horsetails (Quilty, 1994; Hill and Brodribb, 1999), and were likely to be browsed and dispersed by dinosaurs (Bartholomai and Molnar, 1981; Molnar, 1996).

The Cenozoic macrofossil record of the Podocarpaceae is considerable, especially in south-eastern Australia, where the majority of the extant genera have been recorded (Hill and Brodribb, 1999). A few extinct genera (i.e. *Podosporites*, *Willungia* and perhaps *Coronelia*) have also been reported from across high southern latitudes, confirming an extremely diverse and widespread suite of Podocarpaceae during the Cenozoic in the region (Hill and Brodribb, 1999). The origins and relationships of the Podocarpaceae are unclear (Hill 1994) but attempts have been made to explain the phylogeny (Kelch, 1997; Conran *et al.*, 2000; Biffin *et al.*, 2011). Essentially a Southern Hemisphere family though Podocarpaceae macrofossils are found in the Northern Hemisphere (Hill and Brodribb, 1999; Hill, 1994). Although, the earlier Mesozoic and Tertiary floras of the Northern Hemisphere contain no *Nothofagus* or Podocarpaceae (Couper, 1960).

By the onset of the Mesozoic, the evolution of the Podocarpaceae is shown by the genus *Rissikia*, evident from the Triassic (248-206 Ma) of Madagascar, South Africa, Australia and Antarctica (Hill, 1994). Other early southern conifers, such as the Jurassic *Nothodacrium* and,*Mataia* (Townrow, 1967a; 1967b) are most probably podocarps, although, their relationship to extant genera warrants classification (Stockey, 1990). Podocarps were still prominent in the Cretaceous, albeit in Australia they were members of extinct genera (Hill, 1994), e.g. *Bellarinea barklyi* from the Early Cretaceous of Victoria (Drinnan and Chambers, 1986). Mill (2003) discusses the biogeography of Podocarpaceae pertaining to extant/fossil taxa and the palaeogeography of areas presently occupied by podocarps.

Molecular systematic studies using the locus rbcL for the Podocarpaceae show they are monophyletic but with low overall main branch support, although most genera in the phylogeny hold together as clades (Conran *et al.*, 2000). Phyllocladaceae are nested inside Podocarpaceae and *Podocarpus* is one genus with both subgenera positioned as clades (Conran *et al.*, 2000).

Podocarpus were abundant in Antarctic/Australian rift valley during the Cretaceous (Dettmann and Jarzen, 1990; Hill, 1994). A number of extant podocarpaceous genera are recorded as macrofossils in Australia during the early Tertiary (Hill, 1994). The majority of those genera, e.g. *Podocarpus, Falcatifolium, Acmopyle, Dacrycarpus, Dacrydium, Lepidothamnus* and *Phyllocladus*, occur in southeastern Australia, but *Retrophyllum* has been found in the southwest of Australia (Hill, 1994).

There are some shrubby species of *Podocarpus* that do presently occur within open-canopied communities, particularly heath (i.e. *P. spinulosus*, *P. drouynianus* and *P. lawrencei*), but like a number of taxa whose affinities are with rainforests, opinion is divided as to whether these are regarded as particularly tolerant and adaptable remnants of rainforests or are true components of open communities (Kershaw *et al*., 1994; Hill, 1994). Australasia and Malaysia have the greatest diversity of living podocarps, where South America, Antarctica and New Zealand have the greatest diversities of fossil podocarps. The fossil podocarp flora of New Zealand is more recent and probably derived from that of Australia, which has fewer living or endemic fossil genera (Hill, 1994; Mill, 2003; Wagstaff, 2004, Jordan *et al.*, 2011).

3.3 Podocarpaceae, Pinaceae and Pangaea

In the Southern Hemisphere today conifers achieve greatest abundance in wet forests, where there ability to compete successfully with broad-leaved angiosperms is due in part to their production of broad, flat photosynthetic shoots (Hill and Brodribb, 1999; Brodbribb and Hill, 2003). The Podocarpaceae produce large leaves and have superior light harvesting ability than the Pinaceae. The tall closed canopy forests of the equatorial region have remained accessible due to this leaf morphology. The Pinaceae (with the exception of *Pinus krempfii*) are shade-intolerant and have not been able to colonise south of the equator (Hill and Brodribb, 1999; Brodbribb and Hill, 2003).

Fig 2 The unsuccessful colonisation of Pinaceae into Southeast Asia (red), and the successful colonisation of Podocarpaceae (black) (source: Tim Brodribb, University of Tasmania).

The colonization of Southeast Asia and subsequently Australia by Pinaceae has been blocked by the equatorial evergreen rainforests (Fig 2). Pines are physiologically incapable of traversing this region due to shade-intolerance. In contrast, the Podocarpaceae are shade-tolerant and can propagate under a canopy making them capable of colonizing the equatorial zone and beyond (Hill and Brodribb, 1999; Brodbribb and Hill, 2003).

3.4 Migration, adaptation or extinction

Shade tolerance, and the ability to propagate under a canopy, is a common character of the Southern Hemisphere conifers. Possibly, high-latitude rainforest origin and the ability to senesce over seasonal periods of darkness, is associated with the evolution of shade-tolerance in the present members of Podocarpaceae. Relative to the southern Gondwana, the northern Laurasian continents stayed together and allowed latitudinal range shift in response to habitat-suitability (McLoughlin, 2001).

It has been shown from fossil and molecular systematic studies that conifers are a monophyletic group and a common ancestor between the Northern and Southern Hemisphere conifers occurred (Chaw *et al.*, 1997: Stephanovic *et al.*, 1998). The Northern Hemisphere family Pinaceae is shade-intolerant, possibly a result of the ability to migrate in latitude, where the relative lack of restriction did not necessitate significant adaptation to a changing environment. While, the Southern Hemisphere conifers were restricted in distribution and needed to adapt to the changing environment, or go extinct (Enright and Hill, 1995).

Palynological records show that Northern Hemisphere tree populations are capable of rapid migration in response to a warming climate. The Northern Hemisphere fossil pollen record has revealed rapid migration rates of many temperate tree species of 100–1000 m/yr during the early Holocene (McLachlan *et al.*, 2005). The migration rates you would imagine would be comparable to Southern Hemisphere tree populations, but detailed palynology to record past migrations is absent, or of poor quality to record such events here in Australia.

Possibly, the relative lack of land mass in the Southern Hemisphere has necessitated adaptation rather than migration in response to competitive exclusion. The high latitude circumpolar Taiga Forests of the Northern Hemisphere are devoid of arboreal angiosperms. The distribution illustrates the ancestral retreat of gymnosperms away from increased angiosperm competition at lower latitudes (Enright and Hill, 1995). While in the Southern Hemisphere, the lack of migration routes south toward the pole may have caused the remarkable adaptation of the southern conifers in response to increased angiosperm competition (Fig 1: broad leaves and shade tolerance; Enright and Hill, 1995; Brodribb and Hill, 2003; Biffin, *et al.* 2011). Strong selection may account for the extinction of many coniferous genera in the Southern Hemisphere, as recorded in the Mesozoic fossil record (Hill, 1994; 2004).

3.5 *Podocarpus*

Podocarpus was first described in 1807 by L'Heritier. The genus comprises 110 species the majority of which are restricted to lowland and montane forests of warm temperate to tropical areas in the Southern Hemisphere. Seven species are endemic to Australia: *P. dispermus*, *P. drouynianus*, *P. elatus*, *P. grayi*, *P. lawrencei*, *P. smithii*, and *P. spinulosus*.

Podocarpus has two subgenera, subgenus *Podocarpus* and subgenus *Foliolatus*. The subgenus *Podocarpus* have cones that are not subtended by lanceolate bracts and the seed usually has an apical ridge, and a florin ring around stomata (florin ring). While *Foliolatus* has a cone subtended by two lanceolate bracts and the seed usually is without an apical ridge (Mcarthy, 1998).

Distribution of the subgenus *Podocarpus* is in the temperate forests of Tasmania, New Zealand, southern Chile, with some species extending into the tropical highlands of Africa and the Americas (Mcarthy, 1998). While *Foliolatus*, generally has a tropical and subtropical distribution, concentrated in east and Southeast Asia and Malesia, overlapping with subgenus *Podocarpus* in northeastern Australia and New Caledonia (Van Royen, 1979; Silba, 1986; Harden, 1990).

3.5.1 Reproductive biology of *Podocarpus*

Podocarpus elatus has a reproductive cycle of one year and is a dioecious species with separate male and female plants. Information specific to *P. elatus* is limited. The closest phylogenetic species with information available is *P. neriifolius*, which exhibits underdeveloped embryos at time of dispersal, although germination is usually rapid with seed quickly losing viability (i.e. 20-60 days) so that there is no persistent soil-stored seed bank (Enright and Jaffr, 2011). *Podocarpus totara* is a large tree of New Zealand and is a member of the sub-genus *Podocarpus*, while *P. elatus* is a member of *Foliolatus*. *Podocarpus totara* in New Zealand has a reproductive cycle of two years, and strobilus initiation is in September, followed by a nine month period of winter dormancy until emergence during the growth flush in July-August of the following year (Wilson, 1999).

Pollination in *P. totara* occurs in mid-October to mid-November at the megaspore tetrad stage, where female strobili bear only one or two ovules. During December pollen germination and fertilisation occur quickly and the pollen tube carries the body cell which branches out after reaching the archegonia. Embryo maturation is complete by February (Wilson, 1999).

3.5.2 *Podocarpus*: a climatic indicator

Podocarps are important to the study of the effect of long-term climatic change on, leaf morphology (Sporne, 1965; Biffin, *et al.* 2011; Hill and Pole, 1992), physiology (Brodribb and Hill, 2003), taxonomic make-up during the Cenozoic (Hill, 1994), distribution (Ledru *et al.*, 2007) and diversity (Quiroga and Premoli, 2007; Quiroga and Premoli, 2010). The concentration of conifers in wet forest habitats left them vulnerable to Cenozoic climate change and decreases in interspecific diversity have occurred since the Paleogene in all areas where fossil records are available (Hill and Brodribb, 1999).

Podocarpus fossils have been used by palynologists and palaeo-climatologists to reconstruct Quaternary climate in tropical South America even though the factors involved in their modern distribution are not well understood (Enright and Hill, 1995). The moist ecosystems where the vast majority of the genus grow are well defined climatically and their bisaccate pollen grain are unmistakable. Pollen analysis using light microscopy has not yet been able to identify to the species level reliably, so palynological interpretations are the subject to several hypotheses (Enright and Hill, 1995).

For decades, palynologists working in tropical South America have been using the genus *Podocarpus* as a climate indicator (Ledru *et al.*, 2007). The combination of botany, pollen and molecular analysis has proved to be reliable for determining population groups and their regional evolution within tropical ecosystems. The refugia of rainforest communities identified as crucial hotspots has allowed the Atlantic forests to survive under unfavorable climatic conditions and are likely to offer the opportunity for this type of forest to expand in the event of future climate change (Ledru *et al.*, 2007).

Investigation of the long-term responses to climate changes in *Podocarpus parlatorei*, a cold-tolerant tree species from the subtropics in South America, using distribution patterns of isozyme variation has inferred northern expansion of the species during glacial periods

(Quiroga and Premoli, 2007). *Podocarpus parlatorei* is restricted to montane forests within the Yungas, a cloud forest of the subtropics of north-western Argentina and southern Bolivia. It consists of disjunct population groups that are ecologically subdivided according to latitude. These groups were expected to be genetically divergent from one another as a result of historical isolation. The effective number of alleles and observed heterozygosity increased with latitude, with the southern populations tending to be more variable and genetically distinct. A positive association between genetic and geographic distances was detected and reduction in diversity towards the north and high-elevation mountains are consistent with evidence of patterns of forest migration resulting from climate change during the Late Quaternary (Quiroga and Premoli, 2007).

3.5.3 Stand dynamics

Stand dynamic studies on *Podocarpus* species have revealed that on poorly-drained, nutrient-poor and high altitude sites where most of associated species were fairly shade intolerant and light crowned, dense all-aged populations and the presence of numerous saplings beneath the canopy suggested continuous regeneration (Lusk, 1996). Conversely, on more favourable sites, were several of the associated angiosperms were highly shade-tolerant and dense crowned, *Podocarpus* species were less abundant, and there regeneration from seed appeared to be sporadic (Lusk, 1996).

Great longevity of shade tolerant conifers is probably crucial in their persistence in competition with shade-tolerant broad leaved species in undisturbed stands of favourable sites (Lusk, 1996). A review of literature on southern temperate forests (Enright and Hill, 1995; Enright and Ogden, 1995; Lusk, 1996) disputes the hypothesis that heavily shaded, infrequently disturbed habitats are an evolutionary refuge for conifers (Bond, 1989).

Sites likely to have high leaf area indices and infrequent disturbance are more successfully exploited by shade-tolerant angiosperms (Lusk, 1996). Regeneration can take the form of continual recruitment of shade-tolerant seedlings, or in less shade tolerant species it is reliant on small-scale disturbances or topographic features such as rivers and ridgelines to open the canopy (Brodribb and Hill, 2003).

The Myall Lakes and Jervis Bay populations of *P. elatus* are regularly burnt and considerable recruitment is observed post-burning (Chris Quinn, The Royal Botanic Gardens and Domain

Trust, Sydney, *pers. comm.*). *Podocarpus drouynianus* and *P. spinulosus* are probably fire adaptable remnants of rainforest. They resprout from lignotubers and are fire tolerant. It is also shown for *P. lawrencei* that recruitment is favoured after fire (Macdonald, 2004).

3.6 *Podocarpus elatus* R.Br. ex Endl.

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Coniferopsida; Pinales; Podocarpaceae; *Podocarpus*; *Foliolatus*; *elatus*.

Podocarpus elatus is an evergreen conifer that was abundant across the warmer wetter parts of present day Australia before the arrival of Europeans. Indigenous people possibly referred to *P. elatus* as 'Djerren Djerren' in the Eora dialect of the Gadigal nation but there is no known dreaming or song lines pertaining to the species (Clarence Slockee, The Royal Botanic Gardens and Domain Trust, Sydney, *pers. comm.*). *Podocarpus* is derived from two Greek words *pous* (foot) and *karpos* (fruit), referring to the fleshy fruit stems. The species name *elatus*, is Latin for 'tall', refers to how tall the trees grow (http://www.anbg.gov.au/anbg/conifers/*Podocarpus-elatus*.html).

Fig 3 The distribution of *Podocarpus elatus* based on a compilation of collections made for all major herbaria in Australia since 1818. The Clarence River Corridor (Mellick *et al.*, 2011) is a regional genetic boundary between northern and southern populations (indicated by bar).

The species ranges from New South Wales to Queensland and prefers habitat in and around rainforest north of the Beecroft Peninsula, Jervis Bay, NSW (Harden, 1990), in the south, to Cape York Peninsula, QLD, in the north (Fig 3). It prefers subtropical, gallery and littoral rainforest habitats, with rich, moist, non-alkaline soils but the species is usually more successfully established on poorer sites where competition is less vigorous. Plants will tolerate salt spray and frosts to -7 degrees so long as they have good moisture levels and humidity. Maturing in 8-10 years, *P. elatus* is fast growing once established (Harden, 1990). Populations observed along the East Coast of NSW exhibited little recruitment, with most populations being composed of a few mature trees with no seedlings or saplings present.

It is commonly referred to as the Illawarra Plum or Pine, Brown Pine, Turpentine Pine in the timber industry, Yellow or Plum Pine (Harden, 1990). Growing to 40 m with brown bark (fibrous) that is often fissured and scaly on old trees. It is a dioecious species having separate male and female trees. The fruits are a purple black colour and composed of two segments. The edible portion is a grape-like (10mm diameter), sweet with a juniper-like flavour but mucilaginous (the texture is more palatable if refrigerated for a day) that ripens from March to July (Harden, 1990). The inedible part of the fruit is the hard dark seed that is situated externally on top of the swollen stalk. The fruit has high ascorbic acid content (vitamin C), contains minerals, fat, protein, are high in energy and are 61% water. The leaves are lanceolate, oblong to linear (6-18 mm wide, 5-14 cm long) and the midvein is prominent (Harden, 1990). Leaves can grow to 25 cm long on young trees. The male cones are narrow-cylindrical, catkin-like, to 3 cm long, and are in sessile clusters (Harden, 1990). Female cones are stalked, and solitary; scales are few, fleshy, uniting with the stalk to form a fleshy receptacle (Harden, 1990).

Podocarpus elatus was targeted extensively by the early timber-loggers for it is a useful timber which has wide applications from boat building to cabinet making. The wood has a very fine, even texture and a straight grain with faint growth rings, making it desirable for table tops, furniture, musical instruments (piano keys and violin bellies) and wood turning. Commonly referred to as Brown Pine, the yellow wood turns brown in colour shortly after being exposed (Barker *et al.*, 2004). It has not been widely planted as a timber tree due to not being as light and strong as its relative *Araucaria cunninghamii*. It has however been widely clonally cultivated for roadside plantings (David Bateman, Waverly Council, Sydney, *pers. comm.*: Appendix B), as an ornamental shade tree and now more commonly for the native bush food market. There is horticultural development research published on *P. elatus* (Ahmed and Johnson 2000), although the majority of cultivation of the species is now clonal.

3.6.1 Fire and ecotonal specialisation

Bowman's book (Bowman, 2000), titled 'Australian Rainforests: islands of green in a land of fire' emphasises the existence of Australian rainforests is largely owed to the patterns and processes responsible for these sharp boundaries between drier rainforest-types and fire promoting communities. The capacity of the fragmented East Australian rainforests to resist destruction by fire is partially a consequence of the species composition and structure of the surrounding, frequently disturbed drier rainforest communities.

Podocarpus elatus exhibits a comparative advantage in drier communities, fringing more floristically complex core communities. Larger dense populations are found on the southern side of a break in the rainforest canopy with a northern aspect. The established *P. elatus* populations sampled for this study all have a drier northern aspect along a break in the canopy. This drier more frequently burnt rainforest boundary would seemingly select against longer-lived species, though for *P. elatus*, thick bark and waxy leaf cuticle, gravity seed dispersal, shade-tolerance (i.e. rapid and dense propagation under female trees), the ability to senesce as a sapling for long periods and possibly leaf chemistry may aid it in occupying these fire prone areas where other rainforest trees are less able.

Podocarpus elatus is known as Turpentine Pine, due to the high concentration of turpentinol compounds in the foliage, as are found in *Pinus* species. The evolution of these compounds are likely a response to increased herbivory. Turpentinols increase flammability and possibly these compounds in the species may also be a result of frequent burning. It would be interesting to know if *P. spinulosus*, a co-occurring fire adapted podocarp, has similar leaf chemistry to *P. elatus.* Understanding the past expansion/contraction of dry rainforest communities will aid in understanding the seemingly precarious existence of these relatively fragile-wet communities in such a harsh-dry landscape.

Evidence suggests large herbivorous dinosaurs browsed on, and subsequently dispersed, the Australian podocarps (Bartholomai and Molnar, 1981; Molnar, 1996), which may have contributed to the podocarps habitat preference (Hill and Brodribb, 1999), as heavily forested areas would restrict the movement of such browsers. It is worth briefly mentioning that the rapid extinction of mega fauna possibly increased fire frequency due to biomass gain from reduced browsing (Rule *et al.*, 2012), indicating that extinct mega fauna may have had a considerable effect on rainforest fragmentation.

Drier rainforest communities, typified by Araucarian emergents and *Podocarpus* species, are sensitive to affects of fire. Kershaw *et al.* (2005) postulated that dry rainforest communities fringed wetter core rainforest communities in the Australian Wet Tropics (AWT), and expansion and contraction of these communities are seasonal and climate driven. Precipitation gradients are largely responsible for successional community turnover from wet rainforest to dry rainforest and eventually to sclerophyllous forest during dry periods.

During wet periods, wetter rainforest would encroach into previously dry rainforest and the lack of fire would allow dry rainforest to expand into areas previously occupied by sclerophyllous forest. Evidence for the latter can be found throughout the rainforests of northern NSW (central range of *P. elatus*) where old *Eucalyptus grandis* trees tower over wet rainforest communities far from fire prone boundaries.

These boundaries are an important parameter of climate change. With increasing fragmentation (associated with aridification), the abundance of plant communities associated with these boundaries may also increase, as a consequence of increasing surface area. Past changes in species composition and extent of these communities have been showed to be correlated to the glacial cycles of the Quaternary period (Kershaw *et al.*, 2007a).

Podocarpus species are known to senesce for periods up to a decade with very little growth (Brodribb and Hill, 2003). The species exhibits remarkable traits to survive in such a highly diverse, dynamic and competitive environment. Possibly, high-latitude rainforest origin of Podocarpaceae and the need to senesce over seasonal periods of darkness is associated with the evolution of shade-tolerance in the present members of the family.

4. Vegetation change in Australia during the Cenozoic

The Australian landscape has not always been arid, and the central desert was once well-vegetated (McLoughlin, 2001; Martin, 2006). At the start of the Cenozoic (65 Ma), Australia had a warm/humid climate and the vegetation was predominantly warm to cool temperate rainforest (Martin, 2006). The history shaping the eastern Australian rainforest communities involved, high southern latitude of the continent at the end of the Cretaceous period (65 Ma) and its gradual movement north toward the equator; general global aridification over the last 65 My amidst short term fluctuations and the evolution from ancestral Gondwanan lineages of most taxa, but also immigration onto the continent by both plant and animal lineages from different geographic sources at several times (Martin, 1982; Greenwood and Christophel, 2005; Sniderman and Jordan, 2011).

Separation of Australia from Antarctica started at the end of the Paleocene (54.8 Ma) with the formation of a narrow strait, but it was the mid-Oligocene (31.1 Ma) when the first channel was formed between the continents (McLoughlin, 2001). This allowed for the Antarctic Circumpolar Current (ACC) to be created due to the removal of the block caused by the South Tasman Rise, which cooled the continent and increased the size of the west Antarctic ice cap, which removed the available atmospheric moisture and reduced precipitation globally (Martin, 1982). The magnitude of the ACC as we know it today was established about 18 Ma. This started the rapid cooling of Antarctic and the eventual demise of the rich Antarctic flora.

4.1 Cenozoic summary

In the Palaeocene (65–54.8 Ma), the Australian continent was warm/wet, and the vegetation was mostly rainforest, except the northwest where there was limited aridity and central Australia where aridity would have been seasonal (Martin, 2006). Gymnosperms were the predominant vegetation type of south-eastern Australia but angiosperms were dominant in central Australia (Martin, 2006). Podocarpaceous gymnosperms dominated the Palaeocene vegetation due to the cool temperate climate similar to Tasmania and New Zealand today.

The early Eocene (54.8 Ma) was hot/humid ensuing angiosperm diversification and dominance, and the increase of mega-thermal taxa in south-eastern Australia (Martin, 2006). Rainforest gymnosperms were less abundant than the Palaeocene.

In the mid-late Eocene (49–41 Ma) there was a decrease in temperature – the vegetation was mainly rainforest, but in central Australia there were some open vegetation and sclerophyllous taxa (Martin, 2006). Sclerophylly developed as a response to infertile soils, long before the climate became dry (Martin, 2006). *Nothofagus* became the dominant pollen type, especially in the southeast, and Lauraceae the dominant leaf type (Martin, 2006).

There was a rapid cooling during the early Oligocene (33.7 Ma), the result of opening of the seaway between Australia and Antarctica and strengthening of the Antarctic Circumpolar Current (Martin, 2006; Quilty, 1994) Angiosperm diversity decreased and *Nothofagus* pollen became more prominent (Martin, 2006).

During the Oligocene (33.7–23.7 Ma) temperature and angiosperm diversity increased, and the occurrence of swamps in south-eastern Australia were more common, indicating that rainfall was particularly high at this time (Martin, 2006).

The early Miocene (23.7 Ma) saw an increase in temperatures and the vegetation became more variable in south-eastern Australia and in central Australia, rainforest was limited to small pockets. The Miocene remains the warmest and most humid period during the Cenozoic (Martin, 2006).

The mid-Miocene (16.4 Ma) is when regular flows in palaeo-drainage systems over much of western and central Australia ceased, and alkaline lakes of inland basins in central Australia deposited dolomite indicating high rates of evaporation and a well-marked dry season (Martin, 2006).

The climate became colder and drier during the late Miocene (11.2 Ma), reducing the abundance of rainforest in Australia and increasing the fire tolerant Myrtaceae flora especially *Eucalyptus* (Martin, 2006).

A brief warming period and increased precipitation in the early Pliocene (5.3 Ma) caused an expansion of rainforest in river valleys of the western slopes and south-eastern Australia (Martin, 2006).

The climate increasingly became drier during the late Pliocene (3.6 Ma) (Fig 4) and rainforest taxa contracted further to the wetter coastal and highland regions. Grasslands became more prevalent in inland areas and the modern climate was established, but it still was considerably wetter than today (Martin, 2006).

The Pleistocene (1.8–0.01 Ma) fluctuated between drier glacial and wetter interglacial periods. About 0.5 Ma, there was a marked shift to a dry climate (Martin 2006). The present interglacial is drier than the previous interglacial (130 Ka), with the last glacial maximum (LGM: 21 Ka) being particularly adverse. Although conditions have improved, precipitation has not returned to the levels of the previous interglacial period (Martin, 2006).

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held by the University of Adelaide Library.

Fig 4 The build-up of arid cycles during the last 5–6 million years (Bowler, 1982). This figure has been reproduced with permission of the publisher (print copy only, the full reference is on page 53).

The earth's climate has become cooler through the Tertiary (65-1.8 Ma) with frequent oscillations that increased in amplitude (Fig 4) and lead to the series of major ice ages of the Quaternary (Hewitt, 2000).

The surviving rainforest taxa have had to migrate into areas (refuges) that have conserved favourable environmental conditions for survival, and the ranges of many Gondwanan lineages has decreased dramatically. The present genetic structure of populations, species and communities has been primarily formed by the Quaternary ice ages, and genetic, fossil and environmental data combined can greatly help our understanding of how organisms were affected (Bennett, 1997; Hewitt, 2000).

4.2 Quaternary vegetation

The Quaternary is a period in which modern life as we know it have evolved, or have persisted from the Tertiary largely unaffected by environmental change (Hope, 1994; Hewitt, 2000). The Quaternary chronology is not based on widespread evolutionary changes, as are older periods, but instead uses climatic changes, such as the growth of ice sheets and the spread of cold surface water (Hope, 1994; Wright Jr, 1984). Fluctuations of heavy isotope oxygen (^{18}O) from marine sediments and ice cores, records a series of cyclic changes of periods of 100 Ky, being glacial maximums alternating with inter-glacial periods. This record shows in the last 600 Ky there have been six glacials periods (Fig 4).

The two divisions of the Quaternary are the Pleistocene from 1.8 Ma to 10 Ka, and the Holocene, which includes the present inter-glacial. The Pleistocene represents the period of establishment of our current landscapes, climatic patterns and diversity, and the adaptation of the Tertiary biota to these new environments (Hope, 1994). Vegetation history of the Quaternary is of particular importance when comparing extant flora, or recent extinctions, to fossil flora (Hill, 1994; Hill, 2004; Hope, 1994; Wright Jr, 1984).

4.3 East Australian rainforests

World Heritage Listing of the east Australian rainforests (isolated sub-tropical and tropical forests that occur above 300m) was awarded largely due to the rare assemblage of *Lepidozamia hopei, Agathis robusta*, *Prumnopitys ladei*, *Podocarpus grayi* and *Podocarpus elatus* which includes the closest living counterparts of Jurassic-age fossils. The members of Podocarpaceae in the rain forests of tropical Australia are *Podocarpus dispermus, Podocarpus elatus, Podocarpus grayi, Podocarpus smithii, Prumnopitys ladei* and *Sundacarpus amara*. All occur in the Wet Tropics, although *P. elatus* is very rare in the Wet Tropics. It should however be included, as it is more typical of somewhat drier (Araucarian) rainforest (Greenwood and Christophel, 2005).

Tenuous preservation of the Australia Wet Tropics (AWT) rainforest communities under increasing aridification can be attributed to orographic uplift and subsequent increase in precipitation in those areas as a result of the Great Dividing Range (GDR). The vast tracts of rainforest that once occurred throughout Australia contracted to these areas of high precipitation (Kershaw *et al.*, 2007c). Floristic interchange with Southeast Asian rainforests may have occurred (Sniderman and Jordan 2011) but possibly was prevented by a dry corridor in the northern Australian-Sahul Shelf region (Kershaw *et al.*, 2005).

The aridification of the continent, decline of mega fauna and advent of indigenous burning increased the exposure of the relatively fire susceptible drier rainforest communities.. “Araucarian forest appears to have remained intact until it was progressively replaced by sclerophyllous vegetation as a result of increased burning over the last 200,000 years” (Kershaw *et al.*, 2005). Kershaw *et al.*, (2005, 2007a) provides excellent evidence of community turnover in the Australian Wet Tropics (AWT) in response to recent glacial cycles of the Quaternary. His extensive work has provided invaluable records of fossil pollen over the last 230 Ky (Fig 5).

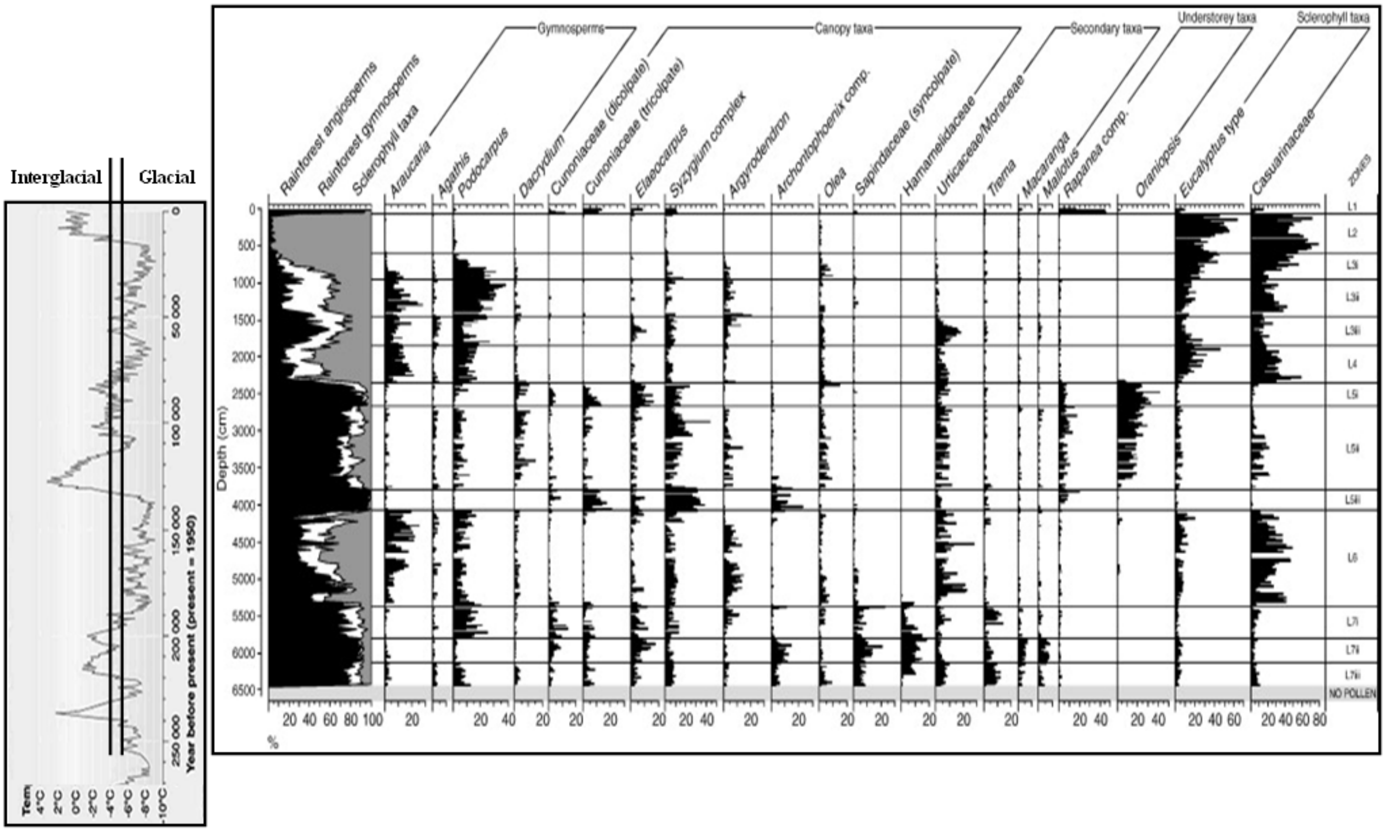

Fig 5 A pollen record of the last 230 Ka from Lynch's Crater (AWT), north-eastern Australia (modified from Kershaw *et al.*, 2007a), showing the relative proportions of the major groups of arboreal forest taxa and percentages of major contributors to these groups. All percentages of individual taxa are based on the arboreal forest taxon sum for individual spectra. Corresponding change in temperature (two glacial cycles) according to Vostok (Antarctica) ice core data (modified from Petit *et al.* 1999) is provided on the left.

During interglacial periods in the AWT the occurrence of *Podocarpus* pollen is dramatically reduced when compared to glacial periods, indicating a preference for cooler drier environments, and/or susceptibility to angiosperm competition. Possibly, the genus persists during periods of angiosperm dominance (interglacials), due to association with drier fringing communities around core rainforest communities, where angiosperms are present yet are not as competitive (Lusk, 1996).

On another front it can be observed that during glacial periods the sclerophyllous taxa become more abundant. This fire promoting vegetation could be encroaching on drier rainforest, and accordingly drier rainforests communities may infringe wetter rainforests during glacial periods.

Kershaw *et al.* (2007a) on completion of the Lynch's crater sequence (Fig 5), maintains the pattern of complex rainforest expansion during wetter interglacial periods is replaced by drier rainforest and sclerophyll vegetation during drier glacials. *Araucaria* and *Podocarpus* (drier rainforest) incur cyclic changes in abundance coinciding with the last two glacial cycles.. During the last glacial period, these genera are replaced by more fire-tolerant elements, as is understood from the recorded increase in fire frequency during this period.

The decline in a more fire sensitive sclerophyll taxon *Callitris*, may suggest an earlier, more regional increase in burning, a feature not inconsistent with the evidence in the offshore fossil record, that regionally Araucariaceae had an initial decline about 130 Ka, after some 10 My of near dominance in the landscape (Kershaw *et al.*, 2007a).

The impact of these changes on the current landscape is that *Araucaria* (dry rainforest) is now restricted to small isolated patches that are unlikely to regain their previous dominance (Kershaw *et al.*, 2005).

The extinction of *Dacrydium* from the continent, whose closest morphological type occurs as in New Caledonia, is clearly related but its presence in the AWT record may suggest it was associated with a different community – a peatland or swamp forest that is now extinct in Australia (Kershaw *et al.*, 2007a).

4.4 Palynology

Pollen analysis has been well-established as a means for reconstructing vegetation history (Hill, 1994, 2004; Brewer *et al.*, 2002). The pollen grains and spores produced by plants are preserved well in anoxic environments (e.g. lake deposits, forest hollows, peat bogs) and may be extracted from these deposits by sampling exposed sections or by taking sediment cores (Brewer *et al.*, 2002). The variation in the pollen assemblage are normally presented in the form of a pollen diagram (Fig 5) with the changes in pollen percentages of each taxon plotted against depth, enables the temporal changes in the representation of individual taxa and the type of surrounding vegetation communities to be determined.

Kershaw *et al.* (1994) indicates that it is not always possible to separate different genera on their pollen morphology. Ledru *et al.* (2007) has found it impossible to classify *Podocarpus* pollen grains at the species-level using light microscopy. Techniques used in forensic palynology (Lynn Milne, University of Western Australia, Perth, *pers. comm.*) made need to be incorporated into current methodology, if species-level identification using light microscopy is important in reconstructing past community structure.

Fossil pollen sampled from sediment cores taken from the bottom of Lake Euramoo (AWT) has revealed that there has been a gradual change in the surrounding vegetation from dry adaptive species to rainforest species (Haberle, 2005). This vegetation change peaked at about 7300 years ago during the Holocene Climatic Optimum and has since been in decline. This is consistent with the habitat becoming wetter until the shift towards competitive dominance of taxa adapted to drought and fire, which may be a response to the intensification of El Nino-related climatic cycles (Haberle, 2005).

In north-eastern Australia, recent pollen spectra from the Ocean Drilling Program (ODP) have sufficient in common with the riverine samples to suggest that rivers are contributing a major pollen component to the offshore sediments (Moss *et al.*, 2005). This would suggest riparian vegetation (gallery forest) and associated taxa would be well-represented in the offshore fossil record. Recent pollen samples from core tops taken from the Grafton Passage on the continental shelf, that was thought to be the major passage for pollen transport to ODP Site 820 (16°38′S, 146°18′E), show significant differences to both riverine/ODP samples and suggest that pollen is dispersed across the continental shelf and through the outer Great Barrier Reef system in an unanticipated fashion (Moss *et al.*, 2005).

4.4.1 *Podocarpus* pollen morphology

Taxonomic resolution (species-level) is limited for *Podocarpus* pollen and understanding of past community structure and relative abundance of species from the fossil pollen record is not reliable (M. K. Macphail, Australian National University, Canberra, *pers. comm.*).

Podocarpaceae pollen mostly have sacci (Wodehouse, 1935), small bladder like structures flanking the corpus (Fig 6). Their likely function is to increase buoyancy to facilitate migration of the pollen grain through the pollination fluid in the micropylar canal, tending to orientate the grain so that the germinal furrow contacts the female gamete (Doyle, 1945).

Sacci structures may also be involved with flight, increasing the dispersal distance of pollen and aiding pollination (Proctor *et al.*, 1996). Sacci are always associated anatomically with the furrow and may function in protecting it in periods of water stress (Pocknall, 1981).

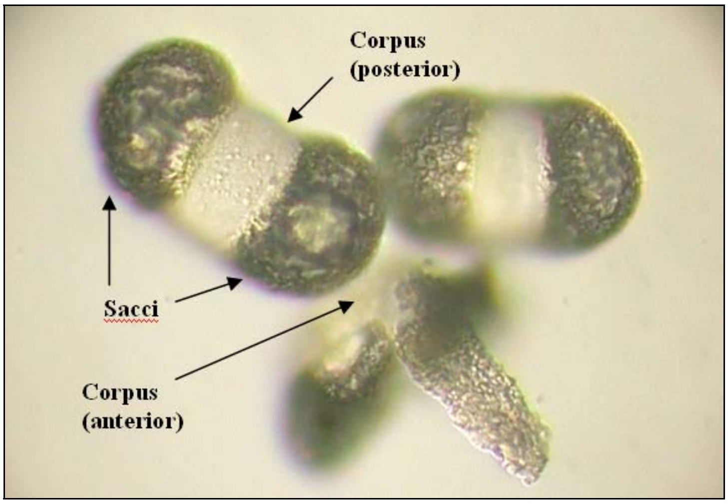

Fig 6 *Podocarpus elatus* bi-saccate pollen (photo: R. Mellick)

Podocarpus pollen is easily identifiable due to distinctive bi-saccate grains (Fig 6). Although bi-sacci is a synapomorphic trait shared with other coniferous flora, it is unique to *Podocarpus* in Australia. The mesic ecosystems where the trees grow are climatically well defined, so the broad distribution patterns can be extrapolated from point locations of fossil remains, due to distinct climatological requirements.

5. Phylogeography and population genetics

Phylogeography is a term coined by Avise *et al.* (1987) to illustrate the spatial distributions of phylogenies. Phylogeography is used to determine common responses of organisms to historical biogeography in an effort to understand speciation, and to comprehend geographic distributions of genealogical lineages including those at the intraspecific level (Avise, 2000). Phylogeography interprets current genetic structure, phylogenetic relatedness of alleles and their spatial arrangement among and within populations to reveal historical components of gene flow (Avise, 2000).

Individual species phylogeography provides identification of geographic areas containing high genetic diversity or distinct evolutionary units for that species (Byrne, 2007). These areas are usually inferred as refugia, although past migration of diversity may have an influence. Phylogeographic data collected from wide-ranging species allows for phylogenetic estimates of gene flow to be made (Lowe *et al.*, 2004). Phylogeography is a critical juncture between established fields and effectively weights the influences of dispersal versus vicariance in shaping the geographic distributions of genetic traits (Avise, 2000).

The field takes a population genetics and phylogenetic perspective on biogeography. An explicit focus on a species biogeographical past sets phylogeography apart from classical population genetics and phylogenetics (Knowles and Maddison, 2002). Past events including population expansion/contraction, bottlenecks, vicariance and migration can be identified. Recently developed approaches integrating coalescent theory, environmental niche modelling and genealogical history of alleles can accurately address the relative roles of these historical events in shaping current patterns (Cruzan and Templeton, 2000).

The field can inform conservation strategies through the assessment of genetic diversity that incorporates an evolutionary perspective, and allows evaluation within a geographical context, so providing integration with other biogeographical information (Byrne *et al.*, 2011). A number of studies have been interested in the spatial and temporal response of organisms to climate driven distributional fluctuation (Bell *et al.*, 2007; Pease *et al.*, 2009; Petit, 2002; Richards *et al.*, 2007; Rissler *et al.*, 2006; Scoble and Lowe, 2010).

5.1 Coalescent theory

The application of coalescence theory to genealogical relationships within species provides information into the process of diversification and the influence of biogeography on distributional patterns (Cruzan and Templeton, 2000).

The coalescent theory is a retrospective model of population genetics that traces all alleles of a gene from a population to a single ancestral copy shared by all members of the population, known as the Most Recent Common Ancestor (MRCA) (Donnelly and Tavaré, 1995). Under conditions of genetic drift alone, every finite set of genes or alleles has a coalescent point at which all descendants converge to a single ancestor (Cannings, 1974; Donnelly and Tavaré, 1995).

Some properties of the coalescent theory are: the larger the number of samples the greater the rate of coalescence (the more lineages there are the greater the probability that two will coalesce), the larger the population size the slower the rate of coalescence, time to coalescence gets longer as the process moves toward the MRCA, smaller sample sizes have a high probability of including the MRCA of the population, and time cannot be measured directly with genetic data but genetic divergence can.

Discourse of the properties and applications of the coalescence theory has been extensive (Donnelly and Tavaré, 1995; Ewens, 1990). As coalescent theory was developed, so were the within-species molecular data sets resulting from the development and availability of molecular techniques (Crandall and Templeton, 1993; Kingman, 2000).

Considering the assumption of selective neutrality (Kimura, 1968) it is possible to model the history of a sample, that is, without regard to the rest of the population. Selection can be accommodated easily if it is strong, while coalescent models of weak selection are more complicated. Chloroplast and nuclear genes will coalesce at different rates due to the difference in inheritance between chloroplast and nuclear DNA as a result of recombination.

The coalescent, as it is typically presented in population genetics, makes all the usual assumptions of the Wright-Fisher model of a population (Donnelly and Tavaré, 1995; Fisher, 1930; Wright, 1931).

5.2 Population genetics

Population genetics is the study of the allele frequency distribution and change under the influence of the four evolutionary forces: natural selection, genetic drift, mutation and gene flow (Hartl and Clark, 1997; Lowe *et al.*, 2004).

It takes account of population subdivision and population structure in space, and attempts to explain such phenomena as adaptation and speciation. Population genetics was a vital ingredient in the modern evolutionary synthesis; its primary founders were Sewall Wright, J. B. S. Haldane and R. A. Fisher, who also laid the foundations for the related discipline of quantitative genetics (Falconer and Mackay, 1996; Hartl and Clark, 1997).

5.2.1 Hardy-Weinberg Principal

The Hardy–Weinberg principle or equilibrium (HWE) states that the genotype frequencies in a population remain constant or are in equilibrium from generation to generation unless specific influences are introduced (Lowe *et al.*, 2004). For example in a random mating diploid organism, AA + 2Aa + aa = 1 genotype proportions are expected.

Those influences include non-random mating, mutations, natural selection, limited population size, random genetic drift and gene flow. Genetic equilibrium is a basic principle of population genetics (Emigh, 1980); it is achieved in one generation, and requires the assumption of random mating with an infinite population size (Hartl and Clark, 1997).

5.2.2 Genetic distance

Genetic distance is a measure of the dissimilarity of genetic material between different species or individuals of the same species (Pollock and Goldstein, 1995). It is a degree of similarity between a pair of individuals, populations or species were values range between zero (identical) and one (completely different) (Lowe *et al.*, 2004). By comparing the percentage difference between the same genes or neutral loci of different species, a figure can be obtained, which is a measure of genetic distance. Depending upon the difference and correcting for known rates of evolution, genetic distance can be used as a cladistic tool.

5.2.3 Heterozygosity

Quantification of within population genetic variation is central in the interpretation of genetic differentiation between populations. Heterozygosity (H_o) is a measure of genetic diversity, being the frequency of heterozygotes for one locus within a population (Page and Holmes, 1998). A heterozygote is an individual with two (diploid) or more (polyploidy) alleles at a locus. In understanding how genetic diversity is partitioned within a population, it is important to determine how many alleles are present at a single locus. Usually the observed (H_o) and expected (H_e) heterozygosities are compared through the determination of allelic frequencies. Deviation away from the expected HWE frequencies indicates that the population is not randomly breeding and some degree of assortative mating is occurring (Lowe *et al.*, 2004).

5.2.4 The fixation index

In 1921 what is known as the fixation index was defined (Wright, 1921). The purpose of which is to quantify the inbreeding effect of population sub-structure (Lowe *et al.*, 2004). The index equals the reduction in heterozygosity expected with random mating at anyone level of a population hierarchy relative to another (Hartl and Clark, 1997). It quantifies genetic differentiation and proves useful because it allows for an objective comparison of the overall effect of population substructure among different organisms (Lowe *et al.*, 2004). F-statistics describe genetic differentiation by partitioning variation into three levels: total population, sub-population and individual (Lowe *et al.*, 2004). F_{ST} and R_{ST} statistics refer to analysis of variance in allele frequencies and in repeat numbers respectively and are used to illustrate quantitatively the degree of genetic structure (Pérez *et al.*, 2002).

5.2.5 Inbreeding depression and heterosis

Inbreeding depression is a term indicating reduced fitness in a given population as a result of breeding of related individuals (Lowe *et al.*, 2004). Although inbreeding to some degree accounts for all the diversity that we see today, it is a double edged sword and breeding between closely related individuals, called inbreeding, results in more recessive deleterious traits manifesting themselves. The more related the breeding pair the more deleterious genes the offspring will have (i.e. increased genetic load), resulting in unfit individuals. Genetic load is a term widely used in domestication and one that will become more familiar to conservation efforts as natural populations become smaller and more fragmented.

The effects of inbreeding depression can be dramatic (Fig 7), depending on the genetic load of a breeding pair or individual (Lowe *et al.*, 2004). In a population where inbreeding occurs frequently, fatal recessives alleles would be rare, due to promptly being eliminated from a population. With infrequent inbreeding, recessive deleterious alleles will be masked by heterozygosity, and so heterozygotes will not be selected against (assuming dominance). Obligate outcrossers, such as trees, are more susceptible to inbreeding depression.

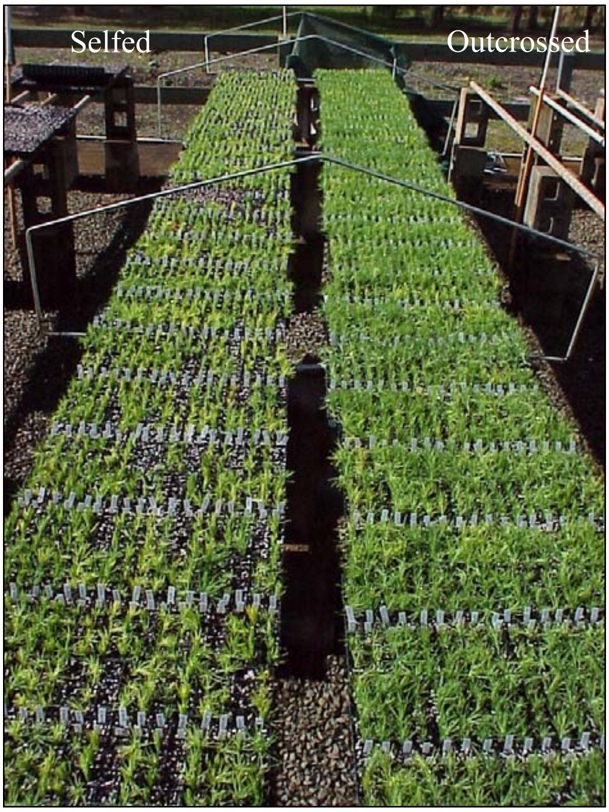

Fig 7 The effect of inbreeding depression (left) and heterosis (right) on the vegetative propagation of the *Pinus elliottii* x *P. caribaea* hybrid (Study and photo: R. Mellick).

Heterosis is the advantageous effects of hybridization (introgression), and to some degree, admixture, between two inbred lines with different deleterious genes. By crossing such lines in a diploid species, the genetic load is dramatically decreased and the fitness of the offspring increases in comparison to the parents. Many domestication efforts have utilized this phenomenon (Fig 7). If an offspring of two highly inbreed lines is selfed, the offspring produced (assuming sufficient fecundity) will largely exhibit genotypic proportions expected under Hardy–Weinberg equilibrium.

6. Molecular analysis

6.1 Nucleic markers including microsatellites

The flanking sequence of nuclear microsatellites (SSRs) has proved informative to evolutionary studies (Rossetto *et al.*, 2008, 2009). Inferring genealogical relationships from SSR allelic size data alone is weak due to the mutational mechanisms involved (Estoup *et al.*, 2002) but the regions flanking the repeat itself provide a tractable source of phylogenetic information that is well suited to cross-population and phylogeographic studies (Rossetto *et al*. 2002; Hey *et al*. 2004). The fact that these flanking regions are conserved to a degree necessary to allow for primer annealing and Polymerase Chain Reaction (PCR), yet more variable as the sequence approaches the SSR (within the amplicon), gives this marker system an advantage over other co-dominant SSR approaches due to being able to score haplotypes from the flanking sequence, and in some circumstances link to repeat length variation, i.e. HapSTR (Haplotype Short Tandem Repeat; Hey *et al.*, 2004).

Microsatellites, or Simple Sequence Repeats (SSRs), are polymorphic loci present in nuclear DNA and organelle DNA that consist of repeating units of one to four base pairs in length (Turnpenny and Ellard, 2005). They are co-dominant molecular markers which have wide-ranging applications in the field of genetics, including kinship and population studies. These markers often present high levels of inter- and intra-specific polymorphism, particularly when tandem repeats number ten or greater (Queller *et al.*, 1993). Microsatellites owe their variability to an increased rate of mutation compared to other neutral regions of DNA. These high rates of mutation can be explained most frequently by slipped strand mispairing (slippage) during DNA replication on a single DNA strand. Mutation may also occur during recombination during meiosis (Blouin *et al.*, 1996). Some errors in slippage are rectified by biomolecular proofreading mechanisms within the nucleus, but some mutations can escape repair.

Commonly it is found that a number of nucleic microsatellite loci distinguish between individuals of a rare or threatened organism. Microsatellite library construction is problematic in conifers due to their large genome size and the high proportion of duplicated DNA (Elsik and Williams, 2001; Scott *et al.*, 1999; Scott *et al.*, 2003). Nucleic microsatellite libraries were developed for *Podocarpus elatus* (Chp 2: Almany *et al.*, 2009) and *P. lawrencii*.

6.2 Plastid DNA markers

Plastid analysis provides a deeper historical observation than nucleic DNA, due to not undergoing recombination and being of a more conserved nature. This conservation allows for historical events to be recorded as signatures in the DNA sequence. Chloroplast genomes are predominantly maternally inherited in angiosperms and paternally inherited in gymnosperms. They are powerful in determining gene flow from seed versus pollen movement and reconstructing phylogeographic colonization. Intergenic spacers and introns of chloroplast DNA are commonly used as markers in phylogenetic studies focusing on species-level relationships in plants. In order to attain informative variation at this low taxonomic level, a large number of nucleotide bases and, therefore, a great many regions must be investigated. The great majority of loci investigated for this project were plastid, and only one polymorphic marker was found (PeB37BGT: Almany *et al*., 2009).

6.3 Cytoplasmic inheritance in *Podocarpus*

The pattern of cytoplasmic inheritance in *Podocarpus totara* confirms a predominantly paternal inheritance of plastids, common in all conifer families, and inheritance of mitochondria is dependant on the mechanism of fertilisation and the structure of male and female gametes found in each family (Owens and Morris, 1990; Wilson and Owens, 2003). Wilson and Owen (2003) inform that Podocarpaceae display a cytoplasmic inheritance mechanism similar to that of the Pinaceae and Taxaceae. Bi-parental inheritance in *Podocarpus elatus* is therefore likely, with cpDNA being paternal inherited and mtDNA being maternal. An extensive search for polymorphic mtDNA markers in the *P. elatus* was warranted due to populations being clumped and maternal structure being likely.

6.4 Interdisciplinary studies

Interdisciplinary studies that use multiple sources of information to infer past genetic structure, such as molecular and palynological data, are now commonly used. The methods are complementary, so that the limitations from one method are often compensated for by evidence from the other methods. For instance the taxonomic resolution using palynological tools is limited, whereas some insights on the dynamics within species can only be obtained using molecular tools. Furthermore, genetic surveys can have a greater spatial resolution yet fossil data provide greater temporal resolution (Petit *et al*., 2002, 2003). Macro-fossil data in most taxa provides more taxonomic certainty, while palynology provides much more fossil data (especially for the Australian Quaternary) but is subject to scrutiny when it comes to reliable species identification.

Petit *et al.* (2002) identified refugia and post-glacial colonisation routes of European White Oaks (*Quercus* spp.) based on both chloroplast DNA (cpDNA) and fossil pollen evidence. Geographic distribution of 32 cpDNA variants belonging to eight *Quercus* species sampled from 2613 populations is presented by Petit *et al.* (2002). Clear cut geographic patterns were revealed in the distribution of cpDNA haplotypes. Combined with palynological data, colonisation routes out of glacial period refugia were able to be identified. Integration of genetic and climatic data (Pease *et al.*, 2009; Scoble and Lowe, 2010) with fossil data will further improve our understanding of the underlying environmental factors that maintain genetic structure.

7. Environmental niche modelling

Environmental Niche Modelling (ENM) techniques endeavour to define for any chosen species, the climatic envelope that best describes the limits to its spatial range, by correlating the current species distributions with likely climatic variables (Beaumont and Hughes, 2002; Heikkinen *et al.*, 2006). It is a process using computer algorithms to predict the geographic distribution of species on the basis of a mathematical representation of their known distribution in environmental space (realised niche). The environment is in most cases represented by climate data (such as temperature and precipitation) but other variables such as soil type, water depth, and land use can also be used (Cunningham, 2004). Understanding the effect of past climates on the distribution of species is integral to elucidating the evolutionary processes of speciation and adaptation. Species distributions over broad latitudinal gradients provide a unique opportunity to understand biological processes that are linked to climate change, such as the climate-induced range shift and the bioclimatic processes responsible for it (Cunningham, 2004).

Environmental niche modelling is primarily a niche-based modelling strategy for predicting the past and potential impacts of climate change on the natural distribution of species, according to their specific environmental requirements. Many of the questions in phylogeographic studies pertain to how environmental variation over space and time shape patterns of genetic divergence (Pease *et al.*, 2009; Scoble and Lowe, 2010). One of these questions is the identification of environmental barriers to dispersal and gene flow.

The development of Geographic Information Systems (GIS) has allowed researchers to investigate these environmental factors through ENM in detail. Furthermore, these methods make it possible to model the spatial patterns of climatic suitability for organisms based on their current distributions and to project these conditions onto mapped estimates of historical and future climates. Allowing for inference of refugial areas based solely on environmental data alone. Environmental niche modelling can answer the question: Is environmental variation causing range disjunction and reproductive isolation of fragmented populations? This can be tested by determining genetic connectivity between populations located in different environmental envelopes.

Plants respond to Quaternary climatic change as species, not as associations or biomes (Byrne *et al.*, 2011). Additionally, speculation has been made that rainforest communities (Hilbert, *et al.* 2007; VanDerWal, *et al.* 2009) and alliances (Mellick, in prep) fluctuate independently in response to climate depending on their species' specific ecology in the context of their abiotic environment. Population isolation/migration and vicariance/admixture, brought about by enduring glacial periods to the brief interglacials like the present (Colinvaux *et al.*, 2000), also add to the evolutionary complexity in highly biodiverse areas.

Species range limits are determined by many evolutionary and ecological processes, from species distribution and abundance to the evolution of niches, furthermore, theory suggests a myriad of processes by which range limits arise, including competitive exclusion, ally effects, and gene swamping (Sexton *et al.*, 2009).

Models are used to attain ecological and evolutionary insights to predict distributions across landscapes, occasionally requiring extrapolation in space and time (Elith and Leathwick, 2009). These ENMs are common tools for assessing the potential impact of climate change on species ranges (Beaumont *et al.* 2008). Prediction of species' distributions is integral to various ecological, evolutionary and conservation applications in science (Elith *et al.*, 2006).

7.1 Inferring historical distribution

The environmental niche model is a method for palaeoclimatic reconstruction built on the assumption of a significant correlation between climate and plant community composition. The goal of this approach is to infer past climatic conditions using plant fossil associations as source data.

Fig 8 Climate-based prediction and constraints on species distributions (modified from Cunningham, 2004).

Modellers are often faced with a problem when the ‘fundamental niche’ (potential distribution) of a species does not correspond with a ‘realised niche’ (actual distribution), and hence either an error in the construction of the fundamental niche (the abiotic thresholds for a species) has been made or that there are genuine factors affecting the actual distribution of the species, such as competition, predation and pathogens (Fig 8). Environmental and historical data are now being combined to understand geographic patterns of genetic diversity in the emerging discipline ‘landscape genetics’ (Scoble and Lowe, 2010). Niche modelling has contributed to such efforts by allowing species–environmental associations to be projected into the past so that hypotheses about historical vicariance can be generated and tested independently with genetic data (Pease *et al.*, 2009). Recent episodes of environmentally mediated divergence are unlikely to be shown in the molecular data, however, utilising environmental data to show changes in the regional genetic disjunctions may contribute necessary information to understand how climate drives divergence.

7.2 Predicting future distribution

Modelling and monitoring studies over the last 20 years has provided considerable evidence that global climate change is already affecting and will continue to affect many species and ecosystems, resulting in declines and extinctions of many species (Dunlop and Brown, 2008). It is usually the most threatened species and habitats that are going to respond first to these climatic changes. Nonetheless, because of the interacting nature of biological and ecological systems, with their positive and negative feedbacks, and the multifaceted nature of the environmental changes in response to climate change (and other pressures), it is not immediately obvious what the overall impacts on biodiversity are likely to be (Dunlop and Brown, 2008).

Generally, studies have supported the prediction that species with narrower climatic niches will be more sensitive to climate-induced range contraction (Bell, 2007). Potential impacts of projected climate change on biodiversity are often assessed using single-species ENM (Heikkinen *et al.*, 2006). Predicting the probability of successful establishment of plant species by matching climatic variables, has considerable potential as supporting methodology to reinforce palynological and phylogeographic studies. In the future, deeper ecological understanding may allow competition to be quantified and included in species distribution modelling. Future biogeographical ranges of species are constructed by applying the models based on climatic variables that best describe the current equilibrium distributions to simulate future distributions under selected climate changes scenarios (Bakkenes *et al.*, 2002; Solomon *et al.*, 2007). Bakkenes (2002) suggests that in reviewing possible future trends, it was found that European plant species, in general, would find their current climate envelopes further northeast by 2050, implying maximum temperature threshold may restrict species' lower latitude range limits.

Added confidence in ENM output can be achieved through projection of the trained environmental envelope onto an ensemble of different atmospheric oceanic global circulation models (AOGCMs) (Chp 5: Appendix; Araujo and New, 2007). Climate models are complex tools. Variability occurs among alternate simulations and no single best model exists. The selection of climate scenarios for impact assessments should not be undertaken arbitrarily - strengths and weakness of different climate models should be considered (Beaumont *et al.* 2008). Having an ensemble assures the predictive errors made may be accounted for somewhat by including a number of different scenarios, and developing an ensemble (an average).

7.3 Correlative and mechanistic modelling

Environmental niche models are correlative (statistical) models. They relate observed presences of a species to values of environmental variables across the geographic extent of the species' range. Some models use absence data, which is very hard to collect confidently, but the most commonly used models use presence-only data, perhaps together with random background data. In contrast, mechanistic (or process-based) models assess the bio-physiological aspects of a species to generate the conditions in which the species can ideally survive, based on a species' traits and observations made in laboratory or controlled field studies (Bresson *et al.*, 2011). Mechanistic modelling defines the abiotic limits of a species range through the physiological tolerances of the species, e.g. *Podocarpus* auxiliary xylem tissue collapses at certain water-stress thresholds and therefore the species distribution is limited by a trade-off between water use and photosynthetic efficiency (Brodribb and Hill, 1999).

The correlative approach compounds the effects of ecology, physiology, competition, history and climatic effects, due to the current distribution being a result of all effects. These cannot be accurately separated and quantified, so mechanistic approaches remain important and are more illustrative of the ecological (realised) niche, as apposed to the environmental or climatic (fundamental) niche of a species, as attained through the correlative approach (Fig 8). It may be possible to combine mechanistic and correlative approaches to alleviate the expense required for mechanistic data collection and convenience involved with correlative modelling. The development of phenotype-genotype correlation between the physiological traits associated with climatic tolerance and a marker suite linked to such characters may allow this. Possibly, selective genotyping (i.e. genotyping the best and worse individuals of physiological quantitative traits), candidate gene analysis and/or quantitative trait loci analysis could achieve the phenotype-genotype correlation required. Then the correlative modelling approach could be applied to the geographic distribution of markers associated with such traits.

8. Aims and objectives

I seek to understand the effect of late Quaternary and future climate change on the distribution, diversity and genetic disjunctions of *Podocarpus elatus* (Podocarpaceae), a long-lived conifer restricted to rainforest. The principle aim of the thesis is to determine if intraspecific diversification in a wide-ranging long-lived species is driven by climate change, and if so, how these climatic-drivers are involved in the evolutionary process.

Chapter one reviews literature about family patterns, event histories, case studies, methodology and interdisciplinary studies, prior to reporting the development of species-specific markers in Chapter two. These chapters also touch upon the problems associated with marker development in conifers (and domestication), cytoplasmic inheritance, and the molecular markers used in the study.

Chapter three aims to evaluate the level of genetic diversity and structure within naturally occurring populations, and to establish if divergence is more strongly influenced by historic or contemporary drivers. The objectives are:

1. Determine genetic structure and how it corresponds to geographic and/or environmental patterns.
2. Evaluate landscape-level habitat fragmentation and if there is loss of gene flow among populations.
3. Identify genetic diversity within populations to see if there are measurable differences across demographic age cohorts.

The fragmentation of the east Australian rainforests and the successional response of communities to past climate change has been reviewed to provide ecological/environmental information pertaining to *P. elatus* and the ecotonal habitat it occupies. Fossil/climatic observations of the Quaternary glacial cycles are reviewed to inform and infer past range shift and its effect on the current genetic structure of *P. elatus*. Molecular information obtained through coalescence-based techniques will provide information on ancestral isolation and migration coinciding with fossil and environmental niche modelling chronology.

Chapter four aims to explore the impact of glacial cycle climatic changes on the distribution of the species: by combining population genetic analysis, Bayesian coalescence-based analysis, the observed fossil record and Environmental Niche Modelling (ENM). The objectives are:

1. Determine if predicted distributional changes and the available fossil record correspond.
2. Evaluate if modelling corresponds to population genetic-based estimates of range contractions/expansions and disjunctions.
3. Identify climatic response of each population group to see if differential range shift response between northern and southern ranges is occurring.

Ancestral demographics will illustrate the natural demography of the species without anthropogenic effects and future ENM will show if future habitat suitability patterns will accommodate these natural demographic patterns. As reviewed, the general decline of rainforest conifers from the fossil record are mainly climate driven and have occurred since the previous interglacial (130 Ka: Kershaw 2005, *et al.* 2007a, Shimeld 1995, 2004).

Chapter five aims to understand ancestral demographics and combine with future ENM to identify conservation strategies for the species. By identifying the species' natural demographic patterns, the future threats to the evolutionary process (i.e. range shift and gene flow) necessary for adaptation to a changing environment will be shown. The objectives are:

1. Determine the ancestral demographic patterns and divergence times for *Podocarpus elatus* and see how they relate to climate change.
2. Evaluate if regional differentiation observed in *P. elatus* is a result of balance between genetic drift and geneflow, or is ancestral polymorphism being maintained in the absence of significant geneflow.
3. Predict the future distribution of *P. elatus* for the IPCC 4th Assessment Report climatic estimates of 2050.

The future design of habitat corridors could take on a broader evolutionary application by linking fragmented habitats along predicted avenues of range shift, and understanding natural gene flow patterns will allow for genotype selection and assisted migration along these avenues. Fire-selection (ecotonal preference) and hybridisation seem to be occurring in the species, and clonal cultivation of male trees constitutes a further threat to the species' future diversity.

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CHAPTER TWO

Isolation and characterisation of polymorphic microsatellite loci from *Podocarpus elatus* (Podocarpaceae).

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PERMANENT GENETIC RESOURCES NOTE

Isolation and characterisation of polymorphic microsatellite loci from *Podocarpus elatus* (Podocarpaceae).

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Keywords: *Podocarpus*, cross-transferability, Podocarpaceae, rainforest, gymnosperm

Abstract

We identified eight polymorphic nuclear microsatellite loci and one putative polymorphic chloroplast locus in *Podocarpus elatus* from an AG-enriched library. The loci were screened across two populations of *P. elatus*, nine individuals of *P. grayi*, two individuals of *P. lawrencei*, and an individual of *P. smithii*. Screening the nuclear loci in *P. elatus* returned an average of 8.25 alleles per locus (from 5-13, average expected heterozygosity 0.561). Seven, six and five of the eight nuclear loci were amplified respectively in *P. grayi, P. smithii*, and *P. lawrencei*. The chloroplast locus amplified in all species and showed intraspecific and species-specific polymorphism which will be useful for phylogeographic and systematic studies.

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Podocarpaceae comprises more than 150 species with 18 extant genera. The main centre of diversity is Australasia, mainly New Caledonia, Tasmania and New Zealand. *Podocarpus* is the largest genus with 110 species worldwide. There are seven endemic Australian *Podocarpus* species. Four are trees closely associated with mesic rainforest communities and the other three are dry-adapted shrubs. The extant species are only a small representation of the once highly diverse Podocarpaceous palaeo-flora (Hill 1994).

Microsatellite library development in gymnosperms has been complicated by large genome size (Leitch *et al.* 2001) and repetitive DNA regions (Fischer & Bachmann, 1998). Scott *et al.* (1999) reported microsatellite library enrichment efficiencies of up to 50% for seven angiosperm species while enrichment efficiencies of less than 10% was found for gymnosperms.

Here we describe the development and characterisation of a microsatellite library based on *Podocarpus elatus* R.Br.Ex.Endl. and test for cross-transferability in three other *Podocarpus* species: the trees species *P. grayi* deLaub. and *P. smithii* deLaub.; and the shrub *P. lawrencei* Hook.f. These markers will accommodate population and phylogeographic analyses.

Total genomic DNA was extracted from fresh and silica-dried leaf tissue from *P. elatus, P. grayi, P. lawrencei* and *P. smithii,* using DNeasy Plant kits (QIAGEN). The microsatellite AG_n-enriched library was constructed following the SuperSNX24 sequence protocol (Glenn & Schable 2005). Two hundred positive clones were screened for the presence of microsatellite-containing inserts by amplifying and sequencing with M13 universal primers. Forty eight contained microsatellites loci and 33 primer pairs were designed using Primer3 (Rozen & Skaletsky, 2000) and NetPrimer (PREMIER Biosoft International). Fluorescent-labelling required forward primers with an M13 universal sequence (5'-TGTAAAACGACGGCCAGT-3') added to 5' end (Schuelke 2000). The most promising 19 of the 33 primer pairs were synthesised and tested.

PCR amplification was performed using the Veriti™ 96-Well Thermal Cycler (Applied Biosystems) and a protocol modified from Schuelke (2000). Multiplex reactions contained 5µl Multiplex PCR mix (QIAGEN), 0.05µM of each forward primer, 0.2µM of each reverse primer, 0.1µM fluorescent M13 primer (FAM, VIC, NED or PET) and 1µl template (5ng/µl) in a total volume of 10 µl. The cycling profile was: 15min incubation at 94°C; 38 cycles of denaturation (30s at 94°C), annealing (1min 30s at 61°C for 30 cycles and 53°C for the last eight cycles) and extension (45s at 72°C); with a final 30 min incubation at 60°C.

Microsatellite profiles were examined using GENEMAPPER v3.7 (Applied Biosystems) and peaks were scored manually. GenAlEx6.1 (Peakall & Smouse 2006) was used to assess diversity levels for each locus as reported in Table 1. GenePop3.4 (Raymond & Rousset 1995) was used to calculate Hardy-Weinberg proportions, linkage disequilibrium, and inbreeding coefficient (F_{IS}) and significance, using the exact test determined after 500 batches of 5000 iterations each (Table 1). Sequential Bonferroni corrections were applied following Rice (1989). Megagametophyte (haploid) samples were used to identify spurious products for three of the markers.

P. elatus samples included 57 individuals from two populations: 25 from Maiala National Park (QLD) and 32 from Wrattens State Forest (QLD). *P. grayi* samples included eight individuals from Cooktown (QLD) plus an additional individual from McIlwraith (QLD). *P. lawrencei* and *P.* smithii samples included two individuals from Victoria and an individual from McIlwraith (QLD) respectively.

All loci were checked using the web-based sequence BLAST tool (http://blast.ncbi.nlm.nih.gov) (Madden *et al.* 1996), with no significant return besides PeB37BGT, where 14% of the forward flanking sequence was 94% identical to *Passiflora* RNA polymerase RPO sub-unit (chloroplast). Single allele amplification of this marker was observed across all populations, with species specific and intraspecific polymorphism.

All loci were polymorphic within and among two populations of *P. elatus* (N_A=3-13; H_E=0.151-0.777; H_O=0.042-0.920). Of the eight nuclear loci two were at genotypic proportions expected under Hardy-Weinberg Equilibrium (HWE) in both populations, one only in population one and five showed significant departure from HWE across both populations (Table 1). This may be due to being small, localised populations prone to effects of genetic drift. Significant linkage disequilibrium (95% confidence) was found between three pairings (PeA16BGT/PeD9BGT, PeC26BGT/PeD9BGT, and PeA32BGT/PeD13BGT) but none was significant after Bonferroni corrections (Rice 1989). PCRs were repeated for 10% of individuals to determine reliability of genotyping results and all repeats were identical.

Successful cross-transferability of these markers was observed in *Podocarpus grayi*, *P. lawrencei* and *P. smithii*. Locus PeB37BGT showed interspecific but not intraspecific polymorphism, which is encouraging for the use of this marker for systematic studies within the taxa.

These loci have amplified reliably in both sub-genera of *Podocarpus* (Subg.1. *Podocarpus*; *P. smithii* and *P. lawrencei*. Subg.2. *Foliolatus*; *P. elatus* and *P. grayi*) (see Table 2). The loci show intraspecific and interspecific variability within and among *P. elatus* populations and the markers will be used to reveal overall genetic structure of *P. elatus*. This will allow inference of the expansion and contraction of the species distribution in response to recent glacial cycles through comparisons of molecular data and palaeoecological data. The putative chloroplast locus may prove useful for systematic and phylogeographic studies within the genus.

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Table 1: Characteristics of eight microsatellite and one putative chloroplast (PeB37BGT) loci isolated for *Podocarpus elatus* and population genetic values for each nucleic locus obtained from 57 individuals from two populations [25 from Maiala National Park (QLD) and 32 from Wrattens State Forest (QLD)]: locus name, repeat type and frequency, forward (F) and reverse (R) primer sequences, expected fragment size, (T_a) annealing temperature, allelic size range, (Total N_A) total number of alleles. Separate diversity indices calculated in GenAlEx for Maiala National Park (in bold) and Wrattens State Forest (in italics): (N) number of individuals amplified, (N_A) number of alleles, (H_E) expected heterozygosity, (H_O) observed heterozygosity, and inbreeding coefficient (F_{IS}) with significance level. F_{IS} describes the divergence of observed heterozygosity away from expected heterozygosity within populations assuming random mating.

Locus	*Repeat*		**Primer sequences (5'-3')*	*†Size (bp)*	T_a (°C)	*Allele size range (bp)*	*Tot. N_A*	N	N_A	H_E	H_O	F_{IS}	*GenBank Acc. no.*
PeA16BGT	$(CT)_{11}$	F	GCTAAACCTTACCCATCAAT	369	61	431-470	11	**25**	**7**	**0.549**	**0.560**	**0.000**	FJ935795
		R	TATGTGGCAAACTCACTCTGT					*32*	*8*	*0.405*	*0.406*	*0.012*	
PeA32BGT	$(AG)_{31}$	F	TGAAGAGTTGGTAGGCAGTG	431	61	180-212	9	**23**	**6**	**0.401**	**0.217**	**0.475*****	FJ935796
		R	GTGGAGACTATGTGTCATTGGT					*32*	*6*	*0.744*	*0.344*	*0.549****	
PeB31BGT	$(CT)_9$	F	GGCATTGGCTCAACAGA	193	61	211-235	5	**25**	**4**	**0.151**	**0.080**	**0.487***	FJ935797
		R	TCGTGGAGAGGTACTTCATT					*32*	*4*	*0.421*	*0.125*	*0.711****	
PeB37BGT	$(GACGGGCTTCCTGAA)_5$	F	TATTATTCATCAGTATATCACCT	314	57	324-349	3	**21**	**2**				FJ935798
		R	ATTCTCTTTCTATTTCTTCG					*16*	*3*				
PeC19BGT	$(AG)_{21}$	F	GAGACCAGACAAAGATGAAGA	204	61	215-242	9	**25**	**7**	**0.557**	**0.480**	**0.158**	FJ935799
		R	GAGTAAGAGCAAGACACCAAA					*32*	*7*	*0.695*	*0.500*	*0.295****	
PeC26BGT	$(TC)_7TA(TC)_2CC(TC)_3$	F	CATCCACCAGTTTCCCTACG	279	61	286-298	6	**24**	**2**	**0.305**	**0.042**	**0.869*****	FJ935800
		R	ATTTGAACGCACAGAGCATTT					*32*	*6*	*0.497*	*0.188*	*0.632****	
PeD9BGT	$(CT)_4CC(CT)_{10}$	F	GTGCTTGTAAATGAGAGAGAG	360	61	361-377	6	**25**	**6**	**0.768**	**0.520**	**0.341***	FJ935801
		R	GGGCGAGGAGAGAATC					*31*	*4*	*0.624*	*0.226*	*0.648****	
PeD13BGT	$(AT)_7$ $(CT)_7$ $(GT)_{10}$	F	CTACCCGAACATTGCTCTA	344	61	364-377	7	**25**	**5**	**0.666**	**0.480**	**0.298****	FJ935802
		R	CTCCCATCGTTTCCAATT					*32*	*5*	*0.649*	*0.594*	*0.101**	
PeD14BGT	$(GT)_{24}$	F	GAATGGATGGGTAATGGC	278	61	281-315	13	**25**	**9**	**0.765**	**0.920**	**-0.183**	FJ935803
		R	GGAACATAACTTGAATGCTTT					*32*	*9*	*0.777*	*0.844*	*-0.070*	

†Significance values (calculated in GenePop) for deviation from HWE are indicated next to F_{IS}:

Key * P<0.05, ** P<0.01, *** P<0.001.

Table 2: Amplification success across three *Podocarpus* species. *P.* grayi: eight individuals from Cooktown (QLD) plus an additional individual from McIlwraith (QLD). *P. lawrencei*: two individuals from Victoria. *P.* smithii and an individual from McIlwraith (QLD). Loci with * indicate amplification was weak across all samples tabulated. PeB37BGT ** is a putative chloroplast locus. PeD13BGT did not amplify in any samples tabulated, so it was not included.

	P. grayi N=9			*P. lawrencei N*=2			*P. smithii N*=1		
Locus	*N*	N_A	Range (bp)	*N*	N_A	Range (bp)	*N*	N_A	Range (bp)
PeA16BGT	9	4	416-439	2	3	411-419	1	1	409
PeA32BGT*	9	3	204-210	0	0	0	0	0	0
PeB31BGT	9	2	209-211	2	1	205	1	1	213
PeB37BGT**	9	1	318	2	1	342	1	1	315
PeC19BGT	9	4	197-219	2	4	197-233	1	1	199
PeC26BGT*	9	6	291-301	2	2	287-289	1	2	297-299
PeD9BGT*	9	4	365-379	0	0	0	1	2	373-375
PeD14BGT	9	2	263-266	2	1	276	1	2	265-268

CHAPTER THREE

Consequences of long- and short-term fragmentation on the genetic diversity and differentiation of a late successional rainforest conifer.

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CHAPTER FOUR

Palaeodistribution modelling and genetic evidence highlight differential post-glacial range shifts of a rain forest conifer distributed across a latitudinal gradient.

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Mellick, R., Lowe, A., Allen, C.D., Hill, R.S. & Rossetto, M. (2012). Palaeodistribution modelling and genetic evidence highlight differential post-glacial range shifts of a rain forest conifer distributed across a broad latitudinal gradient.
Journal of Biogeography, v. 39 (12), pp. 2292-2302

CHAPTER FIVE

Molecular and habitat suitability models highlight future threats and long-term decline of a common rainforest conifer

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Research paper

Running header: Predicting distributional responses to climate change

Molecular and habitat suitability models highlight future threats and long-term decline of a common rainforest conifer

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ABSTRACT

Anticipating range shift response to future climate change and understanding natural demographic patterns is integral to species conservation. Here we model the climate-induced range shift along a latitudinal gradient in two genetically differentiated population groups of the late-successional rainforest conifer, *Podocarpus elatus*. A total of 405 occurrence records (224 southern and 181 northern records) were used to model historical-current-future distribution using MAXENT (21, 6, and 0 Ka; and 2050 A2). A coalescent-based isolation-with-migration (IM) model was used to estimate population demographic parameters and divergence times between the current genetic disjunctions in the species. The environmental niche models suggest differential range shift (i.e. expansion in the north and contraction in the south) will continue into the future, with a southern range shift also occurring in both regions. The isolation-with-migration analysis infers the origin of the Clarence River Corridor dividing the population groups to be prior to the last glacial maximum (LGM). A later divergence in the south (19 Ka) is indicative of slow consistent habitat contractions in the south since the LGM (21 Ka). The study predicts future distribution of genetic diversity in the species and indicates areas at increased risk of localised extinction. We recommend the Macleay Overlap Zone as a priority area for protection based upon intraspecific diversity and past-current-future habitat suitability.

KEYWORDS, Climate-induced range shift, Environmental Niche Models, Genetic diversity, Isolation-with-Migration, Localised extinction, Population expansion/contraction.

INTRODUCTION

Understanding climate-induced range shifts is key to the conservation of populations (Parmesan, 2006), species (Beaumont and Hughes, 2002; Pye and Gadek, 2004) and communities (Williams *et al.*, 2003, Pennington *et al.*, 2004, McLachlan *et al.*, 2005), particularly in view of projected climate change (Novacek and Cleland, 2001; Solomon *et al.*, 2007; Lenton *et al.*, 2008). For populations to survive they either need to adapt (Bonin *et al.*, 2007; Aitken *et al.*, 2008; Lindner *et al.*, 2008), migrate into suitable conditions along environmental gradients (Parmesan and Yohe, 2003; Thuiller *et al.*, 2008) or a combination of both. Defining the abiotic limitations on a species distribution, using either physiological (Brodribb and Hill, 1999; Bresson *et al.*, 2011) or occurrence data (Waltari *et al.*, 2007; Mellick *et al.*, 2011), refines the understanding of current habitat suitability and the prediction of future range shifts. Although it has been shown that single species do respond independently during climate change, fossil records show that species-community associations can be ancestral (Brodribb and Hill, 2003; Kershaw *et al.*, 2007a) where the overarching abiotic limitations on the community affect the association and co-occurrence of species.

The shift of climatic suitability, localised extinction and ancestral gene flow

Localised extinction risk varies due to species traits and spatial characters of the physical environment owing to the combined and interactive effects of ecology and geography (Thomas *et al.* 2004, Lee and Jetz, 2011). Rapid climate change increases the likelihood of localised extinction by shifting the “climatic envelope” of a population outside of the current geographic range (McLachlan *et al.*, 2005). Survival is dependant on the species’ traits, genomic plasticity (potential to adapt) and/or the rate at which a species can shift their range in response. Northern hemisphere palynological studies have revealed rapid migration rates (100-1000 m/yr) in temperate tree species in response to post-glacial climatic warming (McLachlan *et al.*, 2005). While similar findings have been repeated across northern hemisphere species (Brewer *et al.*, 2002; Petit *et al.*, 2002), the constraints of Australian fossil deposits have made detailed documentation of analogous changes along southern latitudes difficult (e.g. South American studies).

The rapid climate fluctuations of the Pleistocene have had a considerable impact on intraspecific variation of many species, and left distinct genetic signatures (Hewitt, 2000; Carstens and Knowles, 2007). The coalescent analysis of genetic data (Kingman, 2000) allows for the formulation of probabilistic models that can help interpret such genetic signatures into ancestral demographic patterns (Hey, 2010). Multi-locus analysis have proved essential for such queries (Hey and Nielsen, 2004), and accounting for historical climatic processes enables ancestral queries to be adequately addressed (Carstens and Knowles, 2007).

Although present-day gene flow estimates between regional groupings are informative in inferring current demographic patterns (Mellick *et al.*, 2012), recent anthropogenic effects can also influence these estimates. Inferring the ancestral migration parameters through a coalescent approach, such as isolation-with-migration (Hey, 2010), enables the investigation of historical associations, or lack thereof, prior to anthropogenic disturbance.

Habitat fragmentation, population decline and loss of intraspecific diversity

The expansion of human land-use throughout the world has fragmented once continuous habitats into habitat islands. Although they are naturally fragmented, vast tracks of East Australian lowland rainforest were removed by clearing in the last 200 years, increasing the risk of localised extinctions (Bowman, 2000). Fragmentation and isolation increase the risk of population decline\ bottlenecks by increasing the susceptibility of fragmented habitats to invasive species and fire (Ewers and Didham, 2006). Furthermore, fragmentation reduces gene flow\connectivity between populations that consequently reduces diversity and adaptive potential (Young *et al.*, 1996). At first, conservation planning focused more on the pattern (representation) than process (persistence), concentrating resources on the conservation of interspecific diversity rather than the processes responsible for intraspecific diversification (Moritz, 2002). More recently, the amount of intraspecific variation has been considered as a key parameter to determine populations to prioritize for protection purposes (Moritz, 2002; Bonin *et al.*, 2007; Harrington and Gadek, 2009).

An earlier study across the entire distribution of *Podocarpus elatus* R.Br. ex Endl. (Podocarpaceae) found agreement between genetic and contemporary ENM disjunctions across the Clarence River Corridor (Mellick *et al.*, 2011). The increasing abundance of environmental data available worldwide has allowed for the growing use of predictive models to infer likely distributional shifts of species' current environmental envelopes within future climatic models (Elith *et al.*, 2006; Phillips *et al.*, 2006), and coupled-model ensembles (Araujo and New, 2007; Batte and Deque, 2011). Coalescent-based molecular techniques (Beerli and Felsenstein, 2001; Beerli, 2006) inferring sink versus source dynamics can be combined to ENM (Mellick *et al.*, 2012) to identify populations likely to go locally extinct, and those likely to persist during rapid climate change scenarios. By determining the natural demographic processes (i.e. no anthropogenic effects) it will be possible to identify pressures on the current distribution. Here we attempt to combine knowledge of ancestral demographics and future ENM to support the conservation of *P. elatus* by identifying the natural demographic patterns of populations throughout East Australian rainforests. The following questions are important within a conservation and management context because human land-use restricts the natural evolutionary processes of range shift and gene flow necessary for adaptation to a changing environment to occur

1. What are the ancestral demographic patterns and divergence times in *Podocarpus elatus* and how do they relate to climate change?

2. Is the regional differentiation observed in *P. elatus* a result of balance between genetic drift and geneflow, or is ancestral polymorphism being maintained in the absence of significant geneflow?

3. What is the predicted distribution of *Podocarpus elatus* for the IPCC 4th Assessment Report climatic estimates of 2050?

4. Based on these findings, what are the appropriate conservation strategies for *Podocarpus elatus*?

METHODS

Study species

Podocarpus elatus is a late successional, mature-phase conifer, with a wide latitudinal distribution (2500 km, 20^o of latitude) in East Australian rainforest. The species prefers drier rainforest and ecotonal rainforest communities (Harden *et al.*, 2006). It is commonly found with *Araucaria* spp. both currently and in the fossil record (Shimeld, 1995; Longmore, 1997; Shimeld, 2004; Black *et al.*, 2006). The palynological record suggests that in the Australian Wet Tropics, gymnosperms expanded during glacial maxima (Kershaw *et al.*, 2007a), and it is likely that the cool, dry conditions of the last glacial maximum (LGM; 21Ka) also favoured *P. elatus*. However, while southern fossil records support a decline in abundance for *P. elatus* since the LGM (Shimeld 2004, Black *et al.*, 2006, Williams *et al.*, 2006), in the north the co-occurrence of a number of *Podocarpus* species and the classification of pollen to generic-level reduce the interpretative power of the limited deposits available.

Sampling strategy

In previous studies, sampling was designed to include the full distribution range of the species (334 individuals from 32 populations; Mellick *et al.*, 2012). In this study, twenty-eight mature individuals from three populations (Fig 1 and Appendix Table 1) were selected to investigate ancestral gene flow, splitting time and effective population size in relation to two genetic regions separated by the Clarence River Corridor (CRC; Mellick *et al.*, 2011) so to reveal the ancestral demographic processes responsible for the current genetic diversity/structure in *P. elatus*. Revealing the natural (ancestral) demography of a species restricted to the East Australian rainforests will allow for effective conservation by approximating natural evolution in the absence of anthropogenic effects.

The populations were selected because of their location and genetic connectivity in relation to the Clarence River Corridor (CRC) and are: 1) Porrots Brush (seven individuals), at the southern distributional range limit of the species. 2) Bundagen (11 individuals), at the southern side of the CRC biogeographic barrier in the central range of the species. 3) Mount Glorious (10 individuals), at the northern side of the CRC biogeographic barrier in the central range of the species.

Occurrence data and species distribution modelling

Environmental data was used for spatial modelling of the individual genetic clusters. This included 11 variables from the WORLDCLIM 1.4 database (Hijmans *et al.*, 2005) and 405 occurrence records (224 southern and 181 northern records) compiled and verified from all Australian Herbaria and the Office of Environment and Heritage's vegetation survey database (YETI) (http://www.environment.nsw.gov.au/research/VISplot.htm), and Atlas of NSW Wildlife (http://wildlifeatlas.nationalparks.nsw.gov.au/wildlifeatlas/watlas.jsp) databases. We refined the current model using methodology described in Mellick *et al.* (2011). The 11 environmental variables were: annual mean temperature, minimum temperature of the coldest month, mean temperature of the wettest quarter, mean temperature of the driest quarter, annual precipitation, precipitation of the driest month, precipitation seasonality (coefficient of variation), precipitation of the wettest quarter, precipitation of the driest quarter, precipitation of the warmest quarter and precipitation of the coldest quarter.

The refined models were developed using observed 20th Century climate data (Mellick *et al.*, 2011) and were projected onto the estimated climate for the decade centred on 2050. Model training and projection was performed using MAXENT 3.3.3e (Phillips *et al.*, 2006; software available from http://www.cs.princeton.edu/~schapire/maxent, last accessed 4 January 2012). To account for uncertainties in the future climate data, we derived data from 13 coupled atmospheric oceanic global circulation models (AOGCMs; Appendix Table 2) used in fourth assessment report of the Inter-governmental Panel on Climate Change (IPCC) (Solomon *et al.*, 2007) which provided the raw temperature and precipitation variables needed to compute the derived bioclimatic variables used to fit the Environmental Niche Models (ENMs). We chose AOGCM model runs for the A2 greenhouse gas scenario (Nakicenovic *et al.* 2000, Nakicenovic and Swart 2000) because assessments of observed trends in green house gas levels suggest that this scenario closely matches the observed trends (Raupach *et al.*, 2007). Raw AOGCM data was obtained from the Climate Model Inter-comparison Project dataset (https://esg.llnl.gov:8443/home/publicHomePage.do, last accessed 4 January 2012). Using a custom-written program, we downscaled the coarsely-gridded AOGCM data using bicubic spline interpolation (Press *et al.*, 2002) to the same 30 arc second grid as the WorldClim data (Hijmans *et al.*, 2005) used to fit the original MAXENT models. We then computed the 19 bioclim variables for each AOGCM also using a custom-written program. Using the projection function of MAXENT, a projection was made onto each AOGCM and then averaged to produce an ensemble consensus map of predicted climate suitability in the decade centred on 2050. Historical projections are included for comparison (Mellick *et al.*, 2012).

DNA extraction, sequencing and microsatellite analysis

Total genomic DNA was extracted from fresh leaf tissue and silica dried leaf tissue using the DNeasy Plant kits (QIAGEN, Venlo, Netherlands). Ten markers were used in the study; four microsatellite loci, one Hap STR (PeA16BGT) and five sequenced markers.

The sequenced markers included four nucleic and one plastid loci. The proportion of sequence variation was very low, possibly a consequence of large genome size, long generation time and obligate outcrossing (substantial gene flow in the species). This search for polymorphic loci involved randomly selecting 16 individuals (two individuals from eight disparate populations covering the range of the species) and sequencing in a forward and reverse direction for each locus. The sequenced loci used in this study are: PeA16BGT, PeA45BGT, PeB37BGT, PeC26BGT and PeD13BGT (Almany *et al.*, 2009). All loci were checked using the web-based sequence BLAST tool (http://blast.ncbi.nlm.nih.gov) (Madden *et al.*, 1996) with no significant returns besides locus PeB37BGT where 14% of the forward flanking sequence was 94% identical to *Passiflora* RNA polymerase RPO sub-unit (chloroplast). Single allele amplification of this marker was observed across all populations.

Microsatellite loci were isolated and primers developed from the methodology set out in Almany *et al.* (2009). Methodology regarding PCR conditions, optimisation procedures and cross-transferability results are outlined in Almany *et al.* (2009). Microsatellite assessment is outlined in Mellick *et al.*, (2011). Sequencing methodology is outlined in Rossetto *et al.,* (2009).

GENEIOUS 4.8.5 was used to edit and align nuclear DNA sequence data (which included ambiguous codes for heterozygous bases). DNAsp v5 imported these unphased formats and phased the diploid sequence data into haplotype sequence data, and each individual was represented by two haplotype sequences (i.e. homozygous individuals had two identical sequences) for each sequenced nuclear loci.

Hap STR locus (PeA16 BGT) was generated by combining the sequence haplotypes and allele length haplotypes for each individual in the study. The variation in sequence (between priming site and the repeat motif) and microsatellite length are combined to reveal further information (Hey *et al.*, 2004). Phase determination between microsatellite genotype and sequenced haplotype can be problematic (Sorenson and DaCosta, 2011). Subjective determination of phase was possible due to the majority of both sequence and allele size being of one class (i.e. more than 75% of haplotypes were of one size class). So based on the comparable proportion of allele size classes to sequence haplotypes, the phase of each individual was able to be determined.

Coalescence-based analyses

The data set was analysed with the recently developed Isolation-with-Migration (IM) model (Hey, 2010) implemented in the program IMa2, to estimate posterior probability for four parameters in each pairwise comparison. These were the time since divergence (*t)* and effective population sizes (population 0, population 1 and ancestral population 2) between adjacent populations either side of the CRC (Mellick *et al.*, 2011). Additionally, a three-population model was run to investigate ancestral migration based on the tree string developed from the pairwise comparisons (Fig 2). These estimates were compared to Ecological Niche Models to infer the possible effect of climate on intraspecific divergence. Arbitrary mutation rates were applied according to marker type. Microsatellite marker mutation rates (Schlotterer, 2000), sequenced markers mutation rates (Biffin *et al.*, 2010; Biffin *et al.*, 2011), and HapSTR mutation rate was approximated (Hey *et al.*, 2004). One hundred year generation time was approximated according to age cohort representation within the populations under study and regression; determined from cultivated trees of known age (The Royal Botanic Gardens and Domain Trust, Sydney: 1.74 (growth factor) x diameter at breast height (cm) = approximate age in years).

The Isolation-with-Migration model was applied as follows: multiple preliminary runs were conducted to assess convergence and adjust prior ranges (M mode). Markov Chain Monte Carlo (MCMC) runs were carried out with a burn-in of 1 000 000 steps with 50 000 genealogies sampled per locus (for a total of 5 000 000 iterations). Inheritance scalars were set as 1 for nuclear markers, and 0.25 for the plastid marker. A prior file was used to set the final prior parameters based on the upper bound limits of the likelihood distribution for each parameter independently. Exponential prior distribution for migration parameters was used due to the previous evidence of divergence (Mellick *et al.*, 2011) and that divergence is not expected unless gene flow is low (Hey, 2010). To ensure appropriate mixing, runs were tuned until effective sample size values exceeded 200 and the trend-line plots showed no observable trends, as suggested in the program documentation. Joint posterior density calculations (L mode) for four parameters (splitting time and effective population sizes) were obtained for each pairwise comparison, and relative splitting times were used to construct the tree string for the three population model. The methodology is included in IMa2 documentation (http://genfaculty.rutgers.edu/hey/software). Nucleotide substitution for each of the sequenced loci was determined via MODELTEST 3.7 (Posada and Crandall, 1998). The stepwise mutation model (Kimura and Ohta, 1978) was assumed for all microsatellite loci, the HKY mutation model (Hasegawa *et al.*, 1985) was applied for the sequenced loci and the infinite sites mutational model was applied to the HapSTR locus (Nielsen and Wakeley, 2001; Hey *et al.*, 2004).

RESULTS

Last glacial maximum to 2050 habitat suitability dynamics

Modelling based on the separate regional clusters identified by STRUCTURE inferred current geographic separation of environmental modelled ranges for northern and southern regions (Mellick *et al.*, 2011). Model performance and assessment is outlined in Mellick *et al.* (2012).

The ENMs (Fig. 1) predict that the climatic envelope of *P. elatus* populations south of the CRC will continue to contract coastward (A2 2050 scenario) as it has consistently since the last glacial maximum (LGM, 21 Ka), and will shift south beyond the present distributional limit along the highlands of NSW and Victoria. The climatic envelope supporting northern populations is predicted to expand by 2050, following a contraction since the Holocene Climatic Optimum (HCO, 6 Ka) and a significant expansion since the LGM. Furthermore, the northern ENM infers expansion beyond the northern distributional limit and south beyond the CRC this century. The distance between the two-modelled ranges is at its broadest at the LGM. In 2050 the north predicted range slightly overlaps the south predicted range, characteristic to a lesser extent to what we inferred during HCO. The current range north and south of the CRC is shown by the Pre-industrial model (PI, 0 Ka). Thus the disparity with respect to climate driven population expansion and contraction processes between the northern and the southern genetic clusters (Mellick *et al.*, 2012) is inferred to continue into the future.

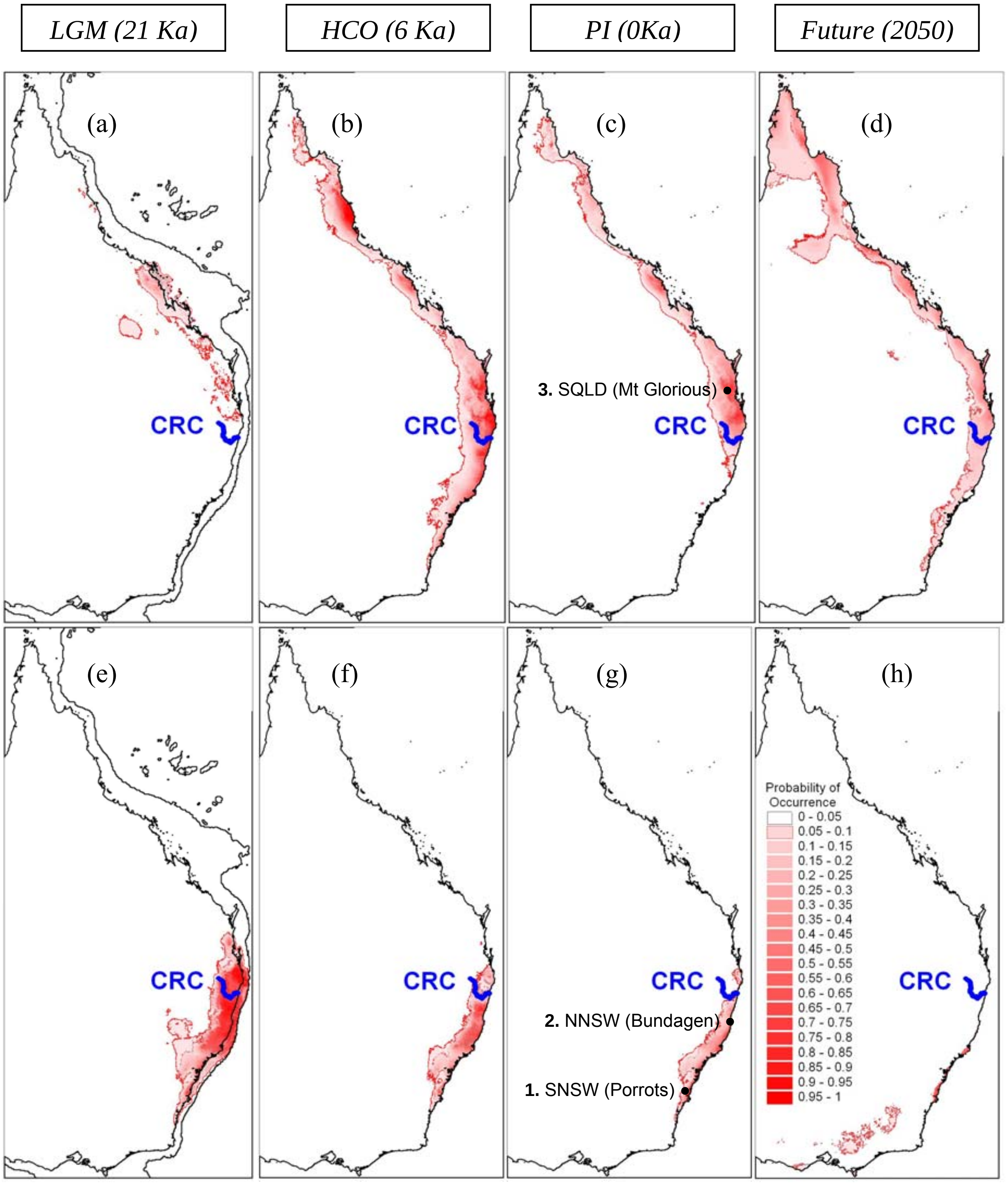


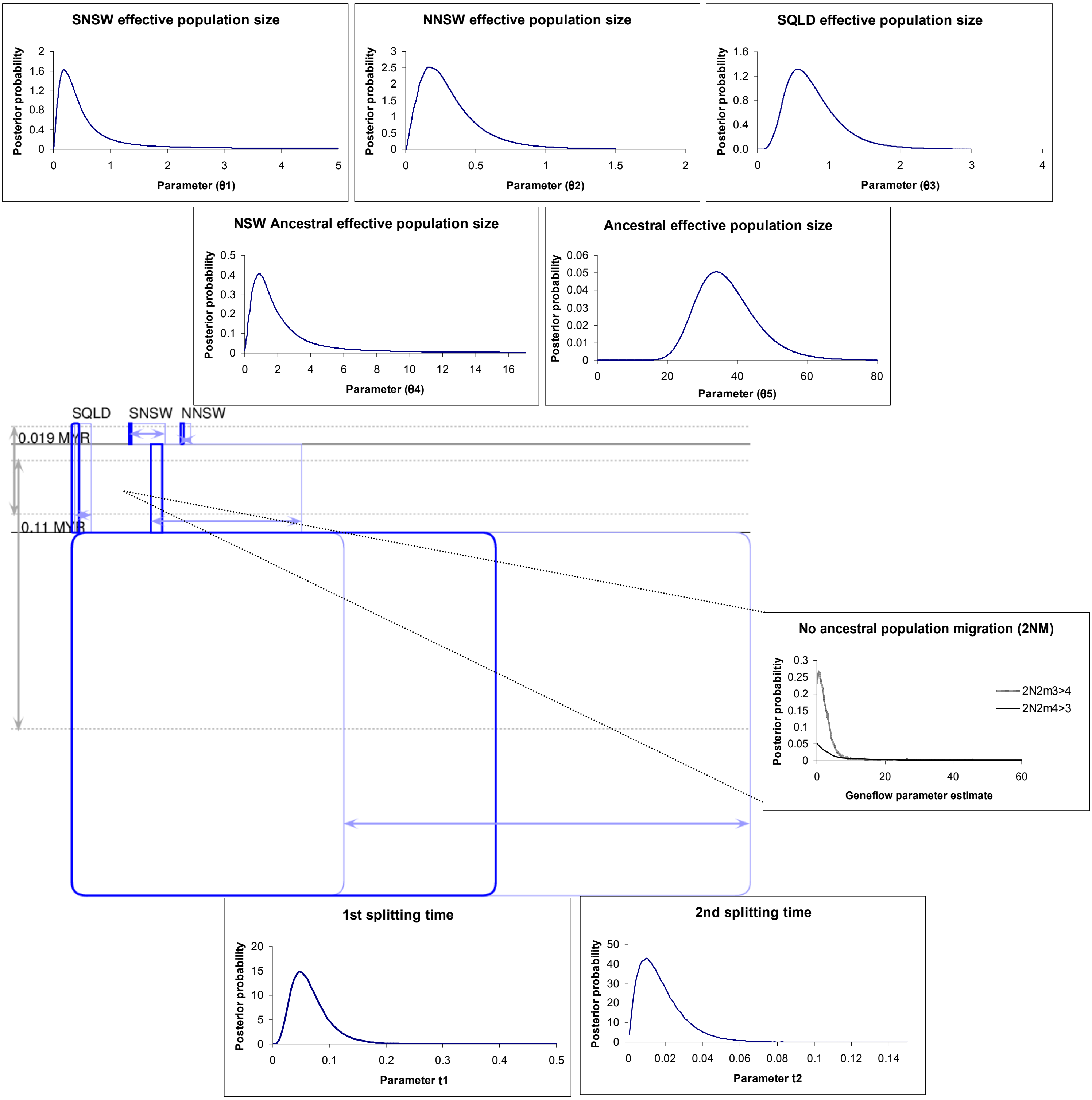
Figure 1 Environmental Niche Models for *P. elatus* north (a, b, c and d) and south (e, f, g and h) of the Clarence River Corridor biogeographic barrier in eastern Australia for 21 ka Last Glacial Maximum (a and e), 6 ka Holocene Climatic Optimum (b and f), 0 ka Pre-industrial (c and g), including the location of the three populations under study, and A2 2050 future time periods (d and h). Red borders around projected distributions do not reflect probability of occurrence.

While, non-natural variables used in Environmental Niche Modelling such as land-use planning, artificial vegetation boundaries and land clearing (VanDerWal *et al.*, 2009), have been shown to improve distribution prediction (Franklin, 1995) it is unrealistic to assume that land-use in the future is predetermined or will remain static. This is due to the uncertainty of variables that affect habitat suitability, such as physical properties of the landscape, resource demand, available technology, and population density, along with an array of laws, policies and attitudes of people toward their physical environment. For this reason we have concentrated on the natural drivers of distributional change in response to climate, and have inferred the future distribution of the *P. elatus* unaffected by the artificial boundaries imposed on the landscape.

Population demographic parameters and divergence

Over-parameterisation as a result of small number of markers necessitated exclusion of current migration parameters from the three-population isolation-with-migration (IM) model. This was not considered as an issue, since a previous Migrate-n analysis (Mellick *et al.*, 2012) already inferred current migration patterns in the microsatellite data set. This considerable parameter reduction provided adequate model stability to infer ancestral migration over the CRC during a period of climatic cooling/drying prior to Last Glacial Maximum (21 Ka). A dramatic decline in effective population size is inferred during the ancestral split at around 110 Ka, where posterior probability peaked (*t1*: Fig 2). No recent bottleneck was detected in the microsatellite data (Mellick *et al.*, 2011), yet when combined here in a multi-locus analysis, which included additional conserved flanking sequence and HapSTR data, a dramatic decline in population size over the last glacial cycle is inferred (Fig 2). The rapid mutation of microsatellites means the markers are useful in detecting recent bottlenecks but lack the genetic signature of more ancestral declines.

A later split between southern populations one and two is shown to have occurred close to the LGM at 19 Ka during which time another decline in effective population size was shown. No significant ancestral migration was shown over the CRC with either exponential or uniform migration prior distributions (Fig 2).



DATA	SNSW θ1	NNSW θ2	SQLD θ3	NSW Ancest θ4	All Ancest θ5	2N2m3>4	2N2m4>3	t1	t2
MLE	0.1825	0.1673	0.5655	0.89	34.04	0.56	1.87	0.0475	0.0095
HPD95Lo	0.0075	0.01425	0.1905	0	21.8	0	0	0.0175	0.0015
HPD95Hi	2.918	0.7748	1.556	12.11	54.44	25.97	2135	0.1475	0.0435
rescaled population size (individuals) and time (millions years) parameter units									
MLE	939	837	2831	4443	170406			0.105	0.019
HPD95Lo	38	71	954	0	109132	*No information*		0.035	0.003
HPD95Hi	14605	3878	7787	60636	272530			0.295	0.087

Figure 2 The history of three *Podocarpus elatus* populations representing the northern (SQLD) and southern (SNSW and NNSW) ecotypes either side of the CRC. In the tree: time is represented on the vertical axis with the sampled population names given above; the 95% highest posterior density intervals are shown with arrows in gray for population sizes (box widths) and splitting times (dotted lines). Posterior probability distributions are shown for *Podocarpus elatus* effective population sizes, splitting times and migration [no information about population migration (2NM) in either direction between SQLD (3) and ancestral NSW (4) was detected].

DISCUSSION

Ecological Niche Models (ENMs) and Isolation-with-Migration (IM) analyses were in concordance with respect to ancestral climate driven divergence in *P. elatus*, and the contraction in the southern distribution in response to post-glacial warming observed in the fossil record (Shimeld, 1995, 2004; Williams *et al.*, 2006; Black and Mooney, 2007). The coalescent analysis infers the divergence either side of the CRC occurred prior to the Last Glacial Maximum, and was likely the consequence of a significant decline in effective population size.

Ancestral demographic decline and changes in habitat suitability

Regional differentiation observed in *P. elatus* is inferred to be a result of ancestral polymorphism being maintained in the absence of gene flow (Fig 2). Our data supports the origin of the CRC to be prior to the LGM, and that it has been maintained since by climatic drivers (possibly a consequence of a phenological barrier to dispersal). Splitting times coincide with decreasing precipitation after the height of the last interglacial (110 Ka), and around the LGM (19 Ka).

Coalescent analysis shows a series of demographic events that aligned with climatic fluctuations, which explain the current differentiation, but importantly they identify a significant decline in abundance that pre-dates the LGM (Fig 2). Long-term absence of significant gene flow and decreasing effective population size, is in agreement with the observed decline in the fossil record of *P. elatus* (Shimeld, 1995, 2004; Williams *et al.*, 2006) and other podocarps (Hill, 1994; Kershaw *et al.*, 2007a; Kershaw *et al.*, 2007b) since the last interglacial (130 Ka). Bottleneck events are likely to have contributed to the low diversity and abundance of rainforest conifers in Australia (Shapcott, 1997; Peakall *et al.*, 2003; Kershaw *et al.*, 2007a). The decline of swamp forest and *Dacrydium* in Australia between 130 and 4.5 Ka was associated with a change to warmer or more seasonal conditions, as well as an increase in fire frequency (Kershaw *et al.*, 2007a, 2007b). This decrease in abundance is replicated by the southern ENM, which also suggests this trend to continue into the future (Fig 1: 21 Ka – 2050).

The 2050 predictions show the climatic conditions supporting the southern populations will contract coastward into microhabitat pockets and extend south mainly along the southern highlands (Fig 1). The climatic conditions supporting the northern populations will expand south approximately 600 km (i.e. 15km/yr), and north along the coast. These estimates show the climatic envelope for the northern genetic region is shifting south, which is in general agreement with the poleward movement of species in response to a warming environment (Hughes, 2000). The southern ENM infers uniform steady decline in habitat suitability since the last glacial maximum as supported by genetic and palynological evidence (Shimeld, 1995, 2004; Black *et al.*, 2006; Williams *et al.*, 2006). In particular, Shimeld (1995) shows the steady decline of *P. elatus* pollen from the height of the last interglacial (130 Ka).

Drier rainforest communities, typified by Araucarian emergents and *Podocarpus* species, are sensitive to the effects of frequent fires. Kershaw *et al.* (2004) postulated that dry rainforest communities fringed wetter core rainforest communities in the AWT, and expansion and contraction of these seasonal communities are climate driven. Precipitation gradients are largely responsible for successional community turnover from wet rainforest to dry rainforest and eventually to sclerophyllous forest during dry periods, and are likely to affect the frequency and intensity of fires encroaching on rainforests. The seasonality and sensitivity of dry rainforest communities to fire/competition may have also played a role in the decline of these communities that included *P. elatus* (Black *et al.*, 2006; Kershaw *et al.*, 2007a; Mooney *et al.*, 2010).

Macleay Overlap Zone: an important area of admixture

We identified the Macleay Overlap Zone (MOZ: Burbidge, 1960) as the centre of genetic diversity and as a critical area of habitat overlap, where the species is likely to survive across climatic cycles. The MOZ is an important crossroad to floristic exchange between the tropical, subtropical and temperate rainforests (Muellner *et al.*, 2008; Stork *et al.*, 2009; Sniderman and Jordan, 2011). This was supported by haplotype distribution patterns (Mellick *et al.*, 2012) which suggested the MOZ to be an area of admixture between northern and southern population groups. Thus we identified the MOZ (Pop 3) as an important repository of ancestral polymorphism for *P. elatus* (Fig 2).

Successional expansion waves in opposing directions would have resulted in the MOZ populations to be of high genetic diversity. The co-occurrence of southern/northern haplotypes, and, that the majority of unique haplotypes occur in the MOZ (Mellick *et al.*, 2012), suggests alternate expansion phases between the regions and sustained habitat suitability coincides with the overlap zone. The geography and climatic junction of the MOZ has ensured overlap and mixing of the differentially adapted genomes which may have ensured long term survival of *P.*

elatus. This emphasises the importance of range size in relation to conservation. Narrow endemics with small ranges, are more vulnerable due to reduced adaptive potential caused by genetic drift and inbreeding, while, broadly distributed species are likely to experience higher levels of admixture between dispersed refugia during expansion phases, which reduces genetic load and ensures increased adaptive potential.

Conclusions: is *Podocarpus elatus* more threatened than its current distribution suggests?

Historical-current-future modelling (Fig 1) suggests that the two population groups respond differently to climate change, but that both are subjected to expansion/contractions cycles indicative of community turn-over (Kershaw *et al.*, 2007a). This emphasizes the sensitivity of this species to climatic fluctuation, and when considering its broad latitudinal range, the likelihood that this species might be temporally restricted to survive in micro-habitats (ecotones). *Podocarpus elatus* is a late successional tree that has generation periods of up to 600 yrs which would allow for the species to persist out of ideal habitat for recruitment for long periods. Spatial displacement to dynamic boundary communities (Harden *et al.*, 2006), may facilitate survival of populations during periods of increased competition, yet may expose the species to more frequent burning and possible localised extinction.

The ecology of these ecotonal communities (e.g. nutrient poor areas, fire prone, less competition) in comparison to wet core rainforest systems are better suited to *Podocarpus* (Lusk, 1996, Brodribb and Hill, 2003). The micro-environmental character of these ecotonal areas may encapsulate certain ecological requirements for *P. elatus* that the bordering rainforest communities lack, such as infrequent burning and increased light availability. The sheltered ecology of ecotonal micro-habitats may have facilitated range expansion into areas of low climatic suitability. These fragmented habitats, considering the species' out-crossing nature, are especially vulnerable to the effects of isolation, genetic drift and increased inbreeding, which result in low overall levels of coniferous diversity (Shapcott, 1997; Peakall *et al.*, 2003; Mellick *et al.*, 2011).

Our inter-disciplinary approach has combined molecular, climatic and fossil data to infer changes in species demography over the last glacial cycle. Although *P. elatus* is not currently considered rare, our data shows that it is in significant long-term decline, and its survival across its distributional range might be tightly linked to the availability of microhabitats (ecotones). These are likely to become increasingly scarce under current anthropogenic disturbance (e.g. clearing, weeds and changed fire regimes). *Podocarpus elatus* populations in littoral/gallery rainforest habitat in the coastal south (Mellick *et al.*, 2012) are under further threat of sea-level rises (i.e. storm surges) anticipated this century (Rahmstorf, 2007). Considering the recent decline and localised extinction of other rainforest podocarps (i.e. *Lagarostrobos* and *Dacrydium*: Hill, 1994; Shapcott, 1997; Kershaw *et al.*, 2007) we suggest that *P. elatus* should be considered at future risk.

Range shift is part of the natural adaptive process (Moritz, 2002), but during previous climate fluctuations, range shift has been unhindered by human land-use. In order to conserve the natural genetic constitution and adaptive potential of species, protected avenues for this predicted range shift will need to be integrated into the human land-use matrix. Predictive distributional modelling and, the understanding of gene flow dynamics provide a means to interpret current distribution patterns and potentially anticipate, and accommodate rapid migration rates as a result of anthropogenic induced climate change. Our data suggest that conservation and management should be focused around the MOZ; where most of the haplotype diversity resides and where sustained habitat suitability in response to post-glacial warming occurs. Conservation strategies may involve the extension of habitat corridors to accommodate future range-shift, and assisted migration of genetically rich stock under threat of localised extinction into areas of high habitat suitability (i.e. the Macleay Overlap Zone).

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CHAPTER FIVE APPENDIX

Table 1 The three *P. elatus* populations used in the study, including the number of individuals sampled (*N*) in each population and location (decimal latitude and longitude). The Clarence River Corridor (Mellick *et al.*, 2011) occurs between population two and three indicated by a solid line. Allelic richness (R_S), inbreeding co-efficient (*f*) and significance level were estimated from six microsatellite loci (Mellick *et al.*, 2011). Unbiased diversity (*uh*) with standard error was estimated from five sequence loci (Mellick *et al.*, 2012). (***P*<0.01****P*<0.001)

	POPULATION	*N*	*LAT*	*LONG*	R_S	*f*	*uh*
1	Porrots Brush (SNSW)	7	-34.6548	150.813	3.74	0.286***	0.194 ± 0.050
2	Bundagen (NNSW)	11	-30.4315	153.075	3.86	0.100	0.247 ± 0.052
3	Mt Glorious (SQLD)	10	-27.3230	152.757	4.14	0.200**	0.200 ± 0.050

Table 2 Details of the 13 AOGCMs used for projecting MaxEnt models onto future climate conditions.

Climate Model Inter-comparison Project 3(CMIP3) identifier	*Originating institution or collective*
BCCR-BCM2.0	Bjerknes Centre for Climate Research, Norway
CGCM3.1(T63)	Canadian Centre for Climate Modelling and Analysis, Canada
CNRM-CM3	Météo-France / Centre National de Recherches Météorologiques, France
CSIRO-Mk3.5	CSIRO Atmospheric Research, Australia
ECHAM5/MPI-OM	Max Planck Institute for Meteorology, Germany
ECHO-G	Meteorological Institute of the University of Bonn, Germany; Meteorological Research Institute of the Korean Meteorological Agency, Korea
GFDL-CM2.1	Geophysical Fluid Dynamics Laboratory, NOAA, Dept. of Commerce USA
GISS-ER	Goddard Institute for Space Studies NASA USA
INGV-SXG	Instituto Nazionale di Geofisica e Vulcanologia, Italy
INM-CM3.0	Institute for Numerical Mathematics, Russia
IPSL-CM4	Institut Pierre Simon Laplace, France
MIROC3.2(medres)	Center for Climate System Research (The University of Tokyo), National Institute for Environmental Studies, and Frontier Research Center for Global Change (JAMSTEC), Japan
MRI-CGCM2.3.2	Meteorological Research Institute, Japan

CHAPTER SIX

CONCLUSION

Sine labore nihil

Rohan Mellick

Conclusion

Overall significance

The distribution histories of two genetically differentiated regions of the wide spread Australian rain forest species *Podocarpus elatus* were compared using molecular (cpDNA and nDNA), environmental (ENM) data and the available fossil record. Species distributional modelling of the separate regions has shown distinct differences between the environmental niches of each region, including a differential response to post-glacial warming. An additional region of overlap between the south and the north coinciding geographically with the Macleay Overlap Zone was identified in *P. elatus*.

The multi-disciplinary approach suggests the regional divergence can be explained by differential range shift responses between these two genetic clusters (i.e. ecotypes). The lower diversity and distributional pattern of the northern populations could suggest that their current distribution is the result of an earlier northern expansion from a narrow genetic pool. The Clarence River Corridor (CRC) is located close to a latitudinal boundary between southern uniform and northern summer rainfall seasonality (Bowman, 2000). Coalescence-base analyses inferred the divergence either side of the CRC to be historical (prior to Last Glacial Maximum; LGM), where stronger seasonality in the south is likely to have perpetuated this divergence. Although the species occupies a broad environmental envelope, the degree of admixture between the regional clusters has been sufficient to impede further divergence and possibly speciation.

The species is a member of Podocarpaceae, one of three southern conifer families that were once dominant in the landscape. The relatively few remaining podocarps are important, not only due to their ancient ancestry but because their physiology is relatively unchanged. Their traits are extremely versatile (e.g. leaf morphology) and adaptable to environmental change, from seasonally frozen high-latitude rainforests in the past, to the current flame forest/rainforest boundaries of the Australian landscape.

Podocarpus elatus-like macrofossils (i.e. similar leaf shape and size) are represented in the Antarctic flora (Hill, 1994; Quinn and Price, 2003). The surviving podocarps are remnants of a world dramatically different from the one they occupy today. It should be appreciated that this lineage, and their climatically-tolerant traits, have endured two mass extinction events and numerous ice ages that have resulted in the end of many other lineages.

Although *Podocarpus elatus* is common to the East Australian rainforests, its existence is closely linked to habitats that are threatened by future climate change. Seeing localised extinction and population decline has been documented in other rainforest podocarps (Shapcott, 1997) and drier rainforest conifers (Peakall *et al.*, 2003; Pye and Gadek, 2004), we should consider *P. elatus* as potentially at future risk.

The differential response of the species may be illustrative of differing climatic drivers between the southern subtropical and northern tropical rain forests. These differing climatic conditions may underline long-term divergent processes between the two regional groups. In the shorter term and at a regional scale, anthropogenic habitat clearing is also likely to contribute to the differences measured between regions and among generational cohorts.

This study identifies the need for conservation of natural range shift and migration processes to ensure adaptive potential, and suggests that the future design of habitat corridors could take on a broader evolutionary application by linking fragmented habitats along predicted avenues of range shift. Furthermore, understanding natural gene flow patterns will allow for genotype selection and assisted migration along these avenues.

Contribution to knowledge

We now have a latitudinal gradient of intraspecific variation of a late-successional conifer over the extent of the mainland east Australian rainforests. The Clarence River Corridor (CRC), a dry river valley in the central range of *Podocarpus elatus*, was identified as a biogeographic barrier. Agreement between the climatic data (niche models) and regional distribution either side of the CRC indicated the genetic divergence was climate-driven, as suggested by fossil record observations (Shimeld, 1995, 2004; Williams *et al.*, 2006; Black and Mooney, 2007; Kershaw *et al.*, 2007).

The landscape genetic approach to understanding divergence and evolution was applied to this study. The occurrence data of genetically differentiated regions allowed for regional niche models to be developed. The palaeodistribution of the separate genetic regions was inferred by projecting these models on to past climatic estimates. Specifically, the Last Glacial Maximum

(LGM: 21 Ka), the Holocene Climatic Optimum (HCO: 6 Ka) and Pre-industrial (PI: 0 Ka). These climates are indicative of a cool dry climate, a warm wet climate, and a climate without significant anthropogenic effect. Although indigenous people did affect the landscape by firestick farming methods, these practices were localised and of a relative small impact (linneansocietynsw.org.au/1%20Mooney%20Palaeofire.pdf; Mooney *et al.*, 2010). The suggestion that these farming methods transformed the landscape is now thought to be inaccurate (Bowman, 2000). It has been recently hypothesised that the decline of mega fauna may have increased the frequency of fire due to reduced browsing (Rule *et al.* 2012).

The distribution of *P. elatus* occupies distinctly different climatic regions that have responded differentially to post-glacial warming. This may explain the intraspecific divergence in the species and possible existence of a phenological barrier to dispersal. Latitude and its effect on precipitation seasonality may influence the phenology of each population group separated by the Clarence River Corridor (CRC).

At the LGM (21 Ka) the two niches were geographically separated, with the northern niche restricted to small areas around central Queensland, and the southern niche being considerably larger and covering the current NSW and Macleay Overlap Zone (MOZ) ranges. In contrast, during the HCO (6 Ka) the niches overlapped with the northern niche expanding dramatically since the LGM. During this time the southern niche contracted, while the north expanded. This differential range shift challenges the general understanding that species respond uniformly across their range to climate, although it does not seem surprising along such a fragmented distribution and broad latitudinal range as *P. elatus*.

The location and aridity of the CRC would have restricted dispersal during expansion phases of each latitudinally distributed region. These successional waves of diversity in opposing directions resulted in the populations flanking the barrier to be genetically distinct and of high diversity, with the majority of unique alleles found close to the barrier. Rainforest herpetofauna (Burns *et al.* 2007; Schauble and Moritz 2001) show genetic divergences over the CRC, which suggests the differential climatic drivers identified in *P. elatus* may support broader rainforest associations.

Palaeodistribution modelling provided information on habitat suitability and inferred periods of isolation and migration. Coalescence-based analysis of genetic data and inference of ancestral demographics corroborates the palaeodistribution modelling, and fits the decline of the species from the fossil record in the southern range since the last interglacial (130 Ka). This long-term decline linked to precipitation has been shown in other systems (Martin 2006; Kershaw *et al.* 2007). The northern occurrences and the noted problems associated with generic-level classifications of fossil pollen, has emphasised the informative southern fossil record in observing the past climate driven dynamics of *P. elatus.* Of particular note, two adjacent deposits on the central coast of NSW have observed a steady decline of the species since the height of the last interglacial (130 Ka: Shimeld, 1995, 2004), and in the southern niche modelling this trend is found to continue from the LGM (21 Ka to 2050). The genetically poor northern region of *P. elatus* also lacks any unique diversity and consequently is inferred to have been established through recent expansion and founder events. The expansion of the northern region and increased habitat availability during the HCO cannot be detected in the southern fossil record. The species is currently more abundant in the south where seasonal rainfall patterns and ecology (e.g. more suitable fire regimes and less competition from fast growing rainforest angiosperms) have better suited *P. elatus*.

Relative to other rainforest conifers and in particular the other Australian podocarps, the species has high diversity, which is probably a consequence of its wide range. It is likely the species has persisted in dispersed refugia (probably at altitude in the north and on the coast in the south) during adverse climatic conditions in the past. The distributional pattern of these refugial areas was largely influenced by rainfall patterns and the character of relief along the Great Dividing Range (GDR) – with areas of rain shadow interrupting upland areas of moist forest along the extent of the mountain range. The data suggests that periods of contraction and isolation, following periods of expansion and admixture are likely to account for the historical divergences we see in *P. elatus*.

Problems encountered

The rarity of populations, variable population size and cohort representation meant that sampling was not balanced (either among populations or age cohorts). To avoid the grouping of small populations into aggregated populations and the potential biasing of population-level estimates, sub-sampling was utilised to answer questions specific to the aims of the separate chapters of this thesis.

The limitation of the southern hemisphere fossil record for the Quaternary period has necessitated supplementary methodology to investigate the distributional changes in *P. elatus*. Genus-level identification of fossil pollen has complicated the identification of species-level dynamics in response to climate. Due to other co-occurring *Podocarpus* species with different boundary-habitat preference, distinguishing the dynamics between species (among habitats) is important. Unfortunately, only the southern region deposits can infer the abundance of *P. elatus* confidently due to the only other co-occurring *Podocarpus* species being a small shrub, and contributing relatively little to the fossil pollen record (Shimeld 1995, 2004).

Environmental Niche Modelling (ENM) was used to supplement the Quaternary fossil record – ENM is based around the assumption that the current environmental-effects on the distribution of a species are representative of past environmental-effects. By developing a climatic envelope of the present distribution and projecting this envelope onto historical and future climatic ensembles, inference about distributional changes in response to climate was made.

Distinguishing between allopatry (vicariance) and parapatry (colonisation) is difficult and is one of more challenging questions in biogeographical research. The two genetic regions of *P. elatus* were identified and assumed to have diverged at some stage in the past. Understanding the character of this divergence, i.e. recent divergence or ancestral distinction, was difficult, although application of coalescent analysis has made it possible to determine the lack of migration between the regions prior to the LGM has maintained ancestral distinction in the absence of significant geneflow. Support for a combination of allopatry and parapatry was inferred through ENM of the separate genetic regions of *P. elatus*.

Future directions

DNA analysis of extinct populations extracted from samples over a 100 years old

New molecular insights are being obtained through the analysis of ancient DNA (aDNA), although the system is rather precarious and has a number of contingencies (Cooper and Poinar, 2001). I extracted DNA of 14 *Podocarpus elatus* herbarium specimens, from three populations now extinct in the Australian Wet Tropics (AWT), to compare the genetic variation of extant and extinct AWT *P. elatus* populations. Although the amplification of many of the isolated loci (Almany *et al.*, 2009) were not reliable from the extracted DNA, a few loci including cpDNA locus PeB37BGT, returned reliable results. No polymorphism between extant and extinct *P. elatus* populations of the AWT was detected.

Putative hybridisation, trait convergence and synapomorphy

The co-occurrence of and putative hybridisation between *Podocarpus elatus* and *P. spinulosus* in the southern range is of evolutionary interest. The species are members of the same subgenus *Foliolatus* and co-occur along fire prone rainforest boundaries. Occasionally putative *P. elatus* x *spinulosus* individuals are found (Peter Wilson, The Royal Botanic Gardens and Domain Trust, *pers. comm.*). Two individuals have been collected (1. Patonga, Pittwater; 2. Target Beach, Jervis Bay) and foliage dried for DNA extraction. Future research would be to determine if there is shared genetic variability between the species and, if so, is it a consequence of incomplete lineage sorting or hybridisation? These hybrid individuals are mallee trees (with seemingly reduced lignotubers), yet, they have foliage very similar to the dry-adapted shrub, *P. spinulosus*. Seeing both species co-occur in these fire prone areas and possibly hybridise: is *P. spinulosus* derived from *P. elatus*, and is this derivation and evolution a result of fire selection toward fire adaptive traits in a rainforest tree?

It would seem the two fire-adapted podocarps, the east Australian *P. spinulosus* and the west Australian *P. drouynianus*, are sister species, because of their morphological similarity and ability to re-sprout from a lignotuber, but there is question to whether they are adaptable remnants of rainforests or are true components of open communities (Kershaw *et al.*, 1994; Hill, 1994). Conran *et al.* (2000) attempted to resolve the species relationships in *Podocarpus*, where the two fire-adapted podocarps were grouped together as sisters with no bootstrap support using cpDNA marker rbcL (Conran *et al.*, 2000). Considering the fire-prone nature of the areas that *P. elatus* and *P. spinulosus* co-occur, and the putative hybridisation, it could be hypothesised that the two species are phylogenetically closer than systematic studies suggest. Fire would seemingly be a strong selection event in ecotonal environments, which *P. elatus* is a custom.

The evolution of fire adaptation and the development of a lignotuber in *Podocarpus* may be a result of convergent evolution among east and west Australian species, rather than synapomorphic character derived from a parent species. This pattern of trait convergence among east and west species is observed in many plant genera. It may be hypothesised and explored that the phenotypic similarity in the disparate fire-adapted species is more likely an adaptive response to environmental change rather than an inherited synapomorphic trait from a common ancestral tree species.

Clonal cultivation of male trees

Podocarpus elatus has been extensively used in cultivation for roadside plantings in the southern more urbanised region. It is resilient to desiccation, has a long leaf-life that ensures aesthetic appeal, the male trees are low maintenance due to no fruit-fall and it has a thick waxy cuticle that provides protection from toxic compounds. Unfortunately, to capture desirable variation the cultivated stock is clonally propagated (David Bateman, Waverley Council, Sydney, *pers. comm.*: Appendix B).

Clonality of male trees may have decreased the diversity of the species' natural pollen cloud, and with the prevailing south-southeast wind direction, northern natural variation may be affected by southern clonal cultivation. The main direction of gene flow is toward the north (Mellick *et al.*, 2012); so potentially the diversity of the species (or at least of some natural populations) could be homogenising through cultivation and clonal propagation of southern male trees.

Adaptability and reproductive response to fire

Podocarpus elatus exhibits traits shared by both fire adapted species and fire intolerant species. Commonly referred to by foresters as Turpentine Pine, the foliage when crushed has a distinctive turpentine fragrance similar to the fire promoting species of Myrtaceae. Although universally accepted as a rainforest tree (typically fire intolerant) the species is frequently referred to as being fire tolerant (Chris Quinn, The Royal Botanic Gardens and Domain Trust, Forestery, Bulahdelah, Jan 2008, *pers. comm.*). It is known that *P. lawrencii*, a shrub that lacks a lignotuber, recovers from low-intensity fire, and may well benefit from reduction in ground cover (Edward Biffin, University of Adelaide, Adelaide, *pers. comm.*). The occurrence of fire promoting oils in the foliage of plants is known to be an adaptation toward fire tolerance. A comparison in leaf chemistry between *P. elatus* and the co-occurring fire-adapted *P. spinulosus* would be interesting in this regard. More research is needed into the adaptability and reproductive response of the species to certain fire frequencies and intensities.

Species worldwide are a result of natural evolution that has afforded them a genetic constitution and an ability to survive environmental extremes. The consequences of our interference (artificial selection through anthropogenic effects) in these processes may be a reduction in the adaptive potential and natural genetic constitution to deal with environmental pressures. We have complex evolutionary processes occurring in the east Australian rainforest habitats, the majority of which are surrounded by a human land use matrix with little means of altitudinal or latitudinal range shifts in response to anthropogenic-induced climate change. Understanding the manner of intraspecific divergence and adaptation along a latitudinal temperature gradient within these ‘captive’ rainforests is integral to their conservation.

The old trees on previously cleared land in the southern sample are suffering dieback, and there is no regeneration or site restoration to reduce the risk of localised extinction. Old trees are reservoirs of diversity for the species and contribute distinct variation to the species’ gene pool. The species is a dioecious obligate outcrossing tree and recruitment success around old female trees may be a consequence of genetic load and the level of population inbreeding (Mellick *et al.*, 2011).

The northern populations are of lower genetic diversity, which may account to some extent for the recently observed localised extinctions in the Australian Wet Tropics, possibly a consequence of a relatively recent founder-event. Populations in the south are at threat due to climate change, as inferred form Environmental Niche Modelling (ENM) and observed in the fossil record, but also as a result of increasing urbanisation, clearing and habitat destruction. Furthermore, littoral/gallery rainforest habitat in the coastal south (Mellick *et al.*, 2012) is under further threat of sea-level rises (i.e. storm surges) anticipated this century (Rahmstorf, 2007).

Long-periods of climatic change has made naturally disjunct populations of *Podocarpus* genetically and climatically divergent from one another, suggesting that local genetic seed, but arguably not where genetically impoverished, should be used in the restoration of degraded habitats. The approach of using locally sourced seed to revegetate areas may be appropriate for the southern populations, but due to the northern genetic impoverishment, success will depend on seed viability and, once established, on resilience to predicted environmental change.

The interest of this research is to understand how the distribution of *P. elatus* has responded to recent climatic change of the Quaternary, and the likely response to future climate change. Using fossil, molecular and ENM, a sturdy overall indication of how past climate change has affected biogeographic distribution of *P. elatus* has been obtained. Due to the study being based on separate independent sources of evidence, the conclusions made are robust and will provide a basis to interpret the environmental patterns and biological processes that have led to present-day genetic structure in *P. elatus*, and possibly other species associated with similar mesic habitats. The information acquired will improve our theoretical understanding of the response of endangered habitats to climate change, as well as providing application toward future conservation policy.

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APPENDIX

APPENDIX A:

APPENDIX B:

APPENDIX C:

Molecular Ecology Resources Primer Development Consortium, Almany, G.R., De Arruda, M.P., Arthofer, W., *et al.*(2009) Permanent Genetic Resources added to Molecular Ecology Resources Database 1 May 2009 -31 July 2009
Molecular Ecology Resources, v. 9 (6), pp. 1460-1466
10.1111/j.1755-0998.2009.02759.x

APPENDIX B:

Clonal propagation trial of *Podocarpus elatus*

Condition One

Date: 9.1.02

Cutting type: Semi-hardwood

Number of Cutting: 120

Hormone Treatment: Clonex Purple

Container Used: Hyco 40 Cell Tray

Propagation Mix Type: Five parts Perlite (Medium Grade), three parts Coco Peat

Result: 112 Cuttings

Result Date: 29.4.02

Additional Treatment/Comments: Cuttings were basally wounded and very healthy

Condition Two

Date: 9.1.02

Cutting type: Semi-hardwood

Number of Cutting: 18

Hormone Treatment: Clonex Purple

Container Used: Community Pot

Propagation Mix Type: Two parts Debco Commercial Propagation Mix, one part Perlite

Result: 18 Cuttings

Result Date: 29.4.02

Additional Treatment/Comments: Cuttings were basally wounded and very healthy.

All the clones had excellent health, although the condition using the Debco Mix gave more vigorous roots. In *Podocarpus elatus* terminal vertical growth is necessary for a straight single stem, otherwise the cuttings have a tendency to grow in a groundcover-type habit.

Propagation of *P. spinulosus* has also been successful using the above treatment. For further details contact David Bateman (Waverley Council, Sydney, Australia).

APPENDIX C:

Software used in the study

Environmental Niche Modelling and Geographic Information Systems

MAXENT 3.3.3E

MAXENT (Phillips *et al.* 2004) is the most widely used Environmental Niche Modelling software. It is a machine learning technique that uses pseudo-absence, as inferred random background environmental data. The software estimates a species niche by determining the probability distribution of maximum entropy. If the environmental data is continuous it can be included under numerous feature types (e.g. product, hinge, quadratic) thereby adding further constraints to the estimation of probability distribution by restricting it to be within the variance for each environmental predictor.

Recent explanations of MAXENT statistics (Elith *et al.* 2010), shows that the software algorithm minimizes the relative entropy between two probability densities (one estimated from the occurrence data and one from the landscape) defined in covariate space. It does not constrain the estimated distribution to the exact empirical average, but to within the empirical error bounds of the average value for a given predictor, in a procedure called 'regularization' (Phillips *et al.* 2004).

MAXENT 3.3.3e (Phillips *et al.* 2006`; software available from http://www.cs.princeton.edu/~schapire/maxent`, last accessed 4 January 2012).

ARCGIS v9

ArcGIS is a geographic information system (GIS) for working with maps and geographic information. We used the software to edit environmental data for input into MAXENT, to create distributional maps and figures for the study. It is used for compiling geographic data, analysing mapped and geographic information.

http://www.esri.com/software/arcgis/

Molecular data acquisition and assessment

GENEMAPPER

GeneMapper Software was used to collect genotyping data for the microsatelites markers used. It is a software package that provides DNA sizing and allele calls for electrophoresis-based genotyping systems (e.g. 310 ABI Genetic Analyser). The multi-application functionality of GeneMapper includes loss of heterozygosity, microsatellite, and SNP genotyping analysis. Manual over-riding features enable dubious calls to be addressed.

https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=600798

MICRO-CHECKER

Micro-Checker can test the genotyping success of microsatellite data from diploid populations. The genotyping procedure may result in the incorrect assignment of microsatellite genotypes, where primer-site mutations result in non-amplified alleles (null alleles), and PCR artefacts cause large allele drop-out (i.e. short allele dominance) and stuttering. The program aids the identification of various genotyping errors, and can also detect typographic errors.

http://www.microchecker.hull.ac.uk/

MODELTEST

MODELTEST is program for the selection of nucleotide substitution models that best fit the mutation patterns of the loci under investigation. The program implements three different model selection frameworks: hierarchical likelihood ratio tests (hLRTs), Akaike information criterion (AIC), and Bayesian information criterion (BIC). The program also assesses model uncertainty and calculates parameter importance.

http://darwin.uvigo.es/software/modeltest.html

GENEIOUS

GENEIOUS was used to align and edit sequence data. It is a suite of cross-platform bioinformatics software applications. The basic version is free for academic use, and a commercial Pro version includes plug-in features, such as heterozygote detection.

http://geneious.com/

Molecular data analysis

BOTTLENECK

BOTTLENECK computes for each locus and for each population sample the distribution of the heterozygosity expected from the observed number of alleles, given the sample size under the assumption of mutation-drift equilibrium. This is obtained through simulating the coalescent process under the Stepwise Mutational Model, and the Infinite Allele Model. This enables expected heterozygosity to be calculated, which is compared to the observed heterozygosity to establish whether there is a heterozygosity excess and hence a past bottleneck event.

http://www.ensam.inra.fr/URLB/bottleneck/pub.html

GENALEX

Genetic Analysis in Excel (GenAlEx) is a Microsoft Excel based cross-platform software for population genetic analysis, which includes genetic data analysis of codominant, haploid and binary genetic data. Analysis and data transformation features provided the data and input files for numerous other programs used in the study.

http://www.anu.edu.au/BoZo/GenAlEx/

STRUCTURE

This MCMC-based software accommodates multi-locus genotypic data to investigate population structure. It infers the presence of distinct populations, assigning individuals to populations, studying hybrid zones, identifying migrants and admixed individuals, and estimating population allele frequencies in situations where many individuals are migrants or admixed. It can be applied to most of the commonly-used genetic markers, including SNPs, microsatellites, RFLPs and AFLPs.

http://pritch.bsd.uchicago.edu/structure.html

NETWORK

Network was used to reconstruct phylogenetic networks for the sequenced haplotypes. Algorithms are designed for non-recombining haplotypic markers.

http://www.fluxus-engineering.com/sharenet.htm

GENEPOP

GENEPOP computes exact tests for Hardy-Weinberg equilibrium, for population differentiation and for genotypic disequilibrium among pairs of loci. The software was also used to convert file formats used by other programs in the study.

http://genepop.curtin.edu.au/

FSTAT

FSTAT is windows-based software that estimates and tests gene diversities and differentiation statistics from codominant genetic markers. It computes both Nei and Weir and Cockerham estimators of gene diversities and *F*-statistics, and tests them using randomisation methods.

http://www2.unil.ch/popgen/softwares/fstat.htm

DNASP

DNA Sequence Polymorphism (DnaSP) is the package used to phase sequence haplotypes. DnaSP can estimate several measures of DNA sequence variation within and between populations as well as linkage disequilibrium, recombination, gene flow, neutrality, gene conversion parameters, and also test for neutrality.

http://www.ub.edu/dnasp/

Coalescent-based analysis

MIGRATE-N

Migrate estimates effective population sizes and migration rates between populations. Migrate uses maximum likelihood or Bayesian inference and is well suited to microsatelite data, using a stepwise mutation model or a brownian motion mutation model.

http://popgen.sc.fsu.edu/Migrate/Info.html

ISOLATION-WITH-MIGRATION (IMa2)

IMa2 fits an isolation-with-migration model to sequence, microsatellite and HapSTR data from closely related populations or species. The software estimates current and ancestral population sizes, migration rates, and divergence times.

http://genfaculty.rutgers.edu/hey/software

ISOLATION-WITH-MIGRATION FIGURE (IMfig)

IMfig is a program (written in Python) illustrates a Isolation-with-Migration model that has been estimated from a data set. IMfig reads an output file generated with the IMa2 program.

http://genfaculty.rutgers.edu/hey/software