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Discontinuous distributions of iconic New Zealand plant taxa and their implications for Southern Hemisphere biogeography

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
in
Plant Biology

at Massey University, Palmerston North,
New Zealand

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"With regard to general problems of biogeography, the biota of New Zealand has been, perhaps, the most important of any in the world. It has figured prominently in all discussions of austral biogeography, and all notable authorities have felt obliged to explain its history: explain New Zealand and the world falls into place around it." Gareth Nelson (1975)

=:

Abstract

New Zealand has long been regarded as a key to understanding discontinuous distributions in the Southern Hemisphere. The archipelago is a fragment of the ancient super continent Gondwana. It has been isolated for 80 million years, has an excellent fossil record, and some of its most ancient biota such as the Southern Beeches (*Nothofagus*) and the Araucariaceae show disjunct distribution patterns with relatives on other fragments of Gondwana. Some of the most controversial problems of Southern Hemisphere biogeography with wide ranging implications involve New Zealand taxa. Three of them have been addressed in this thesis.

The transoceanic relationships of the genus *Nothofagus* have long been regarded as an iconic example of a distribution pattern resulting from the break up of Gondwana. Phylogenetic analyses presented here show that, though most of the extant distribution of the genus is indeed shaped by tectonic events, Southern Beeches have crossed the Tasman Sea between Australia and New Zealand at least twice during the Tertiary period. These results, together with findings of studies on other plant and animal taxa, emphasise the importance of dispersal but at the same time raise the question of whether any New Zealand taxa can be considered Gondwanan relicts. There is no geological evidence for the continuous existence of land throughout the Tertiary in the New Zealand area. However, molecular clock analyses presented in this thesis indicate that *Agathis australis* (New Zealand Kauri) diverged from its closest Australian relative prior to the Oligocene, or period of greatest submergence during the Tertiary. Thus these findings reject the hypothesis of the complete drowning of the New Zealand landmass during the Tertiary. They cannot reject the hypothesis of Stöckler *et al.* (2002) that the New Zealand Kauri lineage has persisted on the archipelago since its separation from Gondwana.

Explanations for forest distribution patterns within the New Zealand islands themselves are diverse. New Zealand *Nothofagus* species show distribution gaps that are not explained by recent environmental factors alone. Early Miocene tectonic events and alternatively Pleistocene climates have been proposed as causes for this disjunct distribution pattern. Phylogeographic analyses reported in this thesis suggest that severe Pliocene and Pleistocene climates as well as Pliocene and Pleistocene tectonic events have shaped present day distribution and diversification of *Nothofagus* species in New Zealand.

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1

Introduction

Thesis structure and scope

The thesis first summarizes what is known about the history of the New Zealand landmass and its vegetation changes in relation to other Southern Hemisphere landmasses. This treatment is then followed by three chapters which represent a series of three self-contained scientific manuscripts. These address three long-standing problems of Southern Hemisphere and New Zealand plant biogeography: (1) Trans Tasman Sea distributions of *Nothofagus*; (2) the relationship of *Agathis australis* to other Pacific *Agathis* species; (3) the nature of the New Zealand beech gaps. Findings from specific taxon studies are discussed in terms of their possible significance for understanding the evolution of the New Zealand forest flora.

Biogeographic context of this thesis

Discontinuous distributions of plant and animal taxa can be found all over the world. The most prominent examples are from the Southern Hemisphere and include distributions of animal taxa such as ratite birds from South America, Africa, Australia and New Zealand (Cracraft, 1974; Craw *et al.*, 1999; Cooper *et al.*, 2001), lungfish (Dipnoi) from South America, Africa and Australia (Darlington, 1957; Schäfer, 1997) and the circum - Antarctic chironomid flies (Brundin, 1966, 1972). Well known among the plants are the distributions of Araucariaceae from South American and the Southwest Asia - Western Pacific region (i.e. Hill and Brodribb, 1999; Kershaw and Wagstaff, 2001) and the Podocarpaceae from South America, Africa, Australasia and some species from Asia and Central America (i.e. Hill, 1995). One of the most quoted examples for discontinuous - or “disjunct “- Southern Hemisphere distributions is the genus *Nothofagus* (Southern Beeches) (i.e. Darlington, 1965; van Steenis, 1971; Mildenhall, 1980; Hill and Jordan, 1993; Pole, 1994; Linder and Crisp 1995; McGlone *et al.*, 1996; Macphail, 1997; Craw *et al.*, 1999; Crisci, 2001; Nelson and Ladiges, 2001; McCarthy, 2003, Sanmartín and Ronquist, 2004; Cook and Crisp, 2005; Renner, 2005; Heads, 2006 and many more).

The importance of these disjunct distribution patterns for understanding processes of global biogeography has long been recognised and their origins have been the focus of investigation for more than a century (Hooker, 1853; Matthew, 1915; Darlington, 1957, 1965; Croizat, 1958; Brundin, 1966, 1972; Banarescu and Boscaiu, 1978; Nelson and Platnick, 1981; Avise *et al.*, 1987; Ronquist, 1997; Craw *et al.*, 1999; Crisci, 2001; Nelson and Ladiges, 2001).

Vicariance, dispersal, and extinction are the three most important mechanisms that shape biogeographic patterns worldwide (Crisci, 2001). Vicariance describes a model of population divergence in which two subpopulations are separated by the development of a barrier - often resulting from tectonic events - that cannot be crossed. Dispersal describes a model where individuals or groups belonging to a population cross a barrier (that might be older than the population), resulting in the development of a new subpopulation.

While the influence of extinction events on present day biogeography is widely acknowledged, the role of vicariance and dispersal is controversial. The interpretation of distribution patterns worldwide has varied throughout history and has been largely influenced by contemporary knowledge. Matthew's (1915) and Darlington's (1957) hypotheses were based on the assumption of geographical stasis. Wegener had proposed his theory of continental drift in 1912 but it was not widely accepted before the 1960s. Thus both Matthew and Darlington claimed that isolated areas like New Zealand had to be reached by long distance dispersal. According to their hypothesis, taxa spread from a centre of origin into the areas they can be found in today. Subsequent extinction of the respective taxon in the regions separating present day areas of distribution would then have resulted in a disjunct distribution pattern. Even after accepting the existence of the continental drift, Darlington (1965) still favoured dispersal as an explanation for the origin of the observed distribution patterns. Banarescu and Boscaiu (1978) were of a similar opinion and claimed that the continental drift, though conceivable, was not essential to explain present day biogeography. Croizat (1958) was among the first to challenge this view. Like Brundin, (1966, 1972) and Nelson and Platnick (1981) he emphasized the importance of vicariance. These Authors have argued that many discontinuous distributions can be explained as formerly connected ancestral populations, which were separated by tectonic events, often linked to the break up of Gondwana. During the last 25 years, a multitude of biogeographic methods have been proposed to analyse distributional

patterns and their relation to geographic events (Crisci, 2001). Each method puts different emphasis on vicariance, dispersal and extinction.

Evaluating the importance of vicariance, dispersal and extinction in shaping extant distribution patterns is essential for our understanding of biogeography. Only with the knowledge of the origin of our present day biota are we able to interpret the information that is contained in the discontinuous distributions that can be observed all over the world.

New Zealand has long been regarded as a model for understanding Southern Hemisphere biogeography (Nelson, 1975). It is among the most isolated fragments of Gondwana, has an excellent fossil record (see Chapter 2), and it is still home to ancient taxa that are known from the old supercontinent. The flora and fauna of New Zealand have been considered passive passengers on a "Moa's Arc" (Bellamy *et al.*, 1990) that rafted away from Gondwana about 80 million years ago (mya) and remained unchanged by immigration. If the New Zealand biota "can be shown to have arrived over the sea [...] then biogeographic hypotheses the world over which involve any kind of land connection must be reconsidered." (Pole, 1994: p. 625)

This thesis reports phylogenetic analyses on plant taxa from the New Zealand flora whose transoceanic distributions have been iconic in Southern Hemisphere Biogeography. These studies have evaluated the relative importance of vicariance and dispersal for explaining disjunct distributions. Trans-Tasman Sea relationships of *Nothofagus* and *Agathis* have been investigated, as have been disjunctions of *Nothofagus* (*Lophozonia* and *Fuscospora* species) within New Zealand.

A combination of fossil and molecular data has been used to date divergence times between disjunct taxa with three molecular clock approaches (Penalized Likelihood, Bayesian Relaxed Molecular Clock, and Strict Molecular Clock). Nested clade analyses (NCA) have also been used to reconstruct the population history of New Zealand Silver Beech (*Nothofagus menziesii*).

Contributions of others to this thesis

The work contained in this thesis is entirely my own except where acknowledged. Several of the chapters were published as co-authored papers. Here I comment on the contribution of the co-authors.

My academic supervisors Peter Lockhart, Steve Wagstaff and Matt McGlone have been co-authors in acknowledgement of contributions to the research. These contributions took the form of comments on project design and draft manuscripts, as well as provision of funding and other resources. Karen Stöckler was co-author recognising the contribution of her unpublished preliminary results obtained in her thesis, which provided a starting point for the research presented in this thesis. She also generously provided unpublished *trn*L intron and *trn*L-*trn*F sequences as well as *Nothofagus* tissue samples. David Havell assisted with logistics for the field work and provided samples. Frédéric Delsuc introduced me to Bayesian molecular dating and provided helpful comments on the draft for Chapter 3. Patrick Mardulyn introduced me to nested clade analyses and provided helpful comments on the draft for Chapter 5. Ragini Mudaliar provided DNA of the Fijian *Agathis macrophylla*, and Federico Sebastiani provided unpublished chloroplast DNA sequences of *Castanea sativa*.

I was responsible for the design of the individual projects and I devised the objectives described in each of the chapters. I undertook fieldwork to collect many of the *Nothofagus* and Conifer accessions, designed and executed the experiments and analyses, and contributed most of all the authors to the writing of submitted manuscripts.

2

The New Zealand plant fossil record

Although oceanic island in character (Winkworth *et al.*, 2002; Trewick *et al.*, 2007), the New Zealand archipelago has a well documented plant fossil record. This chapter gives an overview of the evolution of the New Zealand landmass together with a synopsis of findings from macrofossil and microfossil studies.

2.1 Cambrian – Carboniferous (542 – 359 mya)

Geology and tectonics

Continental crust has been present in the New Zealand region since the Precambrian (more than 540 million years ago (mya), Table 2.1), indicating temporal existence of land. Most of New Zealand's present day landmass was part of a long geosynclinal structure located at the margins of the Gondwanan supercontinent (Fleming, 1979; Figure 2.1). Only Fiordland, coastal Westland, and north-west Nelson were above water (Fleming, 1979).

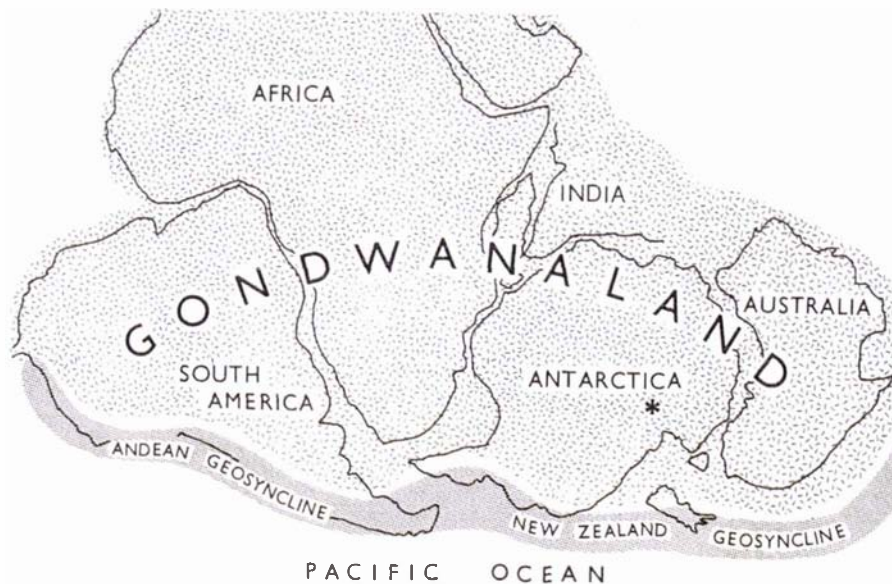

Figure 2.1: Location of the New Zealand Geosyncline relative to Gondwana (from Fleming, 1979)

Table 2.1: Geological time scale (dates from: Stöckler, 2001; Veblen *et al*., 1996; http://www.palaeos.com; http://en.wikipedia.org). BP: before present: radiocarbon years before 1950 AD. Mya: million years ago.

Era	Period		Epoch	began
Cenozoic	Quaternary		Holocene	10,000 BP
			Pleistocene	1.8 mya
	Tertiary		Pliocene	5 mya
			Miocene	26 mya
			Oligocene	38 mya
			Eocene	54 mya
			Palaeocene	65 mya
Mesozoic	Cretaceous	Late	Maastrichian	71.5 mya
			Campanian	83 mya
			Santonian	87.5 mya
			Coniacian	89 mya
			Turonian	91 mya
			Cenomanian	100 mya
		Early	Albian	109 mya
			Aptian	112 mya
			Barremian	122 mya
			Hauterivian	130 mya
			Valanginian	133 mya
			Berriasian	136 mya
	Jurassic	Late	Tithonian	150 mya
			Kimmeridigian	155 mya
			Oxfordian	161 mya
		Middle	Callovian	164 mya
			Bathonian	167 mya
			Bajocian	171 mya
			Aalenian	175 mya
		Early	Toarcian	183 mya
			Pliensbachian	189 mya
			Sinemurian	196 mya
			Hettangian	200 mya
	Triassic	Late	Rhaetian	204mya
			Norian	217 mya
			Cranian	228 mya
		Middle		245 mya
		Early		251 mya
Palaeozoic	Permian			299 mya
	Carboniferous			359 mya
	Devonian			416 mya
	Silurian			444 mya
	Ordovician			488 mya
	Cambrian			542 mya

The flora

The earliest vascular land plant fossils are known from Early Silurian rocks (Edwards and Fanning, 1985). These were herbaceous plants occupying moist habitats such as swamps, lakes, and coastal areas (McLoughlin, 2001).

The Late Silurian and Early Devonian Gondwana was characterised by the cosmopolitan *Baragwanathia* flora, which is named after the earliest known genus of herbaceous lycopod (Anderson *et al.*, 1999). Early land plant fossils suggest that towards the end of the Early Devonian the *Baragwanathia* flora was replaced by the Giant Clubmoss Flora with first tree-like lycopods, which were joined by progymnosperms later in the Devonian (Hill *et al.*, 1999; Anderson *et al.,* 1995; Gess and Hiller, 1995). Throughout the Devonian herbaceous to shrub-sized lycophytes such as *Haplostigma*, *Leclercqia*, *Archaeosigillaria,* and *Protolepidodendron* dominated Gondwanan vegetation, although tree size plants became increasingly abundant (McLoughlin, 2001). By the end of the Devonian, lepidodendralean lycophytes such as *Bumbudendron and Leptophloeum,* and progymnosperms possibly reached heights of up to 20 meters (Gould, 1975).

During the Carboniferous, a period of global cooling and extensive glaciation changed the flora of Gondwana (Ziegler *et al.,* 1997; Scotese *et al.*, 1999; Anderson *et al.*, 1999). Lycophyte floras were progressively replaced by seed fern floras including *Diplothema*, *Eusphenopteris*, *Eonotosperma*, *Nothorhacopteris*, *Fedekurtzia*, *Austrocalyx*, and *Botrychiopsis* (McLoughlin, 2001). Low diversity of the Late Carboniferous *Botrychiopsis* floras suggest tundra vegetation in the periglacial regions of Gondwana (Retallack, 1980; McLoughlin, 2001). The glaciation ended in the Late Carboniferous to Early Permian (Anderson *et al.*, 1999; McLoughlin, 2001).

2.2 The Permian (299 – 251 mya)

Geology and tectonics

During the Permian most continental blocks were united in the mega continent Pangaea. From lithological analyses Scotese *et al.* (1999) inferred global warming and a retreating Gondwanan ice shield, which would have opened new habitats (Anderson *et al.,* 1999).

The flora

The Permian period is characterised by the *Glossopteris* flora, named after an extinct deciduous gymnosperm taxon, which represents the main diagnostic fossil of Permian terrestrial deposits. *Glossopteris* can be found in all Permian Gondwanan deposits between 40° and 90° palaeolatitude (McLoughlin, 2001; Figure 2.2) and was used to support the idea of an ancient Southern Hemisphere landmass long before Wegener's theory of the continental drift was accepted in the 1960s (du Toit, 1937). From its distribution it can be assumed that *Glossopteris* was adapted to seasonal and cooler continental climates. It appears to be an Austral element as opposed to the Tethyan elements of the northern margin of Gondwana that was flanking the Tethys Ocean (McLoughlin, 2001).

The oldest plants identified in New Zealand belong to the *Glossopteris* flora. Representatives of this flora found in Southland/New Zealand include *Equisetites*, *Cladophlebis*, *Sphenopteris*, *Noeggerathiopsis,* and *Glossopteris* (McQueen, 1954; Fleming, 1979).

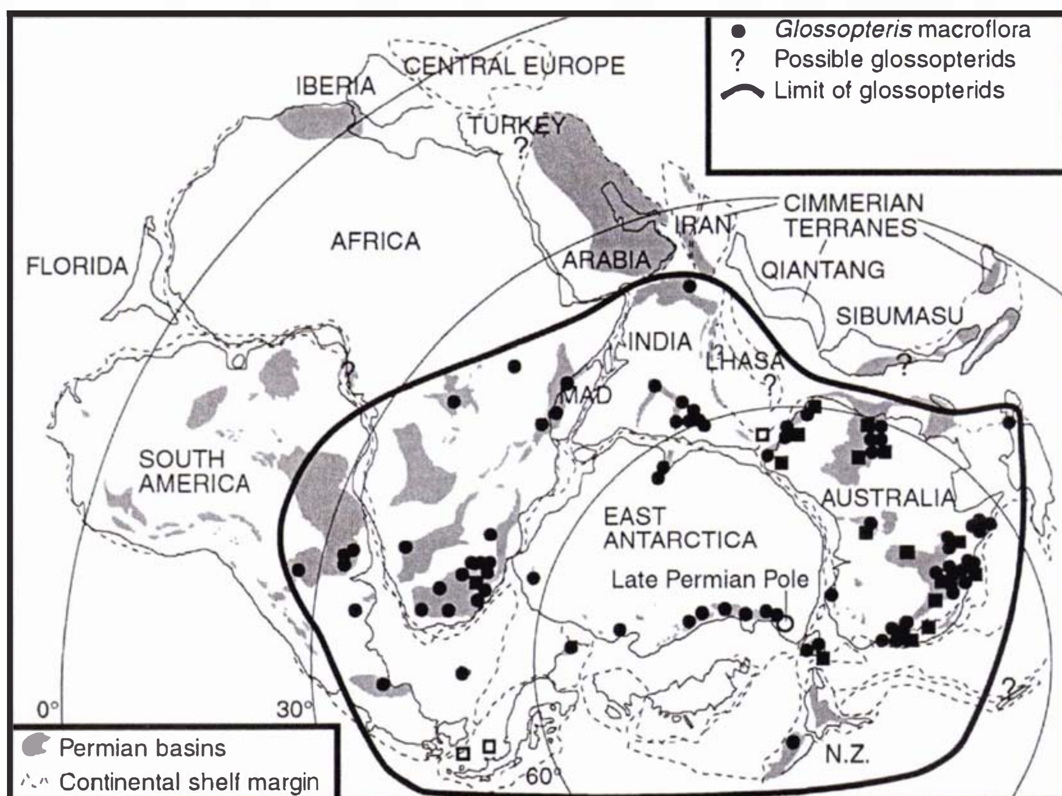

Figure 2.2: Palaeolatitudinal distribution of the Permian *Glossopteris* flora (from McLoughlin, 2001)

2.3 The Triassic (251 – 200 mya)

Geology and tectonics

During the Triassic the New Zealand Geosyncline further subsided and was filled at an increasing rate. The metamorphous and strongly folded greywackes that now form the Southern Alps were formed (Fleming, 1979). The Triassic rocks and fossils of New Caledonia are very similar to the ones of New Zealand, whereas those of eastern Australia show major differences. Fleming (1979) concluded, that the New Zealand Geosyncline and its western border ridge extended to New Caledonia but suggested, that fossil and geological evidence was not sufficient to determine if the ridge was entirely above the sea level.

The flora

The fossil record of the Late Permian – Early Triassic transition indicates a major extinction event. More than 80% of marine genera known from the Permian disappeared and with them went terrestrial taxa such as *Glossopteris* (Anderson *et al.*, 1999; McLoughlin, 2001). The *Dicroidium* flora succeeded the *Glossopteris* flora. As in the Permian, some taxa, including *Dicroidium* species, were widespread indicating only weak provincialism over the Gondwanan super continent (McLoughlin, 2001). However, in the Permian a floristic difference between the northern margins of Gondwana and the southern regions of the supercontinent started to develop that can clearly be identified in Triassic spore-pollen assemblages. Northern parts of Gondwana that were flanking the Tethys Ocean accommodated "Onslow-type" palynofloras that contained Tethyan (equatorial) elements whereas the southern areas were home to the "Ipswich-type" assemblages of plants that were adapted to cooler, seasonal climates (Foster *et al.*, 1994; McLoughlin, 2001). The restructured floras of the Australian/New Zealand sector of Gondwana clearly represent the "Ipswich-type". These were now dominated by early Podocarpaceae (i.e. *Mataia podocarpoides*). Associated taxa included *Linguifolium,* which is also known from Chile and Australia (Retallack, 1980), the fern *Cladophlebis,* which had an almost world wide distribution from the Late Triassic (Rhaetian, 204 – 200 mya) to the Jurassic, as well as *Sphenobaieria* (Ginkoales), *Araucarites* and *Dicroidium odontopteroides* (Oliver, 1950; Fleming, 1979). Further taxa known from the Rhaetian include: *Phyllotheca* (Equisetalian genus), *Chiropteris* (fern-like), *Baiera* (Ginkoales), *Dictyophyllum* (fern), *Thinnfeldia lancifolia* (*Thinnfeldia* is sometimes assigned to *Dicroidium*, however this classification is controversial (Olivier, 1950)), *Sphenopteris otagoensis* and *Sphenoteris owakaensis*,

Taeniopteris spatulata, *Taeniopteris thomsoniana*, *Elatocladus* (resembling Podocarp-typ), and *Brachyphyllum* (Araucariaceae) (Oliver, 1950).

2.4 The Jurassic (200 – 136 mya)

Geology and tectonics

The Jurassic of New Zealand is marked by similar geological conditions as in the Triassic. Gondwana still occupied middle to high latitudes (30°-80°). Only northern South America and northern Africa extended to the tropics. The absence of red-beds, the presence of local coal deposits, and the lack of evidence for glaciation indicates a generally warm climate (Anderson *et al*., 1999; McLoughlin, 2001).

The flora

The Late Triassic – Early Jurassic transition was marked by a major extinction event. This reduced the Gondwanan fauna and flora and led to a worldwide floristic turnover at the end of the Triassic (Fleming, 1979; Anderson *et al*., 1999). *Dicroidium*, which dominated floras across the Southern Hemisphere in the Triassic, went extinct in the Rhaetian (204 – 200 mya). Jurassic Gondwanan floras were distinct from contemporary Northern Hemisphere plant communities, and were characterised by regional differentiation (McLoughlin, 2001). Philippe *et al*., (2004) subdivide Jurassic Gondwana into five different climatic zones (warm temperate, summer wet, winter wet, desert and cool temperate) based on the analyses of the distribution of fossil wood genera. The authors assign New Zealand to the cool temperate zone, which was characterised by a low diversity of fossil woods.

Despite the worldwide floristic turnover connected to the Late Triassic extinction event, Jurassic New Zealand floras appear to be similar to its Triassic vegetation with characteristic plants such as *Mataia podocarpoides* and *Cladophlebis* still present in the fossil record (Oliver, 1950; Fleming, 1979).

Jurassic New Zealand plants identified from the Curio Bay petrified forest are *Osmundites* (fern), *Araucarioxylon* (Pole, 1999) and *Lycopodites* (Oliver, 1950). Macrofossils identified from the Late Jurassic of Waikato Heads include *Palissya*, *Coniopteris* (fern), *Taenioteris spatulata* (possibly a Cycad), *Pterophyllum* (Cycad), *Elatocladus plana* (Gymnosperm), *Equisetites*, and *Carnoconites* (Oliver, 1950; Fleming, 1979). Alford forest, an Early Jurassic site in Canterbury, revealed: tree ferns,

including *Cyathidites;* ferns, including *Todisporites*, *Osmundacidites*; and conifers, including *Callialasporites*, *Araucariacites,* and *Classopollis* (Fleming, 1979). In addition, Oliver (1950) reports the appearance of *Artocarpidium*, *Dictyophyllum*, *Linguifolium*, *Thinnfeldia*, and *Sphenopteris* in the fossil record of New Zealand. *Artocarpidium arberi* was thought to represent the first angiosperm in New Zealand's fossil record and was described from the Waikato Heads beds, which were assigned Late Jurassic age (Oliver, 1950). However, this significantly predates the first widely accepted evidence of angiosperm pollen which was described from the Hauterivian (130 – 122 mya) of southern England and Israel (Brenner, 1984; Hughes and McDougall, 1987). Oliver's fossil is not confirmed by later authors.

2.5 The Cretaceous (136 – 65 mya)

2.5.1 Early Cretaceous (136 – 100 mya)

Geology and tectonics

In the Cretaceous several events influenced the further development of the New Zealand fauna and flora. In the Early Cretaceous the Rangitata Orogeny reached its climax and initiated the disruption of Gondwana in the New Zealand sector. As a result the sediments which had accumulated in the New Zealand Geosyncline formed a mountainous landscape by the early Late Cretaceous (Ballance, 1993). During the Early Cretaceous, New Zealand is thought to have gone through a period of maximum land extension (Fleming, 1979; Mildenhall, 1980). Palaeomagnetic data suggests that during this time New Zealand lay well within the Palaeo-Antarctic circle (Grunow, 1999).

The flora

Despite the geographic location of New Zealand in the Early Cretaceous, vegetation structure and morphology indicate an annual mean temperature of approximately 10°C (Parrish *et al.*, 1998). The fern *Gleichenia* and the podocarp *Microcachrys* are among the extant plants that first appeared in the fossil record of the Early Cretaceous. Besides these extant plants, typically Mesozoic elements, including the ferns *Coniopteris* and *Cladophlebis,* persisted throughout this time (Fleming, 1979)

New Zealand forests were still dominated by Gymnosperms (especially podocarps and araucarians) and ferns, but angiosperms started to diversify and gain abundance (Crane and Litgard, 1989). Early New Zealand angiosperm taxa such as *Tricolpites*

pannosus (Gunneraceae), *Phimopollenites augathellaensis*, and *Liliacidites peroreticulatus* (Liliaceae) are reported from the Albian (109 – 100 mya) (Mildenhall, 1980). Parrish *et al.* (1998) reported the finding of two types of *Agathis* macrofossils from the Middle Clarence Valley near Kaikoura: *Agathis clarencianum* and *Agathis seymouricum*. *Agathis clarencianum* was regarded by the authors as being deciduous. It formed monotypic assemblages in swampy areas. *Agathis seymouricum* was considered by Parrish *et al.* (1998) to be very similar to recent *Agathis australis* in morphology and ecology. From these findings Stöckler *et al.* (2002) concluded that it is a potential member of the same lineage as extant *Agathis australis*. However, the taxonomic status of both fossils is controversial (Pole, pers. com, 2006; see Chapter 4). These Araucariaceae were associated with *Cladophlebis australis*, *Ginkgo*, several podocarp species, cycads (*Nilssonia*), bennettites (*Pterophyllum, Nilssoniopteris, Ptilophyllum, Williamsonia*), pentoxyleans (*Taeniopteris*), and dicot angiosperms (*Archaepetala*, *Carpolithus*) (Fleming, 1979; Parrish *et al.*, 1998).

2.5.2 Late Cretaceous (100 – 65 mya)

Geology and tectonics

In the Late Cretaceous heavy erosion reduced the Rangitata mountains to a peneplain. New Zealand was separated from Australia by the Tasman Sea that started to open about 80 mya, and from Antarctica by the early South West Pacific Ocean that opened at about the same time (Fleming, 1979; Figure 2.3). New Zealand was still positioned in high southern latitudes and the fauna and flora was subject to long winter nights and relatively low temperatures (Mildenhall, 1980).

The flora

Among the first abundant angiosperms reported from the Late Cretaceous were *Nothofagus* (Nothofagaceae) which first appeared in the Early Campanian (83 mya) off the present day coast of south eastern Australia (Dettmann *et al.*, 1990). These early *Nothofagaceae* were of the ancestral pollen type and have been assigned to the fossil species *Nothofagidites senectus* (only found in Australia) and *N. kaitangataensis* (found in both Australia and New Zealand) (McGlone *et al.*, 1996). By the Late Cretaceous, further elements of the present day flora of New Zealand such as *Lycopodium* (*fastigiatum-volubile* group), *Hymenophyllum*, *Blechnum*, *Dicksonia*, *Cyathea*, *Adiantum*, *Pteris*, *Podocarpus*, *Dacrydium cupressinum, Dacrydium* aff. *franklinii,* and *Knightia* had appeared in the pollen fossil record (Mildenhall, 1980). On the family level Chloranthaceae, Loranthaceae, Caryophyllaceae, and Winteraceae

were recorded for the first time (Mildenhall, 1980). Other taxa including *Beilschmiedia*, *Athrotaxis* (Taxodiaceae, genus today restricted to Tasmania), *Cinnamomum* (Lauraceae), *Dryandra* (Proteaceae) (controversial according to Mildenhall (1972)), *Ripogonum*, *Acer,* and *Betula* were not present in the pollen fossil record but appeared as macrofossils (Fleming, 1979; Pole, 1992).

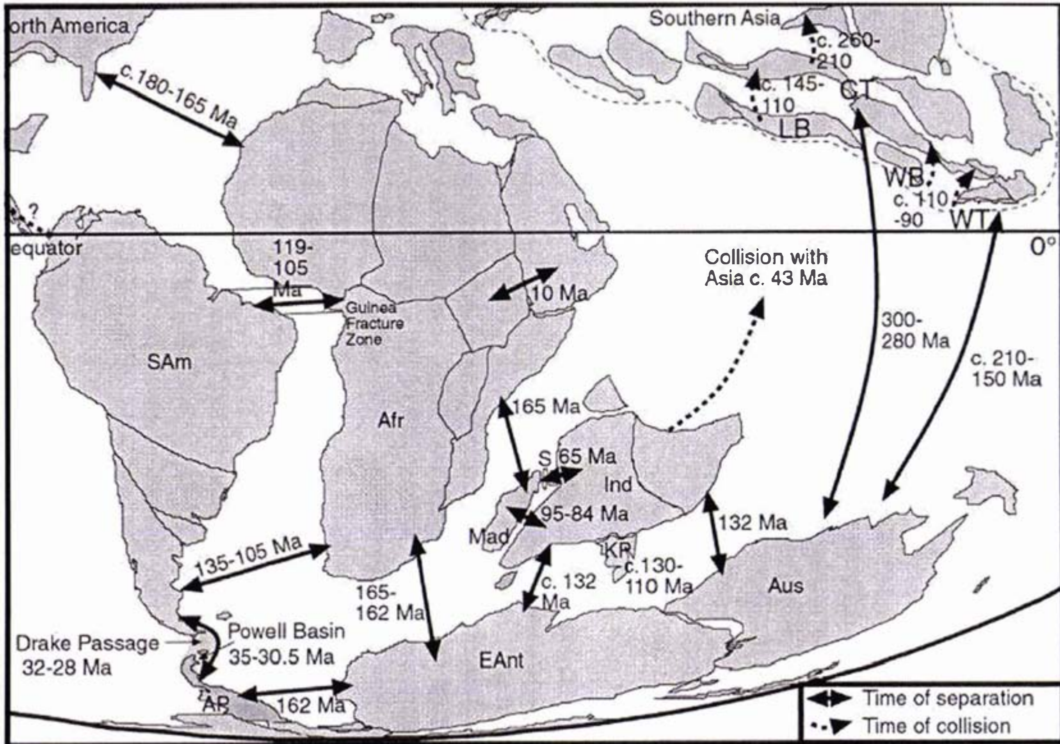

Figure 2.3: Gondwana at the end of the Cretaceous (from: McLoughlin, 2001)

2.6 The Tertiary (65 – 1.8 mya)

By the beginning of the Tertiary 65 mya the Tasman Sea had almost reached its present day size and New Zealand had drifted about 1500 km away from the shelf of West Antarctica. New Zealand continued to drift until about 21 mya but at a slower rate than in the Late Cretaceous. The Cenozoic of New Zealand was characterised by frequent geographical changes and repeated marine transgressions (Fleming, 1979).

2.6.1 Palaeocene (65 – 54 mya)

Geology and tectonics

The geographical structure of the New Zealand landmass remained stabile during the Palaeocene and into the Early Eocene (Kamp, 1986; Figure 2.4). However, during this period first marine transgressions occurred (Fleming, 1979).

Evidence from oxygen isotope analyses suggests that temperatures rose during the Palaeocene to reach a tropical peak in the Early to Mid-Eocene (Fleming, 1979, Figure 2.5). This hypothesis is consistent with the appearance of *Mauritia* (Palmae) and other palms in the New Zealand fossil record (Mildenhall, 1980) and would have also supported the observed diversification of Proteaceae (Fleming, 1979)

The flora

Although angiosperms had already gained abundance, pollen-producing sediments of Palaeocene age indicate that the podocarps remained the dominating plants in New Zealand forests (Mildenhall, 1980). At the Cretaceous/Palaeocene boundary the first pollen of the *Nothofagus* subgenus *Fuscospora* appeared in the New Zealand fossil record (see also Chapter 3). These Nothofagaceae fossil species included *Nothofagidites waipawaensis*, *N. brachyspinulosus, N. lachlaniae* and *N. flemingii* (McGlone *et al.*, 1996). Further first appearances reported from Palaeocene New Zealand include myrtaceous pollen similar to those of *Leptospermum* and *Metrosideros* as well as pollen of *Casuarina*, a genus today confined to tropical and subtropical climate (*Halorhagaciidites*) (Mildenhall, 1980). Mildenhall also reports the pollen of *Dilwynites granulatus* (which has recently been attributed to the extant Araucariaceae genus *Wollemia*) from the Palaeocene fossil record (Dettmann and Jarzen, 2000; see also Chapter 4).

Further taxa are known from the macrofossil record. Pole (1998) reported *Libocedrus* (Cupressaceae) and *Prumnopitys* (Podocarpaceae) fossils from Mount Somers coal mine. These are the oldest macrofossil records of a conifer genus extant in New Zealand.

Figure 2.4: The outline of the New Zealand Archipelago in the Palaeocene (modified from Suggate *et al.*, 1978)

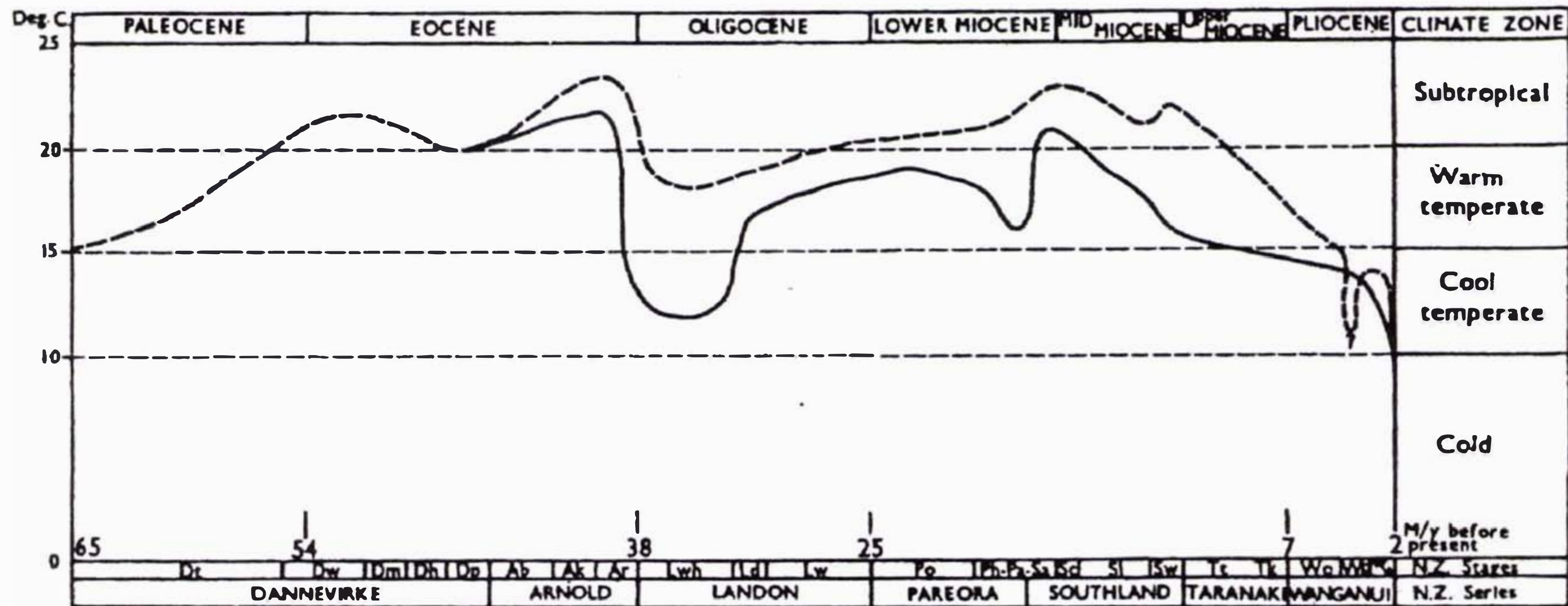

Figure 2.5: New Zealand Cenozoic climates shown on an absolute time-scale. Temperatures from oxygen isotope analyses by Devereux (1967) normalized to the latitude of Wellington (41°S) are shown by a solid line, those estimated from palaeontological evidence, at the same latitude, by a dashed line. (from Hornibrook and Hornibrook, 1971)

2.6.2 Eocene (54 – 38 mya)

Geology and tectonics

In the Eocene the sea continued to transgress on the land. By the Mid Eocene it had flooded over lowlands near Greymouth and subsequently drowned parts of western Nelson. A small isthmus connected an Early Tertiary North Island with the South Island (Figure 2.6). The west coast of the North Island was much further west than today while the East Coast and parts of Northland were submerged. (Fleming, 1979)

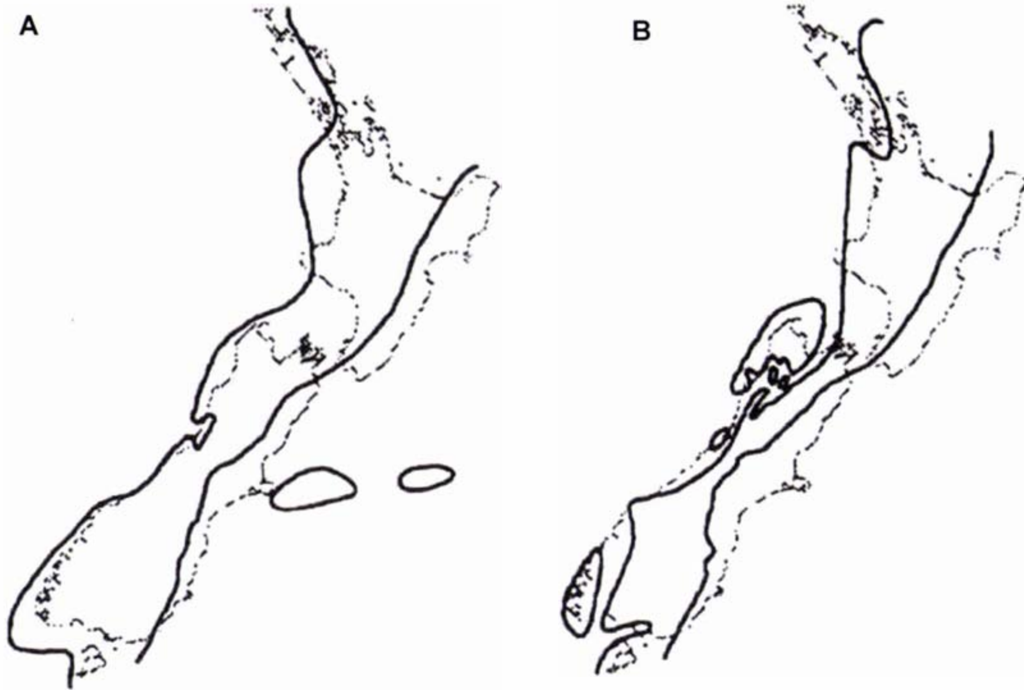

Figure 2.6: The outline of the New Zealand Archipelago in the (A) Mid – (B) Late Eocene (modified from Suggate *et al.*, 1978)

The climate reached a tropical peak in the Early to Mid- Eocene (Figure 2.4), supporting the establishment of Malayo-Pacific and Australian immigrants (Hornibrook and Hornibrook, 1971; Fleming, 1979)

The flora

First pollen of the *Nothofagus* subgenus *Lophozonia* appeared in the Early Eocene, represented by *Nothofagidites asperus* and later *N. suggatei* (McGlone *et al.*, 1996). While the former shows strong similarities to extant *Lophozonia* pollen, the characteristics of the latter are somewhat ambiguous. Dettmann *et al.* (1990) argue that *N. suggatei* pollen could alternatively be poorly preserved *Brassospora* pollen.

In the following period of approximately 8 million years, *Nothofagus* pollen is absent from virtually all New Zealand fossil sites (Pole, 2001). In the Mid-Late Eocene *Brassospora* pollen appeared in the New Zealand fossil record (McGlone *et al.*, 1996; Hill, 2001) Possibly profiting from the moderate temperatures, low variability, and high humidity similar to present day New Guinean mountain forests *Brassospora* beeches evolved into many species of which *N. matauraensis*, *N. emarcidus*, *N. cranwelliae*, *N. vansteenisii*, *N. falcatus*, *N. spinosus, N. longispinosus* and *N. deminutus* have so far been described (McGlone *et al.*, 1996). As a result of this rapid speciation *Brassospora* replaced podocarps and *Casuarina* as dominant trees, marking the most important vegetational change of that time (Pocknall, 1989). With cooling climates in the Late Eocene *Fuscospora* beeches started to gain abundance in the South Island (Fleming, 1979).

Hill (2001) lists several Eocene fossil *Nothofagus* pollen species that are common to south-eastern Australia and New Zealand and all appear first in the Australian, and only later in the New Zealand, fossil record. Among these are *N. asperus* (*Lophozonia*) which had its first record in Australia in the Palaeocene while it was only reported from Late Eocene layers of New Zealand and *N. flemingii* (*Nothofagus*) which was first recorded from the Campanian of Australia and the Mid Eocene of New Zealand. As the Tasman Sea had already reached its present day extent at that time, these examples suggest long-distance dispersal of *Nothofagus* from Australia to New Zealand (McGlone *et al.*, 1996).

The extant conifers *Phyllocladus* (which had been reported from Cretaceous deposits of Australia, the Indian Ocean, South America, and West Antarctica) and *Dacrycarpus* were reported for the first time from the Eocene New Zealand pollen fossil record (Mildenhall, 1980). Further pollen representing Eocene New Zealand genera are Malayo-Pacific elements such as *Beauprea* (Proteaceae), *Quintinia* (Cunoniaceae), *Spinizonocolpites* (Nipa-like mangrove palms), *Anacolosa*, *Banksiaeidites* (representing the Proteaceae *Banksia* or *Dryandra* which are restricted to Australia today), *Cupania* (Sapindaceae, tribe Cupaniae, today restricted to New Guinea, New Caledonia and Queensland), *Dysoxylum*, *Rhopalostylis* (Palmae). The New Zealand endemic *Phormium* (Agavaceae) and *Dracophyllum* (Epacridaceae) which today has its centre of diversity in New Zealand were also reported for the first time (Fleming, 1979). Moist swampland environments hosted *Sphagnum* mosses, Liliaceae, *Gunnera* and Onagraceae (*Jussiaea*) (Pocknall, 1989).

Among the taxa only determined to the family level, Myrtaceae were frequent but not abundant in the Eocene of New Zealand. Pollen resembling that of Euphorbiaceae and early Araliaceae first appeared in the Early Eocene (Fleming, 1979)

The genera *Ripogonum*, *Cinnamomum*, *Acer, and Beilschmiedia* are only reported as macrofossils and are already known from the Late Cretaceous (Oliver, 1950). Macrofossils reported for the first time include the ferns *Blechnum priscum*, *Cyclosorus cretaceo-zeelandicus*, *Sticherus obscurus* and *Pteris pterioides* as well as the conifers *Agathis lanceolata*, *Dacrydium praecupressinum*, *Podocarpus cupressinum*, *P. maitai*, *P. parkeri*, *P. praedacrydioides,* and Aquifoliaceae (*Ilex*/ holly). Mildenhall (1980) reports the monocotyledons *Freycinetia*, *Astelia,* and *Typha* as present in the Eocene whereas according to Fleming (1979) these genera cannot be found before the Late Oligocene.

Antarctic sediments of the same age contain pollen evidence for temperate forests with a dominance of *Fuscospora* beeches with *Brassospora* beeches, Podocarpaceae, Araucariaceae, Myrtaceae, Proteaceae and *Casuarina* as further elements. This remarkable similarity between Early Tertiary Antarctic and New Zealand floras can be interpreted as common inheritance from Gondwana. However another possibility is a more or less frequent gene flow between the two former fragments of the old supercontinent by means of long-distance dispersal.

2.6.3 Oligocene (38 – 26 mya)

Geology and tectonics

The extent of the "Oligocene drowning" that submerged at least most of the New Zealand landmass is still controversial (Pole, 1994; Pole, 2001; Stöckler *et al.*, 2002; Waters and Craw, 2006; see Chapter 4). If not completely submerged (Figure 2.7), New Zealand was reduced to several small islands (Hickson *et al.* 2000). Varying sea levels would have changed the size of non-submerged land throughout the Oligocene (Cooper and Cooper, 1995).

Figure 2.7: The outline of the New Zealand Archipelago in the Mid Oligocene (modified from Suggate *et al.*, 1978)

As Australia separated from Antarctica the circum-polar oceanic currents were established which led to increasing cool westerly winds at the latitude of New Zealand (Prothero, 1994). These winds caused increasing precipitation in New Zealand (Mildenhall, 1980).They might also have contributed to the sudden increase in new, mainly temperate plant taxa (Mildenhall, 1980). The new climate was significantly colder than in the Eocene but possibly warmer than today (Fleming, 1979, Prothero, 1994).

The flora

Oligocene vegetation was uniform all over New Zealand due to its narrow latitudinal range (Crook and Belbin, 1978). Differentiation only began after New Zealand extended its range in the Late Oligocene. The moist climate was optimal for *Brassospora* beeches that dominated Early Oligocene floras and probably reached

their diversity peak. *Casuarina* also profited from this climate. These two taxa were still associated with *Beilschmiedia* (Lauraceae), *Santalum*, various Myrtaceae, Podocarpaceae and Araucariaceae (Fleming, 1979). According to Pocknall (1989) further common families were Sapindaceae, Tiliaceae and. Cyathaceae. *Dacrydium franklinii* was of local importance. Proteaceae (*Isopopogon, Xylomelum, Symphonema, and Beauprea*) were locally common but never as abundant as in the Late Eocene. Palms were common (Fleming, 1979; Pocknall, 1989).

Genera that were first reported from the pollen fossil record of the Oligocene included the genera *Histiopteris*, *Paesia*, *Pteridium* (all Pteridaceae), *Elaeocarpus*, *Myriophyllum*, *Myrsine*, *Fuchsia*, *Laurelia*, and *Coprosma*. *Weinmannia*, an austral genus, is reported by Mildenhall (1980) as first appearing in the Oligocene whereas Fleming (1979) reports the first appearance of the genus from the Early Miocene. Pocknall (1989) reports the genus *Epilobium* from the Late Oligocene but Oliver (1950) only lists it from the Pliocene. New taxa only determined to the family level were Sparganiaceae, Cyperaceae, Juncaceae, Restionaceae, and Asteraceae (Fleming, 1979, Mildenhall, 1980).

Plant macrofossils from the Oligocene include *Athrotaxis*, *Coprosma, Euphorbia, Pomaderis*, *Pittosporum*, *Carpodetus*, *Heimerliodendron, Geniostoma, Rubus*, *Clematis* and *Hoheria* (Oliver, 1950; Fleming, 1979).

2.6.4 Miocene (26 – 5 mya)

2.6.4.1 Early Miocene (26 – 16 mya)

Geology and tectonics

In the Early Miocene an increasing topographic relief caused the withdrawal of the sea from areas that were flooded in the Oligocene. Volcanic activity formed rocks around Coromandel, Great Barrier and Whangarei. However, many regions of the present day New Zealand were flooded in the Early Miocene including the East Cape, north-west Nelson, Canterbury and Fiordland (Fleming, 1979; Figure 2.8).

Figure 2.8: The outline of the New Zealand Archipelago in the Early Miocene (modified from Suggate *et al.*, 1978)

By the beginning of the Miocene New Zealand had drifted far enough north to be under subtropical influence, resulting in an increasing temperature compared to the Oligocene. Marine temperatures reached an average of up to 20°C, 5-7°C warmer than today (Fleming, 1979).

The flora

Forests of the Early Miocene were characterised by *Brassospora* beeches, accompanied by *Casuarina* and in some places by *Eucalyptus* (Campbell, 1985; Pole, 1989). *Fuscospora* beeches, which are characteristic for a more temperate climate, dominated locally in Otago, suggesting latitudinal gradients. Macrofossils possibly representing two *Lophozonia* species *(N. azureus, N. novaezealandiae*) are also known from the southern South Island. In total there were possibly tens of *Nothofagus* species, mostly *Brassospora*, present in New Zealand at that time (McGlone *et al.*, 1996). Higher terrain in Antarctica was already glaciated but coastal regions supported

Nothofagus forests and could still have served as "stepping stones" for vegetation between South America and Australia or New Zealand (Kemp, 1975).

Plants which had their first fossil record in the Early Miocene are *Schizea cf. fistulosa*, *Trichomanes*, *Arthropteris tenella*, *Pteridium aquilinum*, *Histiopteris incisa* (all ferns), Podocarpaceae of the *Dacrydium kirkii-bidwillii* group, *Podocarpus spicatus* and the flowering plants *Geniostoma*, *Macropiper*, *Alectryon*, *Avicennia*, *Cordyline, Corynocarpus* (all Malayo-Pacific elements) *Muehlenbeckia*, *Griselina* (Austral elements), *Coriaria* (Eurasia, New Zealand, South America), *Aristotelia* (Australia, New Hebrides, South America), *Myriophyllum*, *Hydrocotyle*, *Potamogeton, Drosera* (all Cosmopolitan), and *Melicytus cf. ramiflorus* (New Zealand endemic) (Fleming, 1979; Mildenhall, 1980; Campbell, 2002). Pole (1996) also reported the first appearance of the families Euphorbiaceae, Myrsinaceae, and Smilaceae from the Foulden Hills Diatomite in Central Otago.

2.6.4.2 Mid and Late Miocene (16 – 5 mya)

Geology and tectonics

The Mid and Late Miocene brought a further emergence of land. The sea had completely withdrawn from Northland, while East Cape, Taranaki, and Canterbury remained submerged (Figure 2.9).

The flora

The dominance of *Brassospora* continued while *Fuscospora* beeches, Podocarpaceae and ferns as representatives of temperate climate became rare in the Mid Miocene and only regained abundance as temperatures slightly decreased in the Late Miocene (Fleming, 1979).

In the Late Miocene *Fuscospora* beeches and Podocarpaceae mostly dominated over *Brassospora* beeches and *Casuarina* as colder conditions changed the vegetation (McGlone *et al.*, 1996). The general *Nothofagus* diversity decreased as *Fuscospora* became more abundant but not more diverse.

The Late Miocene is also characterised by a significant rise in herbaceous taxa (Mildenhall, 1980). Spores resembling *Lindsaya cuneata*, *Nephrolepis cordifolia* and *Rumohra adiantiformis* (all ferns) are recorded for the first time. New taxa only known

from macrofossils are *Libocedrus plumosa*, *Pittosporum*, *Neopanax*, *Geniostoma* and *Senecio* (Fleming, 1979).

By the end of the Miocene a number of characteristic Tertiary plants have become extinct in New Zealand (Fleming, 1979). Examples are *Ilex* (Aquifoliaceae), which had been known in New Zealand from the Eocene on, *Canacomyria* (Myricaceae), which can today be found in New Caledonia, and *Dacrydium franklinii*, a present day endemic of Tasmania. (Mildenhall, 1980)

Figure 2.9: The outline of the New Zealand Archipelago in the Late Miocene (modified from Suggate *et al.*, 1978)

2.6.5 Pliocene (5 – 1.8 mya)

Geology and tectonics

In the Pliocene most parts of the present day South Island had emerged while most of the southern third of the North Island was below the sea (McGlone, 1985, McGlone *et al.*, 2001). Northland was split into several islands separated by the Ahipara Strait in the north and the Manukau Strait which submerged the Auckland Isthmus (Figure 2.10).

Earth movements intensified resulting in the climax of the Kaikoura Orogeny. Batt *et al.* (2000) concluded from the analyses of the isotopic ages of material exposed close to the Alpine fault in the Southern Alps that the rapid uplift of the Southern Alps began approximately 5 mya. The mountains along the East Cape – Fjordland axis that today form the backbone of New Zealand were the centre of tectonic activity (Walcott, 1979, 1998). The volcanic activity of that time was centred around Northland and Coromandel as well as the western side of the North Island, where the volcanoes of Orangiwharo and Whareorino were active (Fleming, 1979). New Zealand occupied the same latitude as today. Fluctuations of vegetation and climate had already started in the Late Miocene, but they became more rapid during the Pliocene (McGlone *et al.*, 1996).

The flora

As a result of an emerging relief, a flora with both cool and warm loving elements developed in the early Pliocene. In the north of the North Island, *Brassospora* beeches dominated over *Fuscospora* beeches. Other forest pollen types were rare. Further south, temperate plants such as *Fuscospora* beeches, *Dacrydium cupressinum*, *Cyathea* ferns, and Myrtaceae became more abundant. For the last time palms existed as major elements of the forest flora (Mildenhall, 1980).

Indicators for climatic conditions in the Mid Pliocene are equivocal. A wide range of floral characteristics indicate a period of environmental change. The most widespread vegetation type was a podocarp-beech forest with accompanying cool temperate taxa. Some fossil floras from the northern South Island contain abundant representatives of grassland such as Apiaceae, Asteraceae, Cyperaceae, and the genus *Euphrasia*, suggesting periglacial conditions with grassland periodically reaching down to sea level (Mildenhall, 1980; McGlone, 1985). Nevertheless genera that are adapted to a warm temperate climate such as *Dysoxylum*, *Knightia, Agathis,* and others were found south of their present distribution (Fleming, 1979; McGlone, 1985).

Figure 2.10: The outline of the New Zealand Archipelago in the Early Pliocene (modified from Suggate *et al*., 1978)

This floral diversity continued into the Late Pliocene. Podocarp-beech forests in the North were co-dominated by *Brassospora* and *Fuscospora* beeches. Towards the end of the Pliocene the *Brassospora* were replaced by Myrtaceae. The last New Zealand *Brassospora* probably died out about 2 mya (McGlone *et al*., 1996). Of a Mid Tertiary maximum of eleven different *Nothofagus* pollen taxa, only the two extant ones (*Lophozonia* and *Fuscospora*) were left in New Zealand at the end of the Tertiary (McGlone *et al*., 1996).

Oliver (1950) reports broad similarity of Pliocene and present day floras. Of 30 genera found in the Waipaoa Pliocene flora from Poverty Bay, only two taxa, *Platycerium* and *Apocynophyllum*, cannot be found in New Zealand today. Among the genera which were reported from the New Zealand fossil record for the first time were

Grammitis, *Todea*, *Arthropodium*, *Callitriche*, *Colobanthus*, *Chenopodium*, *Salicornia*, *Cardamine*, *Gentiana*, *Eugenia*, *Rubus*, *Dodonea*, *Hebe*, and *Pimelea* (Mildenhall, 1980). Tropical and subtropical taxa such as *Bombax*, *Ephedra*, *Zygogynum* and, as mentioned above, *Brassospora* beeches disappeared due to the cooling climate (Mildenhall, 1980).

2.7 The Quaternary (1.8 mya – present)

2.7.1 Pleistocene (1.8 mya – 10,000 BP)

2.7.1.1 Early and Mid Pleistocene (1.8 mya – 15,000 BP)

Geology and tectonics

The Pleistocene is marked by a succession of several glacial and interglacial periods. The annual mean temperatures during the last glacial maximum were about 4-5°C below those of the present day and there was almost certainly less precipitation (McGlone, 1983; Ogden, 1989; Suggate, 1990; McGlone *et al.*, 1996,). The sea level during the last glacial maximum was 100 – 120 m below the present day sea level (McGlone, 1985). All three New Zealand main islands were connected (Figure 2.11).

The flora

Periods of severe glacial climates eliminated almost all remaining tropical elements in the New Zealand flora. The conifers *Microstrobos* and *Microcachrys*, now restricted to Australia, died out, *Cranwellia*, *Polycolpites reticulatus* and *Acacia* became extinct as well. The Proteaceae were reduced to two species, *Knightia excelsa* and *Persoonia toru.* Alpine plants extended their range with every glacial period while lowland plants were forced into refugia or became locally extinct. In interglacials, some elements of the forest flora expanded their range and occupied most of the landscape (McGlone, 1985; Ogden, 1989; McGlone *et al.*, 1996). Various authors have reported Pleistocene pollen sequences from different sites. Taken together, their findings suggest that the earlier uniform floras have been replaced by a wide range of floral assemblages.

Broader geological and fossil data is mostly only available for the last glacial – interglacial cycle (McGlone, 1985; Wardle, 1988; Suggate, 1990). However, one long pollen sequence of the last 300,000 years is known from Petone Borehole, Wellington (Mildenhall, 1995; McGlone *et al.*, 1996). It shows that *Nothofagus* at this site reached

its lowest abundance during glacial and interglacial maxima while it was dominant during the phases in between.

Figure 2.10: The outline of the New Zealand Archipelago in the Late Pleistocene (Late Otira Glacial) (modified from Suggate *et al.*, 1978)

Pollen sequences are known from the Kaihinu interglacial (penultimate interglacial, 120,000 – 80,000 BP (before present: radiocarbon years before 1950 AD)) from several sites in New Zealand. Pollen data from the west coast of the North Island indicate podocarp forests dominated by *Prumnopitys taxifolia* while mixed conifer hardwood forests (*Libocedrus, Dacrydium cupressinum* and *Nothofagus*) were reported from the east coast. *Nothofagus* was abundant in forests on the central volcanic plateau (McGlone and Topping, 1983; McGlone *et al.*, 1984; McGlone, 1989).

Moar and Suggate (1979) describe pollen sequences from the Kaihinu interglacial to present from several sites in the Westport District, South Island. Kaihinuan and postglacial Aranuian sequences are characterised by either *Nothofagus* or *Dacrydium cupressinum* forests. The composition of *Nothofagus* forests was variable. *Fuscospora* beech assemblages grew together with *Metrosideros*, *Weinmannia*, *Cyathea*, and *Dicksonia* while *Nothofagus menziesii* was associated with *Coprosma*, *Myrsine*, *Nestegis* and *Quintinia*. The *Dacrydium* forests of the west cost of the South Island were similar to extant podocarp forests. On the east coast of the South Island podocarp forests were characterised by *Prumnopitys taxifolia* or *Dacrycarpus dacrydioides* and frost-tender taxa like *Dodonaea* and *Passiflora* (Moar and Suggate, 1996).

The Otira glaciation commenced about 80,000 BP and lasted until 14,000 BP. Newnham (1992) reports a 30,000 year pollen sequence from Northland. This sequence from Otakairangi Swamp near Hikurangi indicates a warm temperate mixed conifer-angiosperm forest with *Agathis*, *Fuscospora* beech, *Dacrydium*, *Libocedrus*, *Prumnopitys* and *Leptospermum* as abundant elements. Further taxa reported from this forest assemblage are *Dracophyllum*, *Ascarina*, *Cyathea*, *Phyllocladus,* and *Metrosideros*. While the abundance of the different taxa changed frequently, a general warm temperate forest assemblage remained the predominant vegetation type throughout the Otira glaciation. At the same time this assemblage is very similar to the post-glacial pre-human vegetation of Northland. This is rather unusual as most of the New Zealand flora, especially in higher altitudes and latitudes, changed significantly in post-glacial times (Newnham, 1992).

Mildenhall (1994) analysed pollen samples from a drill hole at Mangaroa/ Upper Hutt Valley that possibly cover most of the Otirian. These samples indicate scrub and grassland with potential isolated forest stands in favoured locations. Tree taxa reported from this site are *Fuscospora* beech, *Nothofagus menziesii*, *Libocedrus,* and *Phyllocladus*. Scrubland taxa are represented by *Phyllocladus alpinus*, *Halocarpus*, *Myrsine*, *Dracophyllum,* and members of the Asteraceae family. Herbaceous taxa reported from the Upper Hutt Valley are *Acaena*, *Colobanthus*, *Epilobium*, *Gentiana*, *Geranium*, *Libertia*, Asteraceae, Portulacaceae, Ranunculaceae, and Poaceae (abundant) (Mildenhall, 1994). These assemblages are consistent with the assumption of a drier, windier, colder, and less humid climate than at the present time at this location. Similar assemblages have also been reported from the Tongariro region (McGlone and Topping, 1983) and the Waikato lowlands (Newnham *et al.*, 1989).

While scrub and grassland is reported as dominant vegetation of Otirian sites near Westport/ South Island, the existence of sheltered stands of *Nothofagus* is suggested by the abundance of *Nothofagus* pollen (Moar and Suggate, 1979). Similar vegetation has been described from the contemporary North Island south of Waikato. Scrubland was represented by *Phyllocladus, Halocarpus, Dacrydium bidwillii, Coprosma, Leptospermum,* and *Myrsine*. Otirian grassland at the described sites were characterised by Chenopodiaceae, *Cotula, Drosera arcturi, Epilobium, Gunnera, Hydrocotyle, Schizeilema, Oreomyrrhis, Plantago, Stellaria* and *Tillaea* (Moar and Suggate, 1979). The vegetation possibly changed during a slightly warmer interstadial before the last glacial maximum ~25,000 BP. From this time *Nothofagus menziesii* forest is recorded from Shantytown and Paroa in north Westland (Moar and Suggate, 1996). Further south the central west coast was probably completely denuded during the full glaciation as glaciers coalesced and reached the sea (Ogden, 1989).

Based on the findings of Moar and Suggate (1979), McGlone (1983) has suggested, that south of the Waikato Basin the main vegetation type of the last glacial maximum was scrub and grassland. Nevertheless, given the rapid post-glacial spread of forest, he also suggested that forest species survived the glacial maximum in small sheltered locations across New Zealand, including Southland. This view is held by many authors (i.e. McGlone and Topping, 1983; Newnham *et al.*, 1989; Moar and Suggate, 1996; Vandergoes *et al.*, 1997) and is of particular significance for understanding the extant distribution of *Fuscospora* beech in New Zealand. The survival of *Fuscospora* south of the central South Island beech gaps is regarded as problematic by some (Wardle, 1988) because temperatures in that area would have been slightly below the minimum temperature tolerance that *Fuscospora* show today (McGlone *et al.*, 1996).

2.7.1.2 Late Pleistocene (15,000 – 10,000 BP)

Geology and tectonics

About 15 000 BP, temperatures started to rise and glaciers retreated. It was not an uninterrupted rise of temperatures and ice advances in the South Island appeared after this date. The Northern Hemisphere glaciers melted and sea levels began to rise rapidly, separating North and South Island approximately 10,000 – 12,000 BP. The Foveaux Strait was flooded approximately 9500 years ago (Fleming, 1979; McGlone *et al.*, 1996).

The flora

After the beginning of the climatic amelioration forests recovered quickly although the climate was still 2-3°C colder than today and summer drought was common. The transition between scrub/grassland and forest probably took as little as 300 years (McGlone, 1983). Soon forests covered most of the lowland and lower montane regions of the northern and central North Island. These forests were mainly podocarp-hardwood communities dominated by *Prumnopitys taxifolia* (McGlone, 1983; Newnham *et al*., 1989; Vandergoes *et al*., 1997). *Nothofagus* responded slower than the podocarps. However, scattered stands were present. Other trees common were *Libocedrus bidwillii*, *Manoao colensoi*, *Phyllocladus alpinus* and *Halocarpus bidwillii*. The dominance of *Prumnopitys taxifolia* in early forest assemblages was possibly due to its ability to play a pioneering role (McGlone, 1983). Another factor might have been a competitive advantage over species like *Dacrydium cupressinum* because of its ability to tolerate frost and cope with the still relatively dry and cool climate. With further amelioration of the climate *Dacrydium cupressinum* forests replaced the *Prumnopitys taxifolia* forests (McGlone, 1983).

In the South Island, scrub and grassland continued to dominate until about 12,000 BP. At that time a dense scrub with *Phyllocladus alpinus*, *Halocarpus bidwillii*, *Coprosma* and *Myrsine* began to develop (Moar, 1971). The first real podocarp hardwood forests in the South Island with *Dacrydium cupressinum* as dominant plant were reported from Westport/ north western South Island. Further south and also in the drier eastern areas of the North Island scrub and grassland continued to dominate until about 10,000 BP (McGlone, 1983).

2.7.2 Holocene (10,000 years ago – present)

2.7.2.1 Early Post-Glacial (10,000 – 7,000 BP)

This period is referred to as the post-glacial climate optimum (Newnham *et al.*, 1989). There was an increase in temperature and rainfall. The temperature almost certainly exceeded the present day values (McGlone, 1983).

The period from 10,000 BP – 8,000 BP is considered the time of maximum post-glacial forest expansion. Almost all lowlands that had been occupied by scrub and grassland were taken over by forest vegetation. Conifer-broadleaf forests expanded their range rapidly. *Nothofagus* pollen percentages were at their lowest which might reflect their relative abundance compared to conifer-broadleaf taxa rather than their

absolute abundance (McGlone *et al.*, 1996). Only In central Otago forest development was slower than in the rest of the South Island because of the extremely dry conditions (McGlone *et al.*, 1995).

Western areas of the South Island were dominated by *Dacrydium cupressinum* forests which had developed in the late glacial while eastern areas were initially dominated by *Prumnopitys taxifolia - Dacrycarpus dacrydioides* or *Prumnopitys taxifolia - Podocarpus totara* assemblages, which were only slowly modified by expansion of *Dacrydium cupressinum* (McGlone, 1983).

At the same time angiosperms became more abundant in the North Island. Newnham *et al.* (1989) report an increasing amount of angiosperm genera such as *Nestegis, Metrosideros, Alectyron, Laurelia, and Elaeocarpus,* and tree ferns in the pollen record of three sites from the Waikato basin. *Ascarina* pollen curves peaked 10,000 – 9,000 BP. Due to the further amelioration of the climate, cool loving species such as *Libocedrus bidwillii* were reduced (McGlone, 1983).

2.7.2.2 Mid Post-Glacial (7,000 BP to 3,000 BP)

After the climate optimum at the end of the early Post-Glacial temperatures started to decrease, which led to vegetational changes. Especially in the northern South Island, where the establishment of the present day strong westerly airflow led to a cooler, wetter climate, *Nothofagus menziesii* became more common. Subsequently *Fuscospora* beeches spread rapidly at the expense of the podocarps (except for *Prumnopitys ferruginea*) (Dodson, 1978; Vandergoes *et al.*, 1997). Pure podocarp-hardwood forests were replaced by mixed beech-podocarp forests (McGlone, 1983). Exceptions to this New Zealand wide trend occurred in Northland, where *Fuscospora* abundance did not change much, and the southern South Island, where only *Nothofagus menziesii* became more common.

Podocarp-hardwood forest remained dominant in restricted, climatically favoured areas. For example Newnham *et al.* (1989) report findings from pollen sequences of the Waikato lowlands were increasing percentages of *Prumnopitys, Podocarpus, Phyllocladus,* and *Agathis* as well as a decline of *Ascarina* and *Cyathea* suggest a locally drier climate during this period.

2.7.2.3 Late Post-Glacial (3,000 BP to present)

The spread of *Nothofagus* species continued until about 2000 BP. During this period, *Fuscospora* forests extended their range in the south-western South Island and *Nothofagus menziesii* spread further in the uplands and interior of the South Island and in coastal south Westland (McGlone et al., 1996). The reason for the decreasing speed of *Nothofagus* range expansion approximately 2000 BP is controversial (McGlone, 1983; Haase, 1990; Leathwick, 1998; see Chapter 5). The New Zealand flora of that time was very similar to the extant vegetation. However forest was more widespread. Massive deforestation in New Zealand appears at 700 BP, and is attributed to human induced fires (Ogden *et al*., 1998).

3

The trans-oceanic relationships of the genus *Nothofagus**

Abstract

Nothofagus (Southern Beech), with an 80-million-year-old fossil record, has become iconic as a plant genus whose ancient Gondwanan relationships reach back into the Cretaceous era. Closely associated with Wegener's theory of "Kontinentaldrift", *Nothofagus* has been regarded as the 'key genus in plant biogeography. This paradigm has the New Zealand species as passengers on a Moa's Ark that rafted away from other landmasses following the breakup of Gondwana. An alternative explanation for the current transoceanic distribution of species seems almost inconceivable given that *Nothofagus* seeds are generally thought to be poorly suited for dispersal across large distances or oceans.

Here the Moa's Ark hypothesis is tested using relaxed molecular clock methods in the analysis of a 7.2- kilo base pair (kb) fragment of the chloroplast genome. These analyses provide the first unequivocal molecular clock evidence that, whilst some *Nothofagus* transoceanic distributions are consistent with vicariance, trans-Tasman Sea distributions can only be explained by long-distance dispersal. Thus, analyses presented here support the interpretation of an absence of *Lophozonia* and *Fuscospora* pollen types in the New Zealand Cretaceous fossil record as evidence for Tertiary dispersals of *Nothofagus* to New Zealand. Findings from this study contradict those from recent cladistic analyses of biogeographic data that have concluded transoceanic *Nothofagus* distributions can only be explained by vicariance events and subsequent extinction. They indicate that the biogeographic history of *Nothofagus* is more complex than envisaged under opposing polarised views expressed in the ongoing controversy over the relevance of dispersal and vicariance for explaining plant biodiversity. They provide motivation and justification for developing more complex hypotheses that seek to explain the origins of Southern Hemisphere biota.

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3.1 Introduction

An important principle of evolutionary inference is that explanations for the past require an understanding of mechanisms and processes applicable in the present (Lyell, 1970). It is perhaps this sticking point more than any other that has polarised views over the relative importance of vicariance and dispersal for explaining extant plant biodiversity. In 1912, Alfred Wegener put forward a testable hypothesis and mechanism that could explain the transoceanic distribution of animal and plant species. In the 21st century, with many DNA studies now implicating the importance of long-distance dispersal for explaining plant biodiversity (Carlquist, 1996; Renner *et al.*, 2000; Winkworth *et al.*, 2002; Zhang and Renner, 2003; Renner, 2004; Wagstaff, 2004; Cook and Crisp, 2005; Winkworth *et al.*, 2005; Waters and Craw, 2006; Cowie and Holland, 2006), it is disconcerting that there is currently a very poor understanding of the mechanisms of transoceanic dispersal (but see Close *et al.*, 1978; Carlquist, 1996; Hurr *et al.*, 1999; Muñoz *et al.*, 2004; Renner, 2004; Nathan, 2006; articles in "Diversity and Distributions" volume 11, number 2). Indeed, the inference that the seeds of extant *Nothofagus* species are not suited for dispersal across large distances has played a major role in motivating the hypothesis that transoceanic distributions of *Nothofagus* (Figure 3.1) can only be explained by vicariance (Preest, 1963; Craw *et al*, 1999; McCarthy, 2003; Sanmartin and Ronquist, 2004). This hypothesis posits that following the Cretaceous breakup of Gondwana, *Nothofagus* rafted and evolved *in situ* upon different Southern Hemisphere lands. Whilst very attractive, this hypothesis fits somewhat uncomfortably with the findings from analyses of morphological and molecular data. In particular, whilst earlier molecular data have been insufficient for rigorous molecular clock analyses, their interpretation has favoured hypotheses of transoceanic dispersal (Martin and Dowd, 1993; Hill and Jordan, 1993; Manos, 1997).

Based on the sequence of Gondwana breakup, a hypothesis of vicariance most parsimoniously predicts that Australian *Nothofagus* species should be most closely related to South American species. This follows since South America and Australia were connected via Antarctica until approximately 35 million years ago (mya) (Figure 3.1). In contrast, New Zealand is thought to have separated from Australia 80 mya (Wilford and Brown, 1994; McLoughlin, 2001). Thus to explain the close relationship between Australian and New Zealand species by vicariance, it is necessary to argue that extinction of Australian and/or closely related South American species has occurred (Linder and Crisp, 1995). Whilst this explanation is ad hoc, the fossil record does provide evidence for numerous *Nothofagus* extinctions in Australia, South

America, and New Zealand (Hill *et al.*, 1996; Markgraf *et al.*, 1996; McGlone *et al.*, 1996).

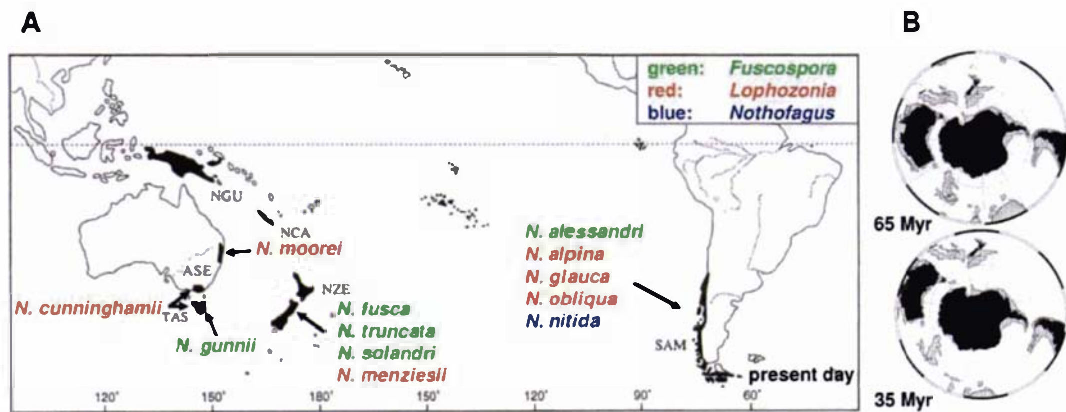


Figure 3.1: Southern Hemisphere maps and present-day *Nothofagus* distribution
(A) Transoceanic distribution of *Nothofagus* subspecies *Lophozonia* and *Fuscospora* and South American species *N. nitida* (subgenus *Nothofagus*). Map adapted from Swenson *et al.*, 2001. ASE, Australia; NCA, New Caledonia; NGU, New Guinea; NZE, New Zealand; SAM, South America; TAS, Tasmania. (B) Relationship of Australia, New Zealand, and South America 65 (Million years (Myr) and 35 Myr before present, reconstructed from http://www.odsn.de/ (link "Plate Tectonic Reconstructions").

However, the fossil record has also been interpreted as indicating multiple events of transoceanic dispersal of *Nothofagus* from Australia to New Zealand. Whilst the extinct "ancestral" *Nothofagus* pollen type occurred in New Zealand prior to the breakup of Gondwana, *Fuscospora* pollen first appeared in New Zealand during the Palaeocene (65 mya) and *Lophozonia* pollen first appeared during the Late Eocene (50 mya; Hill, 2001). Sixty-five mya the Tasman Sea had already reached its present-day size (Wilford and Brown, 1994; McLoughlin, 2001). Hence it is possible that extant New Zealand *Nothofagus* subgenera did not have the opportunity to reach New Zealand via overland migration. Hill (1984) has also described the species *Nothofagus cethanica*, which first appeared in Oligocene macrofossils from Tasmania. This species shares unique features with extant *N. fusca* and *N. truncata* from New Zealand and may share a sister relationship with these species explained by trans-Tasman Sea dispersal (Hill, 1991).

A contribution to the debate over the relative importance of vicariance and dispersal can be made by estimating the divergence times of extant species. However, DNA sequences of insufficient length have prevented robust molecular clock analyses from being undertaken. For this reason, this study reports the sequencing of a 7.2- kb

chloroplast genome fragment covering the gene regions (*trn*L–*trn*F and *atp*B–*psa*I) for 11 species of three *Nothofagus* subgenera (*Lophozonia*, *Fuscospora*, and *Nothofagus*). The aim has been to date divergence of extant species in the subgenera *Lophozonia* and *Fuscospora*. Relaxed molecular clock analyses have been carried out using the methods of Sanderson (2002; 2003) and Thorne *et al.* (1998). The findings are that, whilst vicariance is likely to explain some transoceanic relationships amongst *Nothofagus* species, phylogenetic relationships between trans-Tasman species in both *Lophozonia* and *Fuscospora* can only be explained by Mid to Late Tertiary transoceanic dispersal.

3.2 Methods

3.2.1 Sequence data

Chloroplast DNA sequences (7.2 kb comprising the *atp*B–*psa*I region and the *trn*L–*trn*F region; see Appendix III) were determined for each of 11 accessions of *Nothofagus* (subgenera or pollen groups *Lophozonia*, *Fuscospora*, and *Nothofagus*) sampled in South America, Australia, and New Zealand (see Appendix II) following the protocols described in Appendix I. These genome regions were also determined for *C. sativa* (an outgroup taxon from Fagaceae) and aligned using progressive multiple-sequence alignment: ClustalX version 1.81 (Thompson *et al.*, 1997). This resulted in an unambiguous alignment of 7,269 nucleotide sites. Data are missing for approximately 250 base pairs of the *atp*B gene and the *atp*B–*rbc*L intergene region of *Nothofagus*.

3.2.2 Tree building

Phylogenetic analyses were conducted with PAUP* version 4.0b10 (Swofford, 2003) under the maximum likelihood (ML) criterion. A model sensitivity test was conducted, investigating a range of 60 symmetrical models of DNA substitution corresponding to the 56 implemented in MODELTEST version 3.06 (Posada and Crandall, 1998) plus F84, F84+I, F84+Γ8, and F84+I+Γ8. ML parameters of these models were estimated by PAUP* following the approach used in MODELTEST. These parameters were then used to conduct 60 individual ML heuristic searches in PAUP* with tree bisection-reconnection branch swapping and a neighbour-joining starting tree. ML bootstrap proportions were obtained after 100 replications, using the same search strategy and ML parameters as for the analysis of the original dataset.

3.2.3 Molecular dating

3.2.3.1 The penalized likelihood (PL) method

Divergence dates were obtained using the PL method of Sanderson (2002) as implemented in the program r8s, version 1.60 (Sanderson, 2003) with the TN algorithm. The outgroup was excluded using the "prune" command. The degree of autocorrelation within lineages was estimated using cross-validation as suggested by Sanderson (2002), and the correcting smoothing parameter λ defined accordingly. Divergence dates were estimated on the 60 ML phylograms recovered in the

phylogenetic model sensitivity analysis. Ages for each node across the 60 ML trees were summarized using the "profile" command.

Confidence limits on dating estimates were computed by using nonparametric bootstrapping of the original dataset as suggested by Sanderson and Doyle (2001). The program SEQBOOT of the PHYLIP 3.6 package (Felsenstein, 2002) was used to generate 100 bootstrap resampled datasets of 7,269 sites in length. ML branch lengths of the optimal topology were then estimated under the F84+Γ8 model for each of the bootstrap resampled datasets using PAUP*. Divergence estimates were then calculated for each of the 100 bootstrap replicates using r8s to obtain standard deviations on each node by the "profile" command and the settings described above.

3.2.3.2 The Baysian relaxed molecular clock (BRMC) method

The BRMC approach was applied using the program MULTIDIVTIME as implemented in the Thornian Time Traveller (T3) package (Yang, 2003). First, the program BASEML of the PAML package version 3.13 (Yang, 1997) was used to estimate the ML parameters of the F84+Γ8 substitution model, using the ML topology previously identified. Second, the program ESTBNEW was used to estimate branch lengths of the ML topology and the corresponding variance–covariance matrix. Finally, the program MULTIDIVTIME was used to run a Markov chain Monte Carlo for estimating mean posterior divergence times on nodes with associated standard deviations (SD) from the variance–covariance matrix produced by ESTBNEW. The Markov chain was sampled 10,000 times every 100 cycles after a burn-in stage of 100,000 cycles. A 75 million years (SD=37.5 million years) prior (Dettmann *et al.*, 1990) for the expected number of time units between tip and root and a prior of 200 million years for the highest possible number of time units between tip and root were used. Priors for the substitution rate at root node and the Brownian motion constant (describing the rate variation, i.e., the degree of rate autocorrelation along the descending branches of the tree) were derived from the median branch length. As for the PL method, the outgroup was not included in this analysis.

3.3 Results

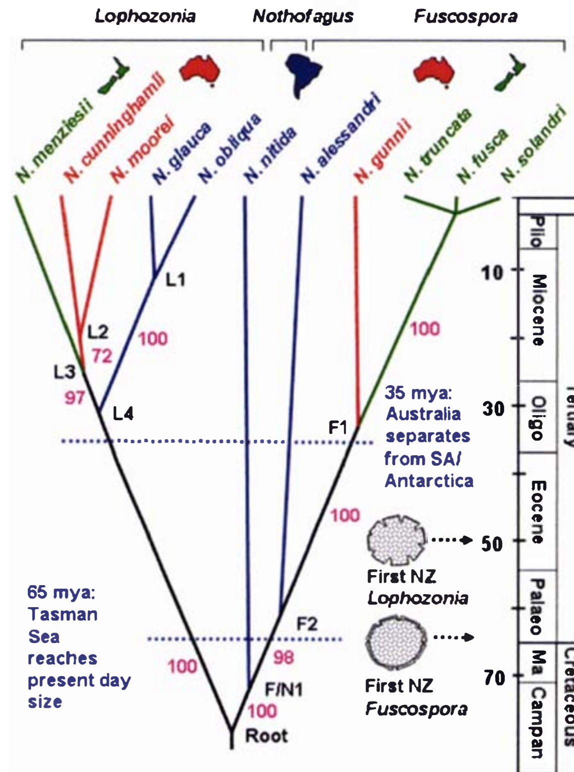
Figure 3.2 A and B shows the optimal maximum-likelihood reconstruction of phylogenetic relationships for chloroplast DNA sequences (7.2-kb comprising the *atp*B–*psa*I region and the *trn*L–*trn*F region; 7,269 nucleotide sites) for *Nothofagus* (subgenera or pollen groups (a) *Lophozonia*, (b) *Fuscospora*, and (c) *Nothofagus*) and outgroup *Castanea sativa* (not shown). In a sensitivity analysis of 60 substitution models, the tree topology shown in Figure 3.2 A and B was always recovered with very little difference in branch lengths regardless of the substitution model used. Of all substitution models evaluated, K81uf+Γ was identified as the best fitting one for the data based on hierarchical likelihood ratio tests and the Akaike Information Criterion. This substitution model and also the F84+Γ8 model were used for further analyses. The latter was included because the Bayesian relaxed molecular clock (BRMC) approach as implemented in the program MULTIDIVTIME only allows the use of the JC and the F84 models. Thus analysis with the F84+Γ8 model allowed a comparison of date estimates to be obtained using different relaxed molecular clock methods. All nodes of the optimal ML tree recovered in the sensitivity analysis received nonparametric bootstrap support greater than 97%, with the only exception being the grouping of *N. cunninghamii* with *N. moorei*, which received 72% support.

Divergence times for the nodes in this tree (Figure 3.2) were estimated using the BRMC method (Thorne *et al.*, 1998; Kishino *et al.*, 2001; Thorne and Kishino, 2002) and the penalized likelihood (PL) method (Sanderson, 2002). For these analyses, a period of 70– 80 million years was used to calibrate the divergence between the three fossil pollen groups representing subgenera *Lophozonia*, *Nothofagus*, and *Fuscospora*. These four pollen groups all first appeared in the fossil record approximately 75 mya (Dettmann *et al.*, 1990). A second constraint of a minimum of 20 mya for the divergence of *N. cunninghamii* and *N. moorei* was also used. This constraint was based on observations reported by Hill (1991) that 20 million years old fossils intermediate between *N. moorei* and *N. cunninghamii* were recorded from Tasmania and that fossils closely resembling *N. moorei* were also present at that time. The inferred ages for the remaining nodes of the tree, obtained under the F84+Γ8 model of substitution are given in Table 3.1 and graphically illustrated on Figure 3.2. The variance on these estimates was low and the values were little influenced by the choice of substitution model (Table 3.2). The robustness of the estimates to calibration error was tested by constraining the divergence of Australian and New Zealand sister taxa to 65 mya (the time when the Tasman Sea reached its present position; thus this date

provided a lower bound for divergence times of trans- Tasman *Nothofagus* disjunctions that might be explained by vicariance). Constraining these two nodes in this way produced unrealistic age estimates for all basal nodes. For example, using the BRMC method, which additionally required a prior expectation to be specified for the age of the root node (which was specified at 75 mya—the time of appearance of all four extant pollen types), 191 mya was estimated as more likely age for the root node. For the PL approach, which does not require specification of a prior, the age of the root node was estimated at 634 mya. Other basal nodes in both the *Fuscospora* and *Lophozonia* lineages were also much older than reasonably expected (see Table 3.1).

A

Bayesian relaxed molecular clock



B

Penalized Likelihood

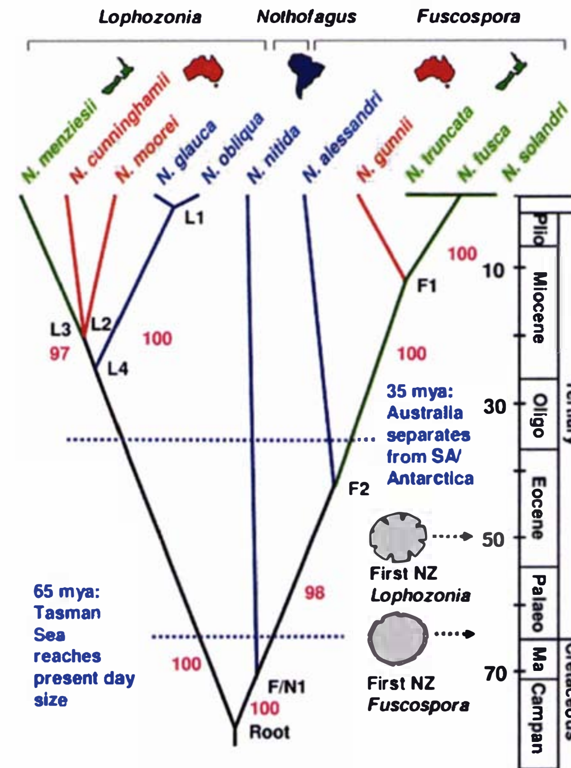


Figure 3.2: ML trees indicating evolutionary relationships for *Nothofagus* species based on the *atp*B–*psa*I and *trn*L–*trn*F regions of the chloroplast genome (7,269 bp). Divergence dates (in mya) were obtained with an F84+Γ8 substitution model using (A) the BRMC approach of Thorne *et al.* [29] and (B) the Penalized likelihood approach (Sanderson, 2002) (B) . For the dates indicated, the age of the root node and that of F/N1 were constrained to 70–80 mya; L2 was also constrained in accordance with fossil data (Hill, 1991) at 20 mya. Violet numbers show bootstrap values. The pollen grains represent the first appearance of the respective pollen type in the New Zealand fossil record. Plio, Pliocene; Oligo, Oligocene; Palaeo, Palaeocene; Ma, Maastrichian; Campan, Campanian. L1–L4, *Lophozonia* 1–4; F1–F2, *Fuscospora* 1–2; F/N1, *Fuscospora/Nothofagus* 1.

Table 3.1: Estimated divergence dates and standard deviations of different *Nothofagus* clades. Dates are based on different calibration dates and estimation approaches and are given in mya. BRMC: Bayesian relaxed molecular clock approach (Thorne *et al.*, 1998). PL Penalized Likelihood (Sanderson, 2002).

node number/ constraint	L1		L2		L3		L4		F1		F2		F/N1		root	
date estimate approach	**BRMC**	**PL**	**BRMC**	**PL**	**BRMC**	**PL**	**BRMC**	**PL**	**BRMC**	**PL**	**BRMC**	**PL**	**BRMC**	**PL**	**BRMC**	**PL**
root: 70-80 mya F/N1: 70-80 mya L2: 20 mya	**15** ± 6	**0.6** ± 0.6	**23**‡ ± 3	**20**‡	**27** ± 4	**20** ± 4	**34** ± 4	**25** ± 4	**33** ± 8	**13** ± 4	**61** ± 4	**42** ± 6	**72**‡ ± 1	**70**‡	**78**‡ ± 1	**80**‡
F1, L3: 65 mya	**37** ± 14	/	**47** ± 9	**40** ± 16	**65***	**65***	**83** ± 7	**118** ± 20	**65***	**65***	**116** ± 18	**213** ± 40	**138** ± 22	**316** ± 64	**191** ± 3	**634** ± 113

*: node fixed
‡: node constrained

Table 3.2: Variation of estimated divergence times (in mya) under 60 symmetrical models of DNA substitution. Dates estimated using PL approach.

node number/ constraint	L1		L2		L3		L4		F1		F2		F/N1		root	
	min	**max**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**
root: 70-80 mya F/N1: 70-80 mya L2: 20 mya	**0.7**	**0.7**	**20**‡	**20**‡	**21.7**	**21.8**	**26.6**	**27.0**	**12.7**	**13.3**	**41.1**	**42.4**	**70**‡	**70**‡	**80**‡	**80**‡

‡: node constrained

3.4 Discussion

The findings from molecular clock analyses using five independent calibrations (for four nodes), suggest that the sister relationships of the Australasian (Australia and New Zealand) species within both *Lophozonia* and *Fuscospora* lineages are too young to be explained by continental drift (as indicated by the inferred ages of nodes F1 and L3). Transoceanic dispersal appears the most likely explanation for the trans-Tasman sister relationships indicated in Figures 3.1 and 3.2. In contrast, the age inferred for node F2, using both relaxed clock methods is compatible with a hypothesis of continental drift as an explanation for the sister relationship between South American and Australasian *Fuscospora* lineages. The age for node L4, which separates Australasian and South American *Lophozonia*, may also be consistent with vicariance. The BRMC method dates it at 34 mya. However, the PL method estimates this node to be only 25 million years old, an age too recent to be consistent with vicariance. Thus the results for node L4 are regarded as equivocal. Nevertheless, southern beeches are likely to have been present in Antarctica 25 mya (Hill and Truswell, 1993), and thus long-distance dispersal across the young southern ocean between South America and Australia via Antarctica may be conceivable.

The robustness of the phylogenetic inferences has been investigated by varying the substitution model (60 symmetric models were used), estimating the variance of age estimates, and evaluating the influence of calibrations on divergence times. With the exception of the root node, the PL method consistently gave more recent age estimates than did the BRMC method. Both methods showed sensitivity to the number of calibration points used, a finding consistent with recent observations on the performance of relaxed molecular clock methods (Pérez-Losada *et al.*, 2004). In general, the date estimates produced by the BRMC approach were more consistent with the fossil record (Hill, 1991). Heads (2006) raises a valid point and general concern that the fossil record can only ever provide minimum age estimates for taxa. A relevant question is therefore whether or not additional calibration points could make date estimates older and thus change the conclusion of trans-Tasman dispersal. Results from this study suggest that this may be unlikely, given the observation that constraining a minimum age for trans-Tasman sister species to 65 mya leads to greatly inflated and unrealistic age estimates for all basal nodes. Hence to explain this finding a further hypothesis of a dramatic and independent slowing in the rate of evolution in *Lophozonia*, *Fuscospora*, and *Nothofagus* lineages would need to be invoked.

Thus the hypothesis that present-day distribution patterns of *Nothofagus* can be explained by continental drift following the breakup of Gondwana and subsequent extinction of some species can be rejected on the basis of the divergence dates presented here. These dates also indicate that present-day *Nothofagus* species in New Zealand are not the direct descendants of the *Fuscospora* and *Lophozonia* southern beeches that reached New Zealand in the Palaeocene and Eocene eras, respectively (Hill, 2001). These findings are consistent with findings of Swenson *et al.* 2001 and have recently been confirmed by Cook and Crisp (2005). They highlight the caution that needs to be taken when interpreting fossil evidence for the apparent first appearance of extant taxa. Fossils that identify specific evolutionary lineages may not necessarily indicate the origins for extant taxa or suggest a continuous presence for these taxa. Similar concerns follow from the findings of molecular analyses for *Ascarina* and *Laurelia* in New Zealand (Renner *et al.*, 2000; Zhang and Renner, 2003).

The strength of the molecular analyses highlights the importance of future research into potential mechanisms of long-distance dispersal, and in particular reinvestigation of the transoceanic dispersal properties of *Nothofagus* seeds. For the reasons that are outlined in the introduction, it seems likely that only once the mechanisms of long-distance dispersal are understood will hypotheses based on DNA divergence time estimates be truly convincing. DNA sequence analyses have also suggest that long-distance dispersal and continental drift are both important for explaining distributions of the conifer *Agathis* (Araucariaceae) in the South Pacific (Stöckler *et al.* 2002; Chapter 4). The findings from the molecular studies on these genera highlight the importance of considering more complex hypotheses of relationship in debates concerning the relative importance of dispersal and vicariance.

4

The drowning of New Zealand and the problem of *Agathis**

Abstract

80 million years ago (mya) the landmass that was to become New Zealand broke away from the Gondwanan super continent. During the Oligocene period (26 - 38 mya) there was a significant reduction in the landmass of New Zealand However, whether or not New Zealand was completely submerged is a matter of controversy and recent debate. If it was completely submerged during the Oligocene, then all extant biota must have arrived by transoceanic distance dispersal since that time. This study reports molecular systematic and molecular clock analyses based on 3011 base pairs of sequence data (*mat*K and *rbc*L coding regions, *trn*D-*trn*T intergenic region) from the chloroplast genome of nine different species from the Araucariaceae family. Analyses of these data suggest that Australian and New Zealand *Agathis* lineages diverged in the late Cretaceous/early Tertiary period. These estimates are consistent with *in situ* survival of the *Agathis australis* lineage in New Zealand following the breakup of Gondwana. They imply the continuous existence of land in the New Zealand area throughout the Tertiary.

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4.1 Introduction

Pole (1994) has suggested that given the extent of the fossil evidence for long-distance dispersal to New Zealand it is not inconceivable, that the entire fauna and flora of the archipelago arrived after the Mid Tertiary. This hypothesis also receives support from the observation that there is little geological evidence for the continuous existence of land for New Zealand throughout the Tertiary (Campbell and Landis, 2003). It is known that during the Oligocene extensive regions of New Zealand were submerged (Cooper and Cooper, 1995). However the extent of the submergence is undetermined. The hypothesis of a complete Oligocene drowning of New Zealand has been considered implausible by some researchers (Craw *et al*., 1999, McCarthy, 2003; Heads 2006) and very likely by others (Waters and Craw, 2006).

Molecular systematic studies on the New Zealand flora and fauna provide one means of testing the Oligocene drowning hypothesis. Recent studies have included those on invertebrates (Donald *et al*., 2005), Fish (Waters and Craw, 2006 and references therein), birds (Cooper and Cooper, 1995; Cooper *at al*., 2001; Ericson *et al*. 2002) and plants (2000; Stöckler *et al*., 2002; Zhang and Renner, 2003; Knapp *et al*., 2005). Findings in many of these studies have indicated post-Oligocene arrival of extant New Zealand lineages. However, the findings in some molecular systematic studies have been interpreted otherwise. An unbroken Gondwanan link with respect to some New Zealand bird fauna has been inferred from reconstructed gene trees for mitochondrial (Cooper *et al*. 2001) and nuclear genes (Ericson *et al*. 2002). Stöckler *et al*. (2002) have also advanced similar arguments to explain trans-Tasman distributions of *Agathis* in the plant family Araucariaceae. Their phylogenetic reconstruction of chloroplast *rbc*L genes identified the New Zealand *Agathis australis* (Kauri) as the sole representative of an early diverged lineage within the genus that is genetically very distinct from its Australian relatives. This finding, together with Early Cretaceous fossils for *Agathis* species in the Clarence valley in the South Island of New Zealand (Daniel 1989; Parrish *et al*., 1998; *A. seymouricum*; see Chapter 2) was interpreted by Stöckler *et al*. (2002) as support for *Agathis* having existed *in situ* in New Zealand since the break up of Gondwana.

Waters and Craw (2006) have recently argued that the evidence of Stöckler *et al*. (2002) is weak in terms of the contribution it can make to the debate on the extent of the Oligocene drowning, because molecular clock analyses were not reported in this study. They have also questioned the relevance of the 90-100 million year old *Agathis*

fossils from the Clarence Valley of New Zealand (Daniel, 1989) that Stöckler *et al.* (2002) used to support their hypothesis. These fossils predate the breakup of Gondwana and might therefore belong to a widely distributed lineage with close relatives in Australia.

This study reports divergence time estimates made using relaxed molecular clock methods for nine different species of Araucariaceae based on three chloroplast regions: two coding regions (*mat*K and *rbc*L) and one intergenic region (*trn*D-*trn*T). These concatenated regions total 3011 nucleotide sites of unambiguously aligned chloroplast genome sequence. Phylogenetic analyses of these data can not reject the hypothesis that *Agathis australis* survived the Oligocene drowning of the New Zealand landmass. Rather, they suggest that at least parts of the New Zealand archipelago remained above sea level throughout the Tertiary Period.

4.2 Methods

4.2.1 Sequence data

Chloroplast DNA sequences (3011 nucleotide sites comprising the *mat*K and *rbc*L genes as well as the *trn*D-*trn*T intergene region) were determined for each of 9 accessions of *Agathis*, *Araucaria* and *Wollemia* (Araucariaceae) sampled from the botanical gardens in Valdivia, Chile (*Araucaria angustifolia*), the Royal Botanical Gardens, Sydney, Australia (*Agathis robusta, Ag. microstachya, Ag. atropurpurea, Araucaria cunninghamii, Ar. bidwillii, Wollemia nobilis)*, the University of the South Pacific, Suva, Fiji *(Agathis macrophylla)* the Esplanade Gardens, Palmerston North, New Zealand (*Agathis australis*), Massey University, Palmerston North, New Zealand (*Prumnopitys ferruginea*) and Rangiwahia Track, Ruahine Range, New Zealand (*Dacrydium cupressinum*) (protocols: Appendix I; herbarium voucher numbers: Appendix II; sequence data: Appendix III).

All *rbc*L sequences as well as the *mat*K gene for the New Zealand endemic podocarps *Dacrydium cupressinum* (Rimu) and *Prumnopitys ferruginea* (Miro), and *Pinus koraiensis* (Korea Pine), a native of Korea, China and Japan were obtained from GenBank. Sequence data for the *trn*D-*trn*T intergene region was also obtained from GenBank for the Korea Pine (Accession numbers: Appendix II). All other sequences were determined as part of my thesis. The sequence data was aligned using progressive multiple-sequence alignment: ClustalX version 1.81 (Thompson *et al.*, 1997).

4.2.2 Tree building

Phylogenetic reconstructions were made with PAUP* version 4.0b10 (Swofford, 2003) using heuristic maximum likelihood (ML) criteria. Trees were initially built for the *mat*K and *rbc*L genes and also the *trn*D-*trn*T intergene region. These data were subsequently concatenated producing an unambiguous alignment of 3011 nucleotide sites. Analyses were then conducted on this combined data matrix as described in Chapter 3.

4.2.3 Molecular dating

All molecular dating analyses, except for the model sensitivity analysis, were conducted separately for each gene and for the concatenated dataset. The model sensitivity analysis was only conducted for the concatenated dataset. Divergence dates for all datasets were estimated using both the Penalized Likelihood (PL) method (Sanderson, 2002, 2003) and a Bayesian Relaxed Molecular Clock approach (BRMC) (Thorne *et al.*, 1998; Kishino *et al.*, 2001; Thorne and Kishino, 2002) following the proceedings described in Chapter 3.

4.2.3.1 r8s calibration points for Araucariaceae

All fossil calibration dates by definition represent minimum ages (Heads, 2006). However, in order to run the program r8s, at least 1 maximum age must be defined. Hill *et al.* (2000) consider it possible that Araucariaceae evolved after the major extinction phase at the end of the Permian Period (299 - 251 mya), which was caused by widespread arid conditions throughout Gondwana. Thus a conservative estimate of 225 mya was assumed for the maximum age of the root. This date is weakly supported by the fossil record, in which pollen grains assigned to Araucariaceae have been recorded from the early Triassic Period (251 - 245 mya; De Jersey, 1968).

In the Araucariaceae phylogeny the *Wollemia / Agathis* clade is sister to the *Araucaria* clade (Figure 4.1). While the oldest unequivocal *Araucaria* macrofossils are known from the Jurassic (Stockey, 1982) the oldest reliable records of *Wollemia* like pollen (*Dilwynites*) only date from the Turonian Period (91-89 mya) (Macphail *et al.*, 1995). The minimum age of the root was therefore set at 89 mya.

The Araucaria sections *Eutacta* (in this analysis represented by *Araucaria cunninghamii*) and *Bunya* (*Araucaria bidwillii*) are known from the Jurassic whereas the oldest reliable records of the section *Araucaria* (represented by *Araucaria angustifolia*) are of Early Cretaceous age (Hill and Brodribb, 1999). Therefore it was assumed that the most recent common ancestor (MRCA) of *A. bidwillii* and *A. angustifolia* is a minimum of 100 million years old while *A. cunninghamii* and *A. bidwillii* are separated by at least 136 million years of evolution.

Fossils assigned to *Agathis* have been recorded from the Jurassic and Cretaceous period. However, they lack sufficient diagnostic characters to allow confident identification (Hill and Brodribb, 1999). The oldest unequivocal *Agathis* record

is known from the Late Eocene Vegetable Creek sediments in Northern New South Wales, Australia (Hill, 1995). The MRCA of *Agathis* and *Wollemia* was therefore assumed to be not younger than 38 million years old.

4.2.3.2 MULTIDIVTIME calibration points

The Bayesian approach to molecular dating allows for additional calibration points. In addition to the calibration dates described above a prior for the age of the root and a prior for the expected rate of substitution at the root node were set. Based on fossil evidence, Miller (1977) suggested that *Agathis* and *Araucaria* diverged in the Late Jurassic / Early Cretaceous. According to Hill (1995) more fossil evidence and phylogenetic analyses are needed to support this conclusion. Nevertheless, the date suggested by Miller (1977) provides a prior expectation for the age of the root. Thus we have assumed the basal node of the tree to be 136 million years old. To take into consideration the uncertainty of this date a prior for the standard deviation of that date was set to 68 million years. The prior for the average substitution rate from root to tip was calculated separately for each dataset (median branch length from root to tip divided by estimated number of time unites from root to tip). In every case a standard deviation of 50% was assumed. The priors for gamma distribution of the rate at root node and the Brownian motion constant (which describes the rate variation i.e., the degree of rate autocorrelation along the descending branches of the tree) were derived from the median branch length.

The variable “big time” (a non-conservative estimate for the number of time units from root to tip) was set at 350 mya. This provided a bounded estimate for the age of the Gymnosperms (Rothwell and Schneckler, 1988).

4.3 Results

4.3.1 Araucariaceae phylogeny

Figure 4.1A shows the optimal ML reconstruction made using concatenated *mat*K and *rbc*L genes and the *trn*D-*trn*T intergene region for nine species from the Araucariaceae (genera *Agathis*, *Wollemia* and *Araucaria*) and outgroups *Pinus koraiensis, Dacrydium cupressinum* and *Prumnopitys ferruginea*. The tree topologies estimated for the *mat*K, *rbc*L and *trn*D-*trn*T datasets were congruent although the trees differed in their respective level of resolution.

These topologies were largely congruent with respect to the trees reported in earlier sequencing studies (Setoguchi *et al*. 1998; Stöckler *et al*. 2002). The only exception concerned the placement of *Wollemia nobilis*. Setoguchi *et al*. (1998) published a phylogeny of a wide range of species from the Araucariaceae. Their results were inferred from the *rbc*L gene using a parsimony approach. In their analyses *Wollemia nobilis* was basal to both the *Araucaria* and *Agathis* lineage. In Figure 4.1A, *Wollemia* is sister to *Agathis*. Possible reasons for this incongruence are taxon sampling, outgroup selection, difference in size of the datasets, and the selected optimality criterion.

To test which of these factors might be most important, Araucariaceae relationships were first reconstructed using the *rbc*L dataset of Setoguchi *et al*. (1998) and a maximum likelihood tree building criterion. Their topology could be reproduced, suggesting that the implementation of ML was not the cause of the observed discrepancy in root placement. Next the impact of outgroup choice was investigated under the maximum likelihood criterion. Most support for *Wollemia nobilis* being sister to the *Agathis* lineage was found when the complete set of outgroups (*Pinus koraiensis, Dacrydium cupressinum* and *Prumnopitys ferruginea)* was used. However, the exclusion of *Pinus koraiensis,* the most diverged outgroup, favoured reconstruction of the Setoguchi *et al.* topology. The robustness of phylogenetic relationships for the *rbc*L dataset of nine species was also investigated using only podocarps as outgroup taxa. As with Setoguchi *et al.*'s dataset, *Wollemia nobilis* was found to be sister to both the *Araucaria* and *Agathis* lineages. The only difference to Setoguchi *et al.*'s topology was the placement of *Araucaria bidwillii* that was grouping with *A. cunninghamii* rather than with *A. angustifolia*. This local instability in the placement of *Araucaria bidwillii* could be corrected when more taxa were added to the dataset. This finding indicates

that for the *rbc*L dataset outgroup choice has significant influence on the phylogenetic position of *Wollemia nobilis* in reconstructed trees for Araucariaceae.

The above analyses were also repeated on the concatenated datasets (*mat*K and *rbc*L genes and the *trn*D-*trn*T intergene region) of nine species which were mainly used in this study. Finally a sensitivity analysis of 60 substitution models (see Chapter 3) was conducted on the concatenated dataset. With these data, and in these analyses, the tree shown in Figure 4.1A was always recovered with very little difference in branch lengths regardless of the substitution model used. Of all substitution models evaluated, K81uf+Γ was identified as the best fitting one for the concatenated data based on hierarchical likelihood ratio tests. This substitution model and also the F84+ Γ8 model were used for further analyses. The latter model was included because the Bayesian relaxed molecular clock (BRMC) approach, as implemented in the program MULTIDIVTIME (see Chapter 3), only allows the use of the JC and the F84 models. This analysis with the F84+Γ8 model allowed a comparison of date estimates to be made using different relaxed molecular clock methods. With few exceptions, all nodes of the optimal ML tree recovered in the sensitivity analysis received nonparametric bootstrap support greater than 95%. The exceptions were the grouping of *Ag. microstachya* with *Ag. atropurpurea*, which received 67% support and the grouping of *Ag. robusta* relative to these two species which received 86% support. To be able to compare the effect of the two different topologies on molecular clock estimates a maximum likelihood tree with the concatenated dataset was also built while constraining the Setoguchi topology. Doing this produced an unresolved trichotomy for *Wollemia*, *Agathis* and *Araucaria* with respect to the outgroups (Figure 4.1B).

4.3.2 Molecular clock analyses

Divergence times for the nodes on the respective optimal tree (Figure 4.1) were estimated for each gene and intergene region separately and for the concatenated dataset using the penalized likelihood (PL) method (Sanderson, 2002) and BRMC method (Thorne *et al.*, 1998; Kishino *et al.*, 2001; Thorne and Kishino, 2002). Due to the relatively small amount of sequence divergence in the individual gene/intergene regions, the standard deviations for the individual datasets could not be calculated accurately using the nonparametric bootstrapping method recommended for r8s by Sanderson and Doyle (2001). Standard deviations for the individual datasets were therefore only calculated using MULTIDIVTIME. The inferred ages of the nodes of the

tree, obtained under the optimal model (r8s) or the F84+Γ8 model of substitution (MULTIDIVTIME) are given in Table 4.1.

The standard deviations for the age of nodes for individual genes/intergene regions were high especially for the completely unconstrained node 2. The variation among date estimates for individual nodes was large between the different individual gene/intergene datasets. The largest variation of divergence time estimates was found for node 2 (New Zealand *Agathis* – Australian *Agathis*). For the concatenated dataset the standard deviations for the date estimates were lower than for the individual datasets (Table 4.2) and the values were little influenced by the choice of substitution model (Table 4.4).The total average age (μ_t) and total standard deviation (σ_t) for each node across all datasets and dating methods are shown in Table 4.3.

Table 4.1: Node age (in mya) and standard deviation estimates (in million years) based on *mat*K and *rbc*L genes and *trn*D-*trn*T intergene region. Unconstraint topology

gene/ intergene region		***mat*k**		***trn*D-*trn*T**		***rbc*L**	
node	constraint in mya	r8s	multidivtime	r8s	multidivtime	r8s	multidivtime
root	89 - 225	225	193 ± 20	225	185 ± 22	225	189 ± 21
1	min 38	110	116 ± 32	182	136 ± 34	191	145 ± 31
2		**34**	**51 ± 27**	**49**	**65 ± 34**	**96**	**91 ± 33**
3	min 136	163	161 ± 17	177	165 ± 20	153	160 ± 17
4	min 100	100	120 ± 16	100	124 ± 19	102	128 ± 19

Table 4.2: Comparison of node age (in mya) and standard deviation estimates (in million years) based the concatenated dataset. Unconstraint and constrained topology

dataset		**Concatenated dataset unconstrained**		**Concatenated dataset Setoguchi topology**	
node	constraint in mya	r8s	multidivtime	r8s	multidivtime
root	89 - 225	225 ± 0	197 ± 18	225 ± 0.1	201 ± 14
1	min 38	159 ± 18	141 ± 23	225 ± 0.1	207 ± 14
2		**56 ± 11**	**61 ± 22**	**78 ± 15**	**92 ± 26**
3	min 136	163 ± 14	159 ± 15	144 ± 7	150 ± 11
4	min 100	103 ± 8	117 ± 13	100 ± 1	112 ± 10

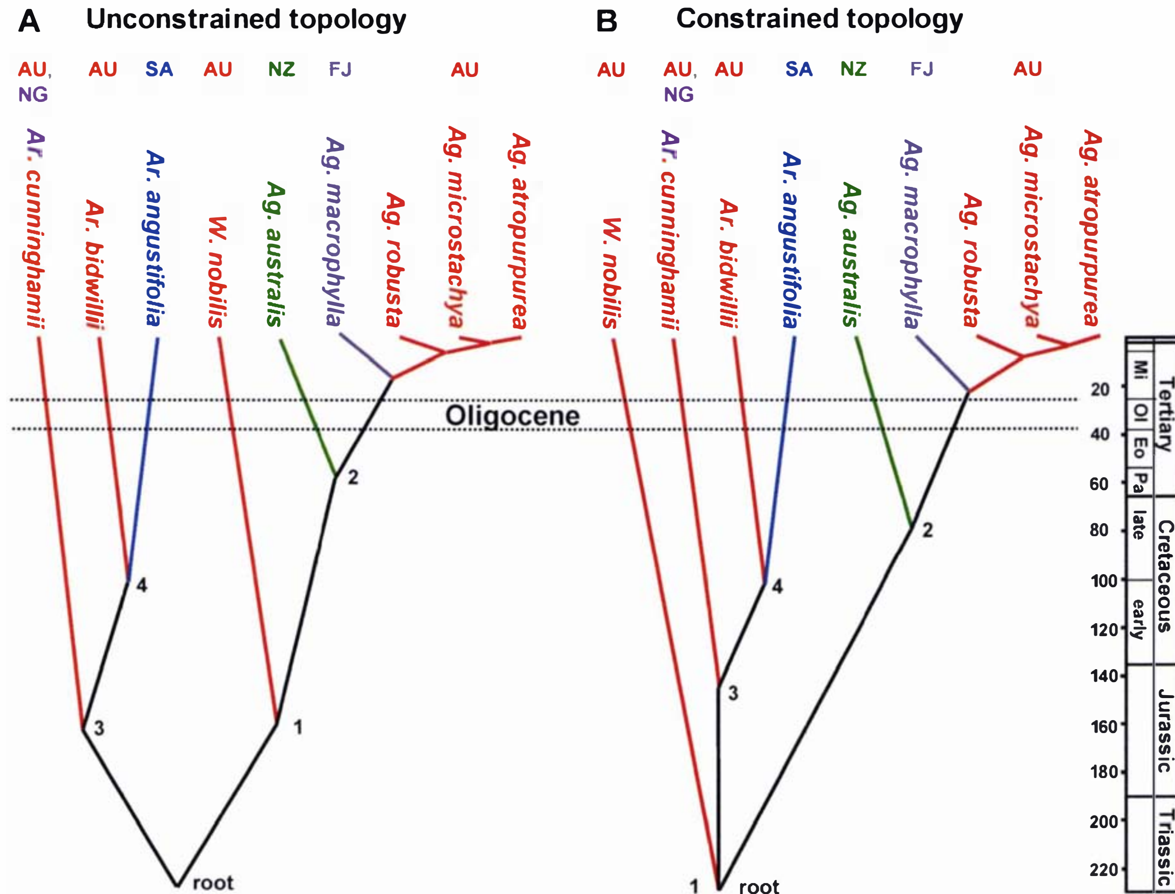

Figure 4.1: ML trees indicating evolutionary relationships for Araucariaceae based on the *rnat*K and *rbc*L coding regions and *trn*D–*trn*T intergene region of the chloroplast genome (3011 basepairs). Divergence dates (in mya) were obtained with an (K81uf+ Γ) substitution model using the PL approach of Sanderson (2002). For the dates indicated, the age of the root node was constrained to 89 - 225 mya; 1, 3 and 4 were also constrained in accordance with fossil data at a minimum of 38, 136 and 100 mya respectively; A) topology estimated using concatenated dataset and no constraint; B) topology constrained to topology of Setoguchi *et al.*, 1998). Abbreviations: AU, Australia; NG, New Guinea; SA, South America; NZ, New Zealand; FJ, Tropical Australasia; Mi, Miocene; Ol, Oligocene; Eo, Eocene; Pa, Palaeocene.

Table 4.3: Average age of nodes across all genes and methods (μ_t) in mya and standard deviation (σ_t) of these estimates in million years (unconstraint topology).

node	constraint in mya	Average age of node across all genes/intergene regions and dating methods (μ_t) in mya	Standard deviation of node age across all genes/ intergene regions and dating methods (σ_t) in mya
root	89 - 225	207	18
1	min 38	150	27
2		**65**	**20**
3	min 136	163	7
4	min 100	113	11

Table 4.4: Sensitivity of estimated node age to 60 symmetric models of DNA substitution. Dates (in mya) estimated using PL approach.

node	constraint in mya	**Model sensitivity, complete dataset with unconstrained topology**	
		min	max
root	89 - 225	225	225
1	min 38	158	161
2		**57**	**60**
3	min 136	157	160
4	min 100	100	100

4.4 Discussion

4.4.1 Topology

The topology of the Araucariaceae phylogeny consistently produced by all datasets and under 60 different symmetric models of DNA substitution and ML tree selection criteria is largely congruent with *rbcL* gene trees found in earlier studies (Setoguchi *et al.*, 1998, Stöckler *et al.*, 2002). The only exception is the placement of *Wollemia nobilis*. Setoguchi *et al.* (1998) published a phylogeny of a wide range of species from the Araucariaceae. Their results were inferred from the *rbc*L gene using a parsimony approach. In their analyses, *Wollemia nobilis* was basal to both the *Araucaria* and *Agathis* lineage. Analyses presented here suggest an alternative root placement, and that this difference can be attributed to features of the Setoguchi *et al.* (1998) *rbc*L dataset. At present it is perhaps most prudent to assume that the phylogenetic relationship between *Wollemia*, *Agathis* and *Araucaria* is close to an unresolved trichotomy. In the analyses presented here, concatenating sequences from three different datasets produced a stable root placement that was recovered irrespective of the outgroup used. However it is difficult to be confident that this more accurately represents the true unknown phylogeny, since concatenating sequences can sometimes create false support due to topological biases in data and model misspecification (Holland *et al.*, 2004). For this reason divergence times were estimated with the concatenated dataset on two alternative topologies.

As with earlier analyses of the *rbc*L sequences, *Agathis australis* (Kauri) was identified in these analyses as the sole representative of an early diverged lineage within the genus, genetically very distinct from the Australian *Agathis* species. *Agathis macrophylla* which is native to the South-West Pacific from the Solomon Islands to Fiji was found basal to the Australian species but diverged much more recently from this group than *A. australis.* The *Araucaria* species used in this analysis represented three anciently diverged lineages within the genus, and this assumption was indicated from the phylogenetic reconstructions.

4.4.2 Divergence time estimates

The aim was to estimate the divergence time of *Agathis australis* from New Zealand and its closest relatives from Australia and Oceania. While the differences in date estimates from the different genes were quite large, all except one estimate predated the putative Oligocene drowning event. The r8s estimate for node 2 based on the *mat*K gene was only 34 million years and therefore Early Oligocene. However all estimates have to be regarded as minimum divergence times given the conservative nature of the calibrations used. When a tree constraint to Setoguchi *et al.*'s topology was used the estimated time for the split between *Agathis australis* and the Australian and Oceanian *Agathis* species became older. (Table 4.2, Figure 4.1) The results indicate that *Agathis australis* separated from its closest living relatives some time in the Eocene. This timing is consistent with the geographical isolation of New Zealand and Australian landmasses and favours a hypothesis of *in situ* survival of *Agathis* in New Zealand throughout the Oligocene. Given that the calibrations used in this study are conservative, a vicariant explanation for the Trans-Tasman Sea distribution of *Agathis* is conceivable. An alternative explanation that lineages diverged, for example in Australia, and that ancestors of *Agathis australis* crossed the Tasman Sea and subsequently became extinct in Australia is less parsimonious, and thus less likely. A definitive analysis of the taxonomic status of "*Agathis seymouricum*", the Cenomonian fossil Araucariaceae from Clarence Valley, is clearly of major importance. If it indeed can be shown to be closest known relative of *Agathis australis* as Daniel (1989) has suggested, then this would be a strong indicator for the continued presence of *Agathis australis* in New Zealand since the Gondwanan breakup. As mentioned above a similar type of origin has been inferred for other New Zealand taxa. Although in all cases this inference was not based on molecular dating, the observation that in ratites (Cooper *et al.*, 2001), in passerine birds (Ericson *et al.*, 2002) and in Araucariaceae the New Zealand species are genetically very dissimilar from their overseas relatives could indicate that this common pattern is explained by similar ancient origins.

Since the divergence dates estimated for trans-Tasman relationships predate the Oligocene marine transgression, these findings bring into question the hypothesis that New Zealand was completely submerged following its separation from Gondwana during the Oligocene (Waters and Craw, 2006).

5

Riddle of the beech gaps*

Abstract

The distribution of *Nothofagus* (Southern Beech) species in New Zealand is characterised by gaps that cannot be explained by present day edaphic or climatic factors alone. Severe Pleistocene climates and glaciation or alternatively early Miocene tectonic events have been proposed as factors that have helped shape the present day distribution pattern of *Nothofagus* in New Zealand. However, the biogeographic history of *Nothofagus* in New Zealand has remained controversial.

This study reports phylogenetic analyses of five chloroplast DNA markers, representing a total of ~2.5 kilo base pairs (kb) of sequence data from the chloroplast genome of 74 accessions from 4 different New Zealand *Nothofagus* species. Divergence time estimates suggest that the early differentiation of extant *Nothofagus menziesii* haplotypes occurred with uplift of the Southern Alps in the late Miocene. Divergence time estimates for taxa disjunct across the Westland gap are consistent with limited gene flow during glacial-interglacial cycles of the late Pliocene and Pleistocene (~2.4 million years - 10 000 years before present (BP)). A genetic disjunction also occurs across the Manawatu gap which might similarly be explained by either glacial extirpation or possibly geographical isolation as a consequence of Pliocene submergence of the southern North Island. Results from nested clade analyses suggest that fragmentation and range expansion have been the main factors influencing the geographic distribution of *N. menziesii* chloroplast haplotypes. In contrast to findings for *Lophozonia* beech, accessions of *Fuscospora* beech showed little or no genetic variation across New Zealand, suggesting a history of recent population expansion for the *Fuscospora* species *N. solandri*, *N. fusca* and *N. truncata*. Thus, although the extant *Lophozonia* and *Fuscospora* beech distributions are somewhat similar in New Zealand these lineages have responded differently to tectonic and climatic change.

*In preparation: Knapp, M.; Stöckler, K.; Mardulyn, P.; Havell, D., McGlone, M.S.; Lockhart, P.J.: Riddle of the beech gaps.

5.1 Introduction

Nothofagus forests form a distinct component of the New Zealand vegetation. 6.25 million hectares of the archipelago are covered by indigenous forest that remains after Maori and European clearances, and 46% of this area is occupied by single *Nothofagus* species with few other associated species. A further 22% comprises mixed forests with one or more of the four New Zealand *Nothofagus* species present (Wardle, 1984). The four New Zealand *Nothofagus* species are members of two different subgenera. *Nothofagus solandri*, *N. fusca* and *N. truncata* belong to the subgenus *Fuscospora*, whilst *Nothofagus menziesii* belongs to the subgenus *Lophozonia* and is the only New Zealand representative of this subgenus.

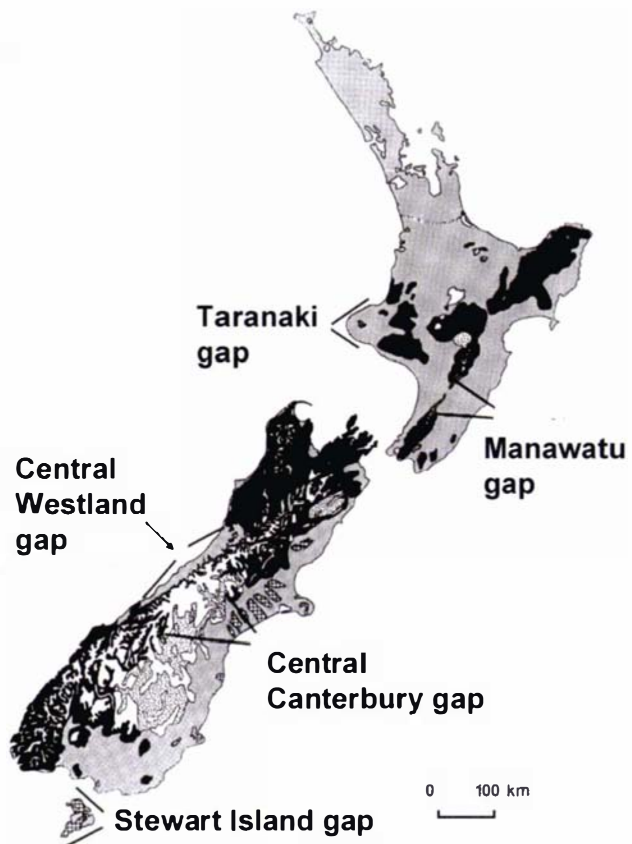

Figure 5.1: Distribution of the genus *Nothofagus* in New Zealand (black regions) and beech gaps (map adapted from McGlone *et al.*, 1996).

The geographic distribution of *Nothofagus* species in New Zealand is disjunct (Figure 5.1; Figure 5.2). Gaps exist where *Nothofagus* does not occur at all or can only be found in small isolated stands (McGlone *et al.*, 1996). In some regions edaphic and/or climatic explanations have been put forward to explain the disjunct beech distribution. However there are also regions where *Nothofagus* is absent without any obvious recent ecological cause (Leathwick, 1998) (Figure 5.1). Among the most obvious of these gaps are the Taranaki gap, where *Nothofagus* is absent from the volcanic area, the Manawatu gap, a 75 km wide partial gap in the northern Tararuas and southern Ruahines with only some isolated stands of *N. solandri*, the Central Westland gap from Mahitahi to the Taramakau River, the Central

Canterbury gap from the Ben Ohau Range to Rolleston Range and the Stewart Island gap (McGlone *et al.*, 1996).

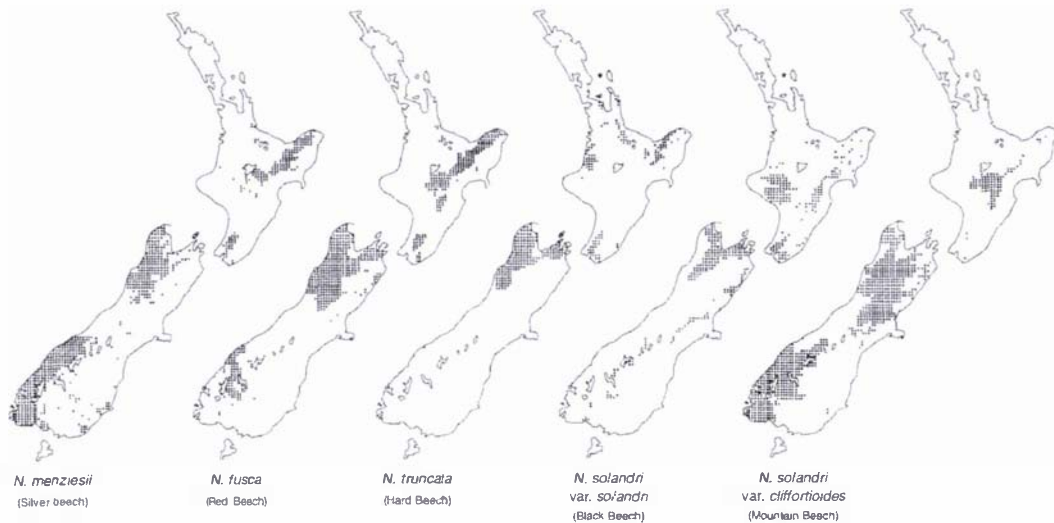

Figure 5.2: Distribution of New Zealand *Nothofagus* species (map adapted from Ogden *et al.*, 1996).

Hypotheses invoking historical causes have been proposed to help explain the Manawatu and central South Island gaps. One of the earliest hypotheses is that *Nothofagus* was excluded from wide areas by extreme glacial climates or glaciation during the Pleistocene and subsequently failed to reinvade due to a combination of climatic and edaphic factors, limited dispersal abilities and competitive pressure from species of the conifer-broadleaf-association that now occupy many of these regions. (Cockayne, 1926, 1928; Wardle, 1963; Burrows, 1965)

McGlone (1985) proposed an alternative hypothesis. He suggested that current gaps were maintained by present day environmental factors, and resulted from the inability of *Nothofagus* species to extend their range in certain habitats after having been excluded. He thus claimed that it was largely the current environment and not the fact of glacial exclusion that was most important. However, he also noted large scale distributional patterns of many higher plant taxa that suggested that tectonic change such as lateral displacement along the Alpine Fault which began in the late Oligocene/early Miocene, formation of sea straits and the uplift of the Southern Alps could be implicated in the formation of these distributions. In particular, he pointed to the endemic-rich areas of the northern North Island, the northern South Island, and the south-western South Island and associated large-scale disjunctions, and asked

whether it was their stable, ancient geology that was responsible for there having retained this diversity.

Others have also contributed to the discussion on biotic and abiotic features of the beech gaps and evaluated the role of both hypotheses regarding the origin of the beech gaps (Wardle, 1988; Rogers, 1989; Heads, 1989; Haase, 1990; McGlone *et al.*, 1996; Heads, 1998; Leathwick, 1998; Wallis and Trewick 2001; Trewick and Wallis 2001; Hall and McGlone, 2006).

This study reconstructs the biogeographic history of New Zealand *Nothofagus* species based on phylogenetic analyses (relaxed clock dating and nested clade analyses) of chloroplast DNA (cpDNA) sequences. Analyses presented here suggest that the biogeographic history of *Fuscospora* and *Lophozonia* differ greatly from each other. However, for both groups our analyses indicate that the Manawatu beech gap and the Central Westland and Central Canterbury gaps are associated with climatic and tectonic events of the Late Pliocene and Pleistocene.

5.2 Methods

5.2.1 Samples

A total of 49 accessions of *Nothofagus menziesii* and 25 accessions of *Fuscospora* beeches (*N. solandri, N. fusca, N. truncata*) were sampled from their respective ranges (Figure 5.2; Figure 5.3; Table 5.1 + 5.3). *Nothofagus moorei* (Australia; *Lophozonia*), *N. cunninghamii* (Australia, Tasmania; *Lophozonia*), *N. obliqua* (South America; *Lophozonia*), *N. gunnii* (Tasmania; *Fuscospora*), *N. alessandri* (South America; *Fuscospora*) and *N. nitida* (South America; *Nothofagus*) were also collected from their native areas, and evaluated as potential outgroups for phylogenetic analyses (see Appendix II).

Table 5.1: *Lophozonia* samples

SAMPLE	COORDINATES	SAMPLE	COORDINATES
NORTH ISLAND		***Haplotype IV (continued)***	
Haplotype I		Maruia River	42 18 34 S 172 12 19 E
Waikanae	40 42 42 S 175 22 30 E	Marble Hill	42 20 51 S 172 13 22 E
Dora Hutt	40 43 53 S 175 23 00 E	Lewis Pass 1	42 21 38 S 172 15 20 E
Holdsworth	40 43 53 S 175 23 00 E	Lewis Pass 2	42 22 42 S 172 16 44 E
Aorangi	41 25 07 S 175 20 40 E	Lewis Pass 3	42 23 07 S 172 23 59 E
Wainuiomata	41 20 25 S 174 58 01 E	Lewis Pass 5	42 28 02 S 172 23 53 E
Haplotype II		Lewis Pass 6	42 31 56 S 172 21 34 E
East Cape	37 45 12 S 178 07 48 E	Lewis Pass 7	42 31 55 S 172 21 27 E
Te Aroha	37 31 29 S 175 44 18 E	***Haplotype V***	
Waihaha	38 42 20 S 175 41 40 E	Buller Gorge1	41 48 57 S 171 36 01 E
Waikaremoana	38 37 18 S 177 01 49 E	***Haplotype VI***	
Kaimanawa East	39 00 00 S 176 09 01 E	Murchison	41 44 30 S 172 24 42 E
Ohakune Skifield	39 19 11 S 175 30 08 E	***Haplotype VII***	
SOUTH ISLAND		Mt Cook	43 44 12 S 170 05 32 E
Haplotype III		Mt Brewster	44 05 03 S 169 24 00 E
Abel Tasman	40 56 40 S 172 53 23 E	Lower Haast	46 08 33 S 167 41 32 E
Mt Duppa	41 15 36 S 173 28 49 E	Haast River	44 01 55 S 169 21 28 E
Mt Arthur	41 11 23 S 172 44 24 E	Haast Pass	44 06 20 S 169 21 32 E
Paenga	41 53 20 S 172 14 20 E	Haast Summit	44 02 15 S 169 22 41 E
Reefton 2	42 11 35 S 171 56 12 E	Homer Tunnel	44 45 52 S 167 59 53 E
Rahu Saddle	42 18 33 S 172 06 49 E	Dusky Sound 1	45 32 10 S 167 13 45 E
Lewis Pass 4	42 26 23 S 172 24 10 E	Dusky Sound 2	45 34 30 S 167 10 31 E
Haplotype IV		Takitimu Range	45 44 49 S 168 04 15 E
Buller Gorge2	41 50 53 S 171 43 32 E	Tuatapere	37 45 12 S 178 07 48 E
Buller Gorge3	41 50 42 S 171 48 36 E	South Coast	46 08 49 S 167 28 46 E
Reefton1	42 04 12 S 171 50 38 E	Catlins	46 30 54 S 169 23 10 E

Table 5.1: *Lophozonia* samples (continued)

SAMPLE	COORDINATES	SAMPLE	COORDINATES
SOUTH ISLAND			
Haplotype VIII		***Haplotype IX***	
Bullock Creek 1	42 05 38 S 171 23 09 E	Buller Gorge 4	41 50 53 S 171 43 39 E
Bullock Creek 2	42 06 01 S 171 24 12 E		
Blackball	42 21 10 S 171 23 55 E		
Roa	42 21 11 S 171 22 33 E		

Table 5.2: *Fuscospora* samples. NT: *Nothofagus truncata*, NS: *Nothofagus solandri*, NF: *Nothofagus fusca*. *: samples with asterisk were only sequenced for the *trn*L-*trn*F region.

SAMPLE	COORDINATES	SAMPLE	COORDINATES
Haplotype A		***Haplotype A continued***	
Thames NT	37 07 46 S 175 33 53 E	Cobb Dam Road NF	41 06 24 S 172 42 19 E
Little Barrier Island NT	36 12 25 S 175 03 57 E	Wangapeka NF	41 25 08 S 172 39 29 E
Havelock NT	41 16 01 S 173 46 55 E	Queenstown NF	44 50 29 S 168 20 54 E
Cobb Dam Road NT1	41 06 01 S 172 43 25 E	Buller Gorge NF*	41 50 43 S 171 48 43 E
Cobb Dam Road NT2	41 06 00 S 172 43 25 E	Bullock Creek NF*	42 06 02 S 171 24 13 E
Urewera NS	38 39 15 S 176 52 28 E	Otira River NF1*	42 47 23 S 171 36 20 E
Sunrise Road NS	39 47 59 S 176 11 59 E	Otira River NF2*	42 47 39 S 171 36 31 E
Kaitoke NS1	41 04 30 S 175 08 54 E	***Haplotype B***	
Kaitoke NS2	41 04 54 S 175 08 37 E	Jackson Bay NS	44 02 45 S 168 43 18 E
Hinewai NS	43 48 36 S 173 01 30 E	Kepler Track NS	45 26 20 S 167 40 54 E
Craigieburn NS	43 09 05 S 171 44 28 E	Te Anau NS	45 46 05 S 167 31 56 E
Nelson Creek NS*	42 24 30 S 171 31 43 E	Hope Valley NF	44 05 36 S 168 20 18 E
Te Aroha NF	37 32 13 S 175 43 57 E	Kepler Track NF	45 26 20 S 167 40 55 E

5.2.2 Sequence data

DNA was extracted from leaf material using the Qiagen DNeasy Plant kit following the manufacturer's protocol (see Appendix I). 2.5 kb of chloroplast DNA sequences comprising the *trn*L intron as well as the *trn*E-*trn*T, *trn*L-*trn*F, *atp*B-*rbc*L *and acc*D- *psa*I regions of the chloroplast genome were determined for all 49 accessions of *N. menziesii,* 20 accessions of *Fuscospora* and one accession of each *Nothofagus moorei*, *N. cunninghamii*, *N. obliqua*, *N. gunnii*, *N. alessandri*, and *N. nitida*. Five accessions of *Fuscospora* (4 *N. fusca*, 1 *N. solandri*) from near the northern edge of

the Central Westland beech gap were only sequenced for the *trn*L-*trn*F region. The sequence data matrix (~2500 base pairs) was aligned using the progressive multiple-sequence alignment procedure implemented in ClustalX version 1.81 (Thompson *et al*., 1997) and edited using BioEdit 5.0.9 (Hall, 1999). The aligned data matrix was analysed with gap characters (i) removed and (ii) recoded. Gaps were recoded as binary presence/ absence data following the algorithm of Simmons and Ochoterena (2000), as implemented in the program "gap recoder" (http://maen.huh.harvard.edu:8080/services/gap_recoder). In order to reduce computation time in tree building analyses, the number of accessions in the alignments was reduced to one accession per haplotype.

5.2.3 Maximum likelihood (ML) trees

Phylogenetic reconstructions based on the cpDNA sequence data were made with PAUP* version 4.0b10 (Swofford, 2003). Phylogenetic trees were built using maximum likelihood (ML). Gaps were treated as missing data, and substitution model parameters were estimated by comparing 56 symmetrical models of DNA substitution with MODELTEST version 3.06 (Posada and Crandall, 1998) (http://darwin.uvigo.es/software/modeltest.html). These parameters were then adopted for ML heuristic searches. Neighbour Joining was used to produce the starting tree in heuristic searches and tree bisection-reconnection branch swapping was used to identify trees with improved scores. Trees were outgroup rooted.

5.2.4 Molecular dating

Divergence dates were obtained using the penalized likelihood (PL) method of Sanderson (2002) as described in Chapter 3 but with the POWELL algorithm (since the available calibration did not occur at the root of the tree; Sanderson/Perrie pers. comm.). A date (minimum age) of 20 million years ago (mya) was used to calibrate divergence of *N. moorei* and *N. cunninghamii*. This calibration was based on observations reported by Hill (1984) that 20 million years old fossils intermediate between *N. moorei* and *N. cunninghamii* have been recorded from Tasmania, and that fossils closely resembling *N. moorei* were present at that time.

5.2.5 Haplotype networks

Haplotype networks, indicating the number of mutational changes along each branch, were constructed for gap removed and gap recoded sequences using statistical

parsimony with the program "TCS" (Clement *et al.*, 2000). All *N. menziesii* accessions and the two most closely related outgroups (*N. moorei and N. cunninghamii*) were included in the data matrix (all species except for *N. cunninghamii* and *N. moorei* exceeded the 95% connection limit with respect to *N. menziesii* and for this reason were not used for rooting the *N. menziesii* TCS network). The TCS network obtained for recoded data with these sequences has nodes IN1, III, IV and V interconnected forming an internal ambiguity (Figure 5.6 A) which could be resolved for the nested clade analysis in four possible ways. Crandall and Templeton (1993) suggest two criteria relevant for selecting the best resolution.

1. rare haplotypes are more likely to be found at the tips, and common haplotypes at the interior, of a TCS network
2. the higher the connectedness of a haplotype the higher its outgroup probability (or in this case the more likely it is to be basal to other haplotypes)

Based on this criterion, the connection between IN1 and haplotype V was removed resulting in the reconstruction shown in Figure 5.7. This haplotype tree and the geographic location information (Table 5.1) for each accession was used for the nested clade analysis. Alternative resolutions of the haplotype network were also investigated and have been discussed.

5.2.6 Nested clade analysis (NCA)

This method tests for non-random geographic distributions of haplotypes. Following an inference key (http://darwin.uvigo.es/download/geodisKey_11Nov05.pdf), it then proposes hypotheses for historical demographic events (population fragmentation or population expansion) or recurrent restricted gene flow among populations as the cause of an identified geographical association (Templeton *et al.*, 1995; Templeton, 1998)

To conduct a NCA on *N. menziesii*, a resolved TCS network was first subdivided into a nested series of clades following the algorithm of Templeton *et al.* (1987) and Templeton and Singh (1993) (Figure 5.7). Subsequently the program GeoDis 2.5 was used to test for non-random geographical associations of haplotypes. Three types of distances were calculated: (i) the 'clade distance', D_c , which measures how geographically widespread the haplotypes within a given clade are; (ii) the 'nested clade distance', D_n , which measures how widespread haplotypes and their nearest sister haplotypes within the next higher level nesting clade are and, (iii) the interior-tip distances ($I\text{-}T_c$ and $I\text{-}T_n$) which compare 'clade distances' ($I\text{-}T_c$) and 'nested clade

distance' ($I\text{-}T_n$) of interior and tip clades which are nested within a common nesting clade. Interior-tip distances indicate how widespread younger clades (tips) are compared to their respective ancestors (interiors) (Templeton *et al* . 1995). The distributions of these three distances under the null hypothesis of no geographical associations within each clade (no correlation between the position of a haplotype in a clade and its geographical position) was determined by recalculating these distances after each of 1000 random permutations of the nested clades vs. geographical locations.

5.2.6.1 Test for influence of sample density on the NCA

To test the influence of sample density on the results of the NCA (Templeton, 1998) 20 fictional samples from actual *N. menziesii* locations were added to the dataset (Table 5.3). 15 fictional samples were assigned Southland and Otago coordinates. 5 fictional samples were assigned to the North Island where *N. menziesii* is less abundant than in the South Island. All fictional samples from Southland and Otago were assigned the haplotype VII as all characterised samples from that region had this haplotype. Depending on their location fictional samples from the North Island were assigned haplotype I or II (Figure 5.2). NCAs were conducted with fictional North Island and/or fictional South Island samples together with the original data using the settings described above.

Table 5.3: Fictional samples from *N. menziesii* locations

SAMPLE	COORDINATES	SAMPLE	COORDINATES
NORTH ISLAND		*Haplotype VII (continued)*	
Haplotype I		test9	44 27 05 S 168 01 00 E
test1	41 03 03 S 175 08 08 E	test10	44 41 45 S 167 37 37 E
Haplotype II		test11	45 16 40 S 168 11 33 E
test2	37 57 51 S 177 46 18 E	test12	45 31 39 S 168 23 58 E
test3	38 09 24 S 177 39 20 E	test13	45 28 21 S 167 37 40 E
test4	38 09 10 S 177 20 04 E	test14	45 40 45 S 167 05 58 E
test5	38 20 25 S 177 20 06 E	test15	46 04 10 S 166 49 36 E
SOUTH ISLAND		test16	46 11 42 S 167 50 36 E
Haplotype VII		test17	46 32 46 S 169 18 11 E
test6	44 03 17 S 168 52 15 E	test18	46 26 08 S 169 15 43 E
test7	44 16 09 S 168 35 01 E	test19	46 01 49 S 167 55 01 E
test8	44 27 18 S 168 49 09 E	test20	45 57 11 S 167 38 08 E

5.3 Results

5.3.1 Sequence variation in *Lophozonia*

N. menziesii sequences display regional patterns in the distribution of haplotypes. Nine different haplotypes were identified among the accessions analysed (Figure 5.3). A northern lineage (haplotypes I-V) and a southern lineage (haplotypes VI-IX) were recognized (Figure 5.3 and 5.5). Within the northern lineage, Haplotype I and II were found in the North Island only. They are separated by the Manawatu Gap. Haplotype III is separated from Haplotype I by the Cook Strait and has the centre of its distribution in the Nelson region. Haplotype IV can be found mainly in the Maruia River Valley, the Buller Gorge and in the Lewis Pass area. Some accessions of haplotype III were also found in this region (Table 5.1). Haplotypes I, II and IV are only separated from haplotype III by one mutation. Haplotype V is a singlet from Buller Gorge which is separated by one mutation from haplotype IV and by two from haplotype III. This northern lineage differs by at least five mutations from a second group of haplotypes (VI, VIII, IX) which belong to the southern lineage and were found in the Paparoa Ranges from the Buller Gorge south, with the exception of the singlet haplotype VI which was found near Murchison, east of the Buller Gorge. Haplotype VIII was represented by four samples from Bullock Creek and the Blackball region and separated by two mutations from haplotype IX, another singlet from Buller Gorge. Haplotype VI was three mutations removed from VIII and IX. Two mutations separated it from haplotype VII which was only found south of the Central Westland and Central Canterbury Beech Gap but was shared by all samples from these regions.

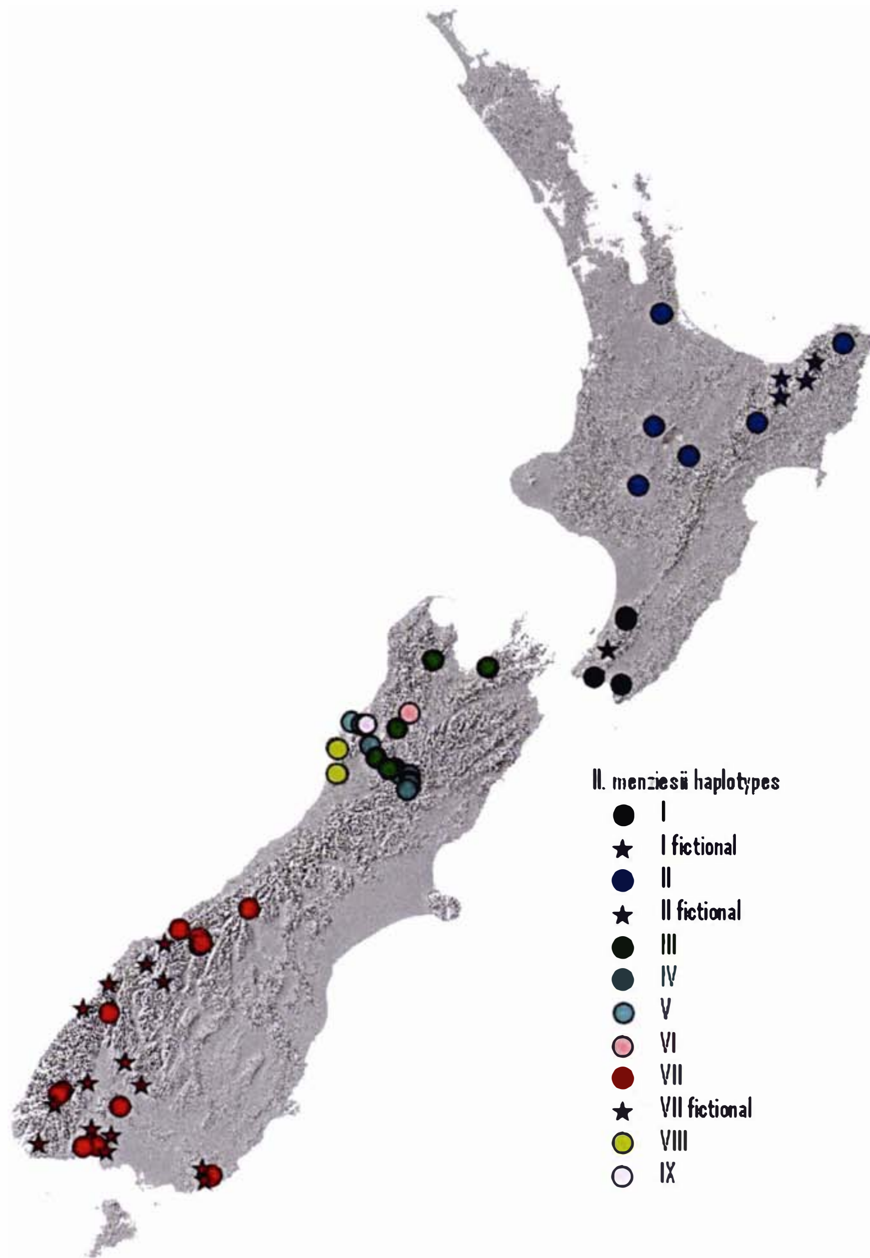

Figure 5.3: Distribution of *Lophozonia* haplotypes. Each circle represents at least one accession; each star represents exactly one fictional accession. New Zealand map from the Land Environments of New Zealand (LENZ) package (Manaaki Whenua Landcare Research).

5.3.2 Sequence variation in *Fuscospora*

New Zealand *Fuscospora* show far less variation than *N. menziesii*. Only two haplotypes could be distinguished (Figure 5.4). These differed from each other by a one base pair indel in the *trn*L-*trn*F region of the chloroplast genome. Haplotype A was found north of the Central Westland and Central Canterbury beech gaps and in one accession from south of these gaps. This haplotype was shared by all three New Zealand *Fuscospora* species. Haplotype B was found only south of the Westland gap. It was shared by both *N. fusca* and *N. solandri*. No *N. truncata* from south of the Central Westland and Central Canterbury beech gaps were sequenced in this study but Thomsen (2002) found that these also share haplotype B. As *N. menziesii* samples from the Paparoa Ranges were found to be intermediate between typically northern and typically southern haplotypes, five more *Fuscospora* samples from Buller Gorge, the Paparoa Ranges and the Otira River Valley near the northern edge of the Central Westland Beech Gap were sequenced for the *trn*L-*trn*F region only to increase the sample density in this area and test if a pattern similar to the one observed in *N. menziesii* could be found in *Fuscospora* beeches. However, they all shared the northern haplotype A.

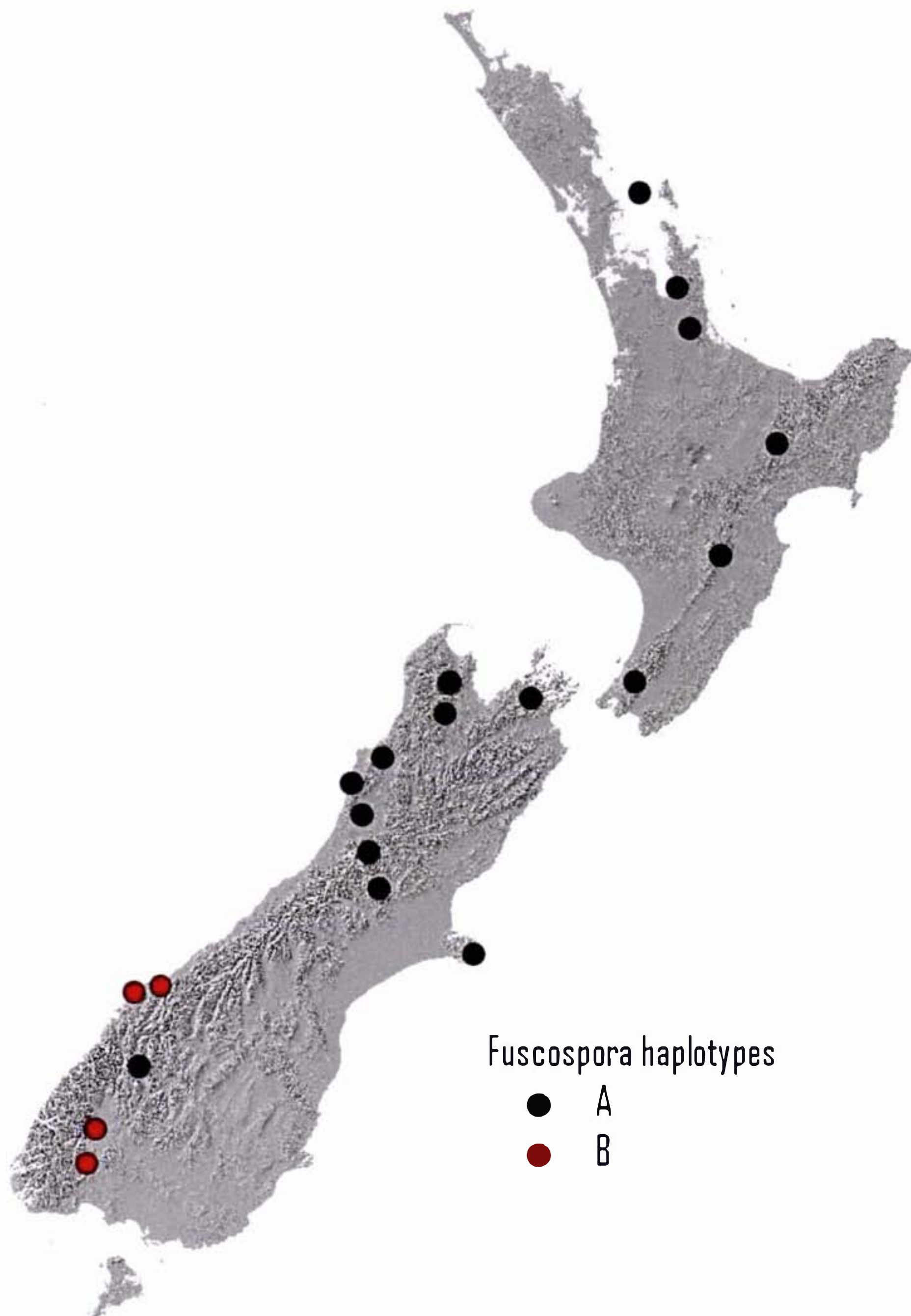

Figure 5.4: Distribution of *Fuscospora* haplotypes. Each circle represents one sample. New Zealand map from the Land Environments of New Zealand (LENZ) package (Manaaki Whenua Landcare Research).

5.3.3 Maximum likelihood tree

Figure 5.5 shows the optimal maximum-likelihood reconstruction of phylogenetic relationships for chloroplast DNA sequences (2.5-kb comprising sequences from the *trn*L intron as well as the *trn*E-*trn*T, *trn*L-*trn*F, *atp*B-*rbc*L *and acc*D- *psa*I regions of the chloroplast genome) for *Nothofagus menziesii* haplotypes. *Nothofagus moorei*, *N. cunninghamii*, *N. obliqua*, *N. gunnii*, *N. alessandri*, *N. nitida*, *N. fusca*, *N. solandri* and *N. truncata* were used as outgroups and are not shown in Figure 5.5. Gaps were coded as missing data. Of all substitution models evaluated, F81+Γ was identified as the best fitting one for the data based on hierarchical likelihood ratio tests. The ML tree shows a major genetic disjunction between taxa from South of the Central South Island gaps and most taxa from more northern regions. Haplotypes IV and V could not be distinguished from haplotype III as these three haplotypes only differ by alignment gaps. Thus the divergence of haplotypes I, II, and III was the only divergence event identified in the northern lineage by the ML analysis.

5.3.4 Molecular clock analyses

Results from molecular clock analyses are summarized in Table 5.4 and Figure 5.5. Due to the relatively small amount of sequence divergence between different *N. menziesii* haplotypes, the standard deviations for the individual date estimates could not be calculated accurately using the nonparametric bootstrapping method recommended for r8s by Sanderson and Doyle (2001). The most basal divergence within extant New Zealand *N. menziesii* was found to be 5.7 million years old. At this time the northern lineage including haplotypes I – V diverged from the southern lineage including haplotypes VI – IX. About 3.6 mya the southern group split into a Buller Gorge/ Paparoa Range group (VIII and IX) and a group dominated by the Southland and Otago haplotype VII. The singlet haplotype VI from near Murchison belongs to the latter group. Haplotype VI and VII separated about 1.5 mya. Haplotypes I – III of the northern lineage diverged approximately 1.2 mya.

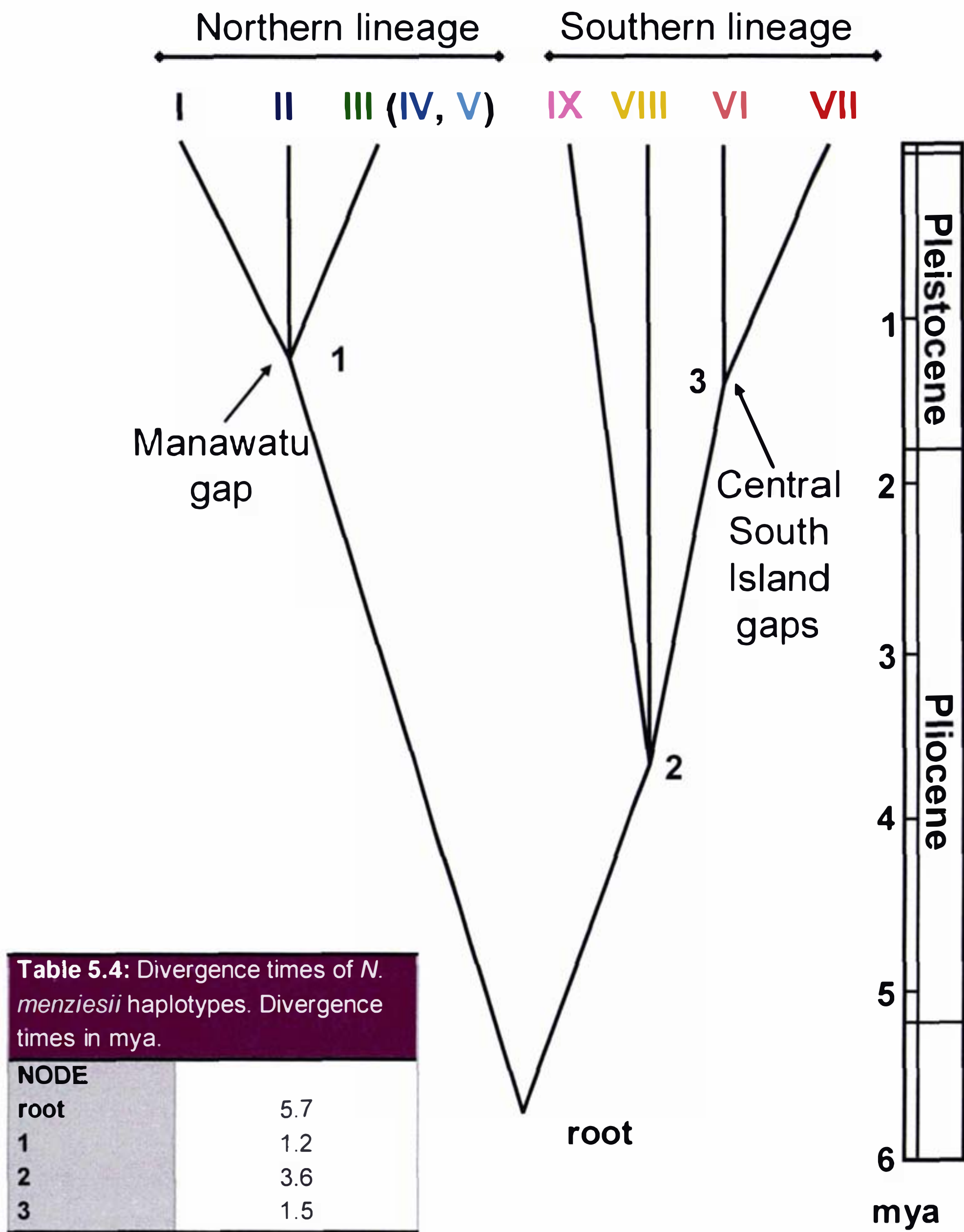

Table 5.4: Divergence times of *N. menziesii* haplotypes. Divergence times in mya.

NODE	
root	5.7
1	1.2
2	3.6
3	1.5

Figure 5.5: ML tree indicating evolutionary relationships for New Zealand *Nothofagus menziesii* haplotypes. The phylogenetic reconstruction is based on sequences from the *trn*L intron as well as the *trn*E-*trn*T, *trn*L-*trn*F, *atp*B-*rbc*L *and acc*D-*psa*I regions of the chloroplast genome (~2500 base pairs) The tree is outgroup rooted (outgroups not shown). Divergence dates (in mya) were obtained using a penalized likelihood approach as implemented in the program r8s (Sanderson, 2002).

5.3.5 Haplotype networks

The TCS networks based on the recoded datasets (Figure 5.6 A) showed higher resolution than the networks built with gaps coded as missing data (Figure 5.6 B). Both networks were congruent with the topology of the ML tree with the exception that for the TCS network based on the recoded dataset haplotypes IV and V were identified as different from haplotype III and the nodes IN1, III, IV and V were interconnected forming an internal ambiguity.

5.3.6 Nested clade analysis

The test for non-random association of haplotypes identified three clades for which inferences could be made about demographic history. The inference key suggested that haplotypes located in the northern North Island and southern North Island (i.e. from either side of the Manawatu gap) were separated from each other as a result of "allopatric fragmentation". Southern North Island and northern South Island haplotypes were similarly related. All these taxa were in turn found to be related to other individuals from the northern South Island through "isolation by distance and restricted gene flow" (limited gene flow among geographically distant populations). The key suggested that a model of "contiguous range expansion" explained the relationships between all haplotypes from the North and South Island. Nested clade design and clade numbers are shown in Figure 5.7. Results of the NCA are summarized in Figure 5.8. Chains of inference for each clade with significant geographical associations are given in Table 5.5.

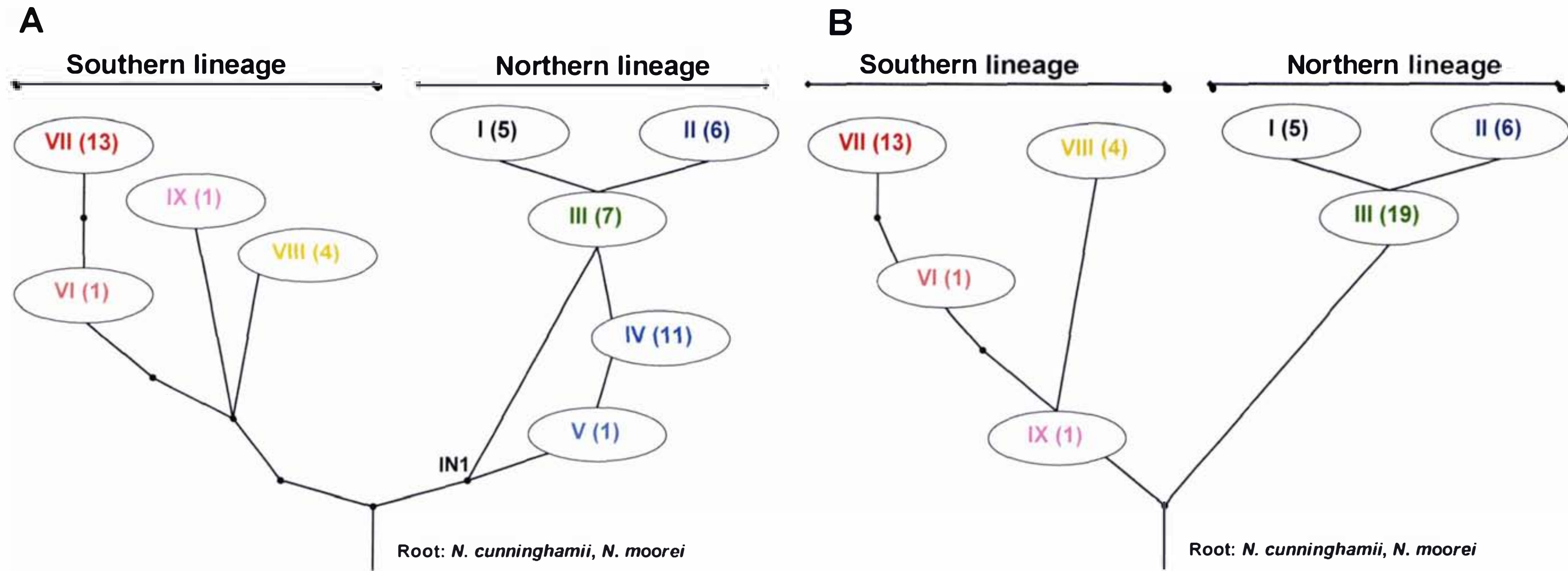

Figure 5.6: TCS networks of *N. menziesii* haplotypes. The phylogenetic reconstructions are based on sequences from the *trn*L intron as well as the *trn*E-*trn*T, *trn*L-*trn*F, *atp*B-*rbc*L *and acc*D-*psa*I regions of the chloroplast genome (~2500 base pairs) The trees are outgroup rooted. **A:** TCS network based on dataset with gaps recoded as binary presence/absence data. **B:** TCS network based on dataset with gaps coded as missing data. Genetic distance between each node (haplotypes and black dots on branches) is one mutation. Numbers in brackets show number of accessions with respective haplotype.

A

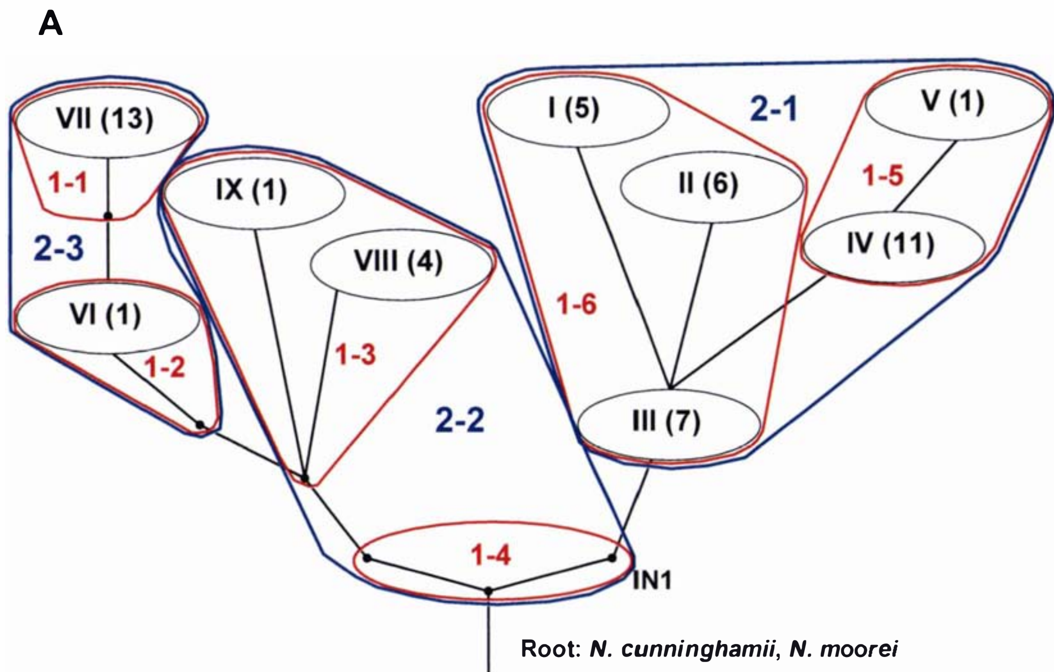

Figure 5.7: Nested clade design for the haplotype network shown in Figure 5.6.A. The topology shown here represents the optimal resolution (topology A) of the ambiguity in the TCS network built from the recoded dataset (Figure 5.6 A) according to criteria proposed by Crandall and Templeton (1993).

Table 5.5: Interpretation of results of the NCA (Figure 5.8) based on topology A (Figure 5.7) using the inference key of Templeton (2005). Total Cladogram (test) based on original dataset plus 15 fictional *N. menziesii* samples from Southland and Otago.

CLADE	CHAIN OF INFERENCE	DEMOGRAPHIC EVENT INFERRED
1-6	1-19-No	Allopatric fragmentation
2-1	1-2-3-4-No	Restricted gene flow with isolation by distance
Total Cladogram	1-2-11-12-No	Contiguous range expansion
Total Cladogram (test)	1-2-11-12-13-Yes	Past Fragmentation followed by range expansion

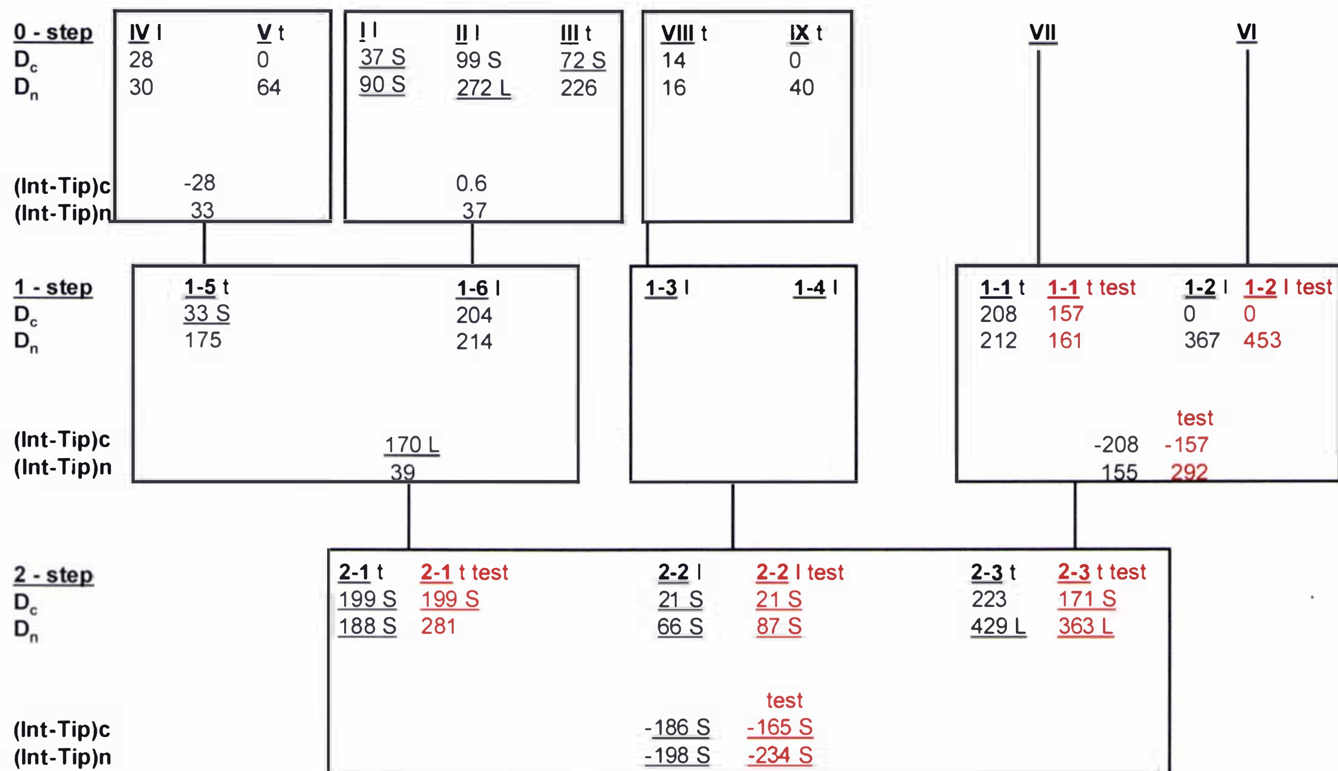

Figure 5.8: Result of the nested clade analysis of geographical distances for the chloroplast DNA haplotypes of *Nothofagus menziesii*. Haplotype numbers as defined in Table 5.1. I or t refer to "interior" or "tip" respectively. Haplotypes nested in a one-step clade are grouped in boxes following the nested design shown in Figure. 5.6. Clade levels increase from top to bottom of the figure. In each box, clade distances (D_c) and nested clade distances (D_n) calculated for each clade within the nested group are shown together with the average difference in distance between interior clades and tip clades for D_c and D_n [(Int-Tip)$_c$ and Int-Tip)$_n$ respectively]. Significantly large or small distance values (at the 5% level) are underlined and characterized with an "L" or an "S" respectively. Red results are based on the test dataset with 15 additional fictional samples from Southland and Otago.

5.3.7 Impact of sample density on NCA inferences

The inferences of the NCA after adding the fictional North Island samples were not significantly different from the inferences made with the original dataset and have therefore not been included in the tables and graphics below. However, the inclusion of fictional samples from Southland and Otago decreased the D_c for clade 2-3 which became significantly small (Figure 5.8). The inference on the "Total Cladogram" level changed from "contiguous range expansion" to "past fragmentation".

5.3.8 Alternative TCS network resolutions and their impact on NCA inferences

Although the criteria suggested by Crandall and Templeton (1993) lead to an unambiguous, resolved TCS network shown in Figure 5.7 (topology A), a further observation suggests a different resolution. Haplotype V shares an ancestral 20-basepair gap with all outgroups as well as haplotypes VI, VII, VIII and IX. This might suggest haplotype V as the most ancestral haplotype of the Northern lineage. Figure 5.9 shows the three other possible TCS network resolutions compatible with the TCS network shown in Figure 5.6.A. The significance of their impact on NCA inferences of demographic history can be described as follow:

Topology B produced the same nesting pattern as the optimal tree (topology A). Inferred demographic events are the same as for the optimal tree, except for clade 2-1 for which "contiguous range expansion" was inferred instead of "restricted gene flow with isolation by distance" as a result of a changed "tip" and "interior" status of the nested clades 1-5 and 1-6.

Topology C produced the same nesting pattern and NCA results as topology A on the "Total Cladogram" level as well as for clades 1-1, 1-2, 1-3, and 2-3. Clades 1-4 and 2-2 are marginally different from the nesting pattern of topology A. Clades 1-5 and 1-6 nested within clade 2-1 differ from the nesting pattern of topology A as haplotype IV is assigned to clade 1-6 instead of clade 1-5. This changed the inference for clade 1-6 from "allopatric fragmentation" to "contiguous range expansion". As this inference is based on a different assemblage of haplotypes within clade 1-6 it cannot be compared with the result from the analyses of topology A. In contrast with inferences based on topology A and topology B, no significant geographic association was inferred within clade 2-1.

With topology D the 1-step clades are identical with the 1-step clades of topology A although both 1-5 and 1-6 are now tip clades (only 1-5 is a tip clade in the optimal tree). Clades 1-1, 1-2, and 1-3 are nested within clade 2-2 while 1-4, 1-5, and 1-6 are nested within clade 2-1. A third 2-step clade does not exist. Similar to results from topology A "allopatric fragmentation" was inferred for clade 1-6. "Restricted gene flow with isolation by distance" was inferred for clade 2-2 while no significant geographic association could be identified for clade 2-1. Inferences for the demographic relationships on the "Total Cladogram" level were also not possible as both nested clades (2-1 and 2-2) were tip clades and a tip/interior status could therefore not be determined. As for topology C, not all results could be compared to the results from topology A. All three alternative phylogenetic reconstructions and the nested clade designs for the respective topologies are shown in Figure 5.9. Chains of inference for each clade with significant geographical associations from each individual topology are given in Table 5.6.

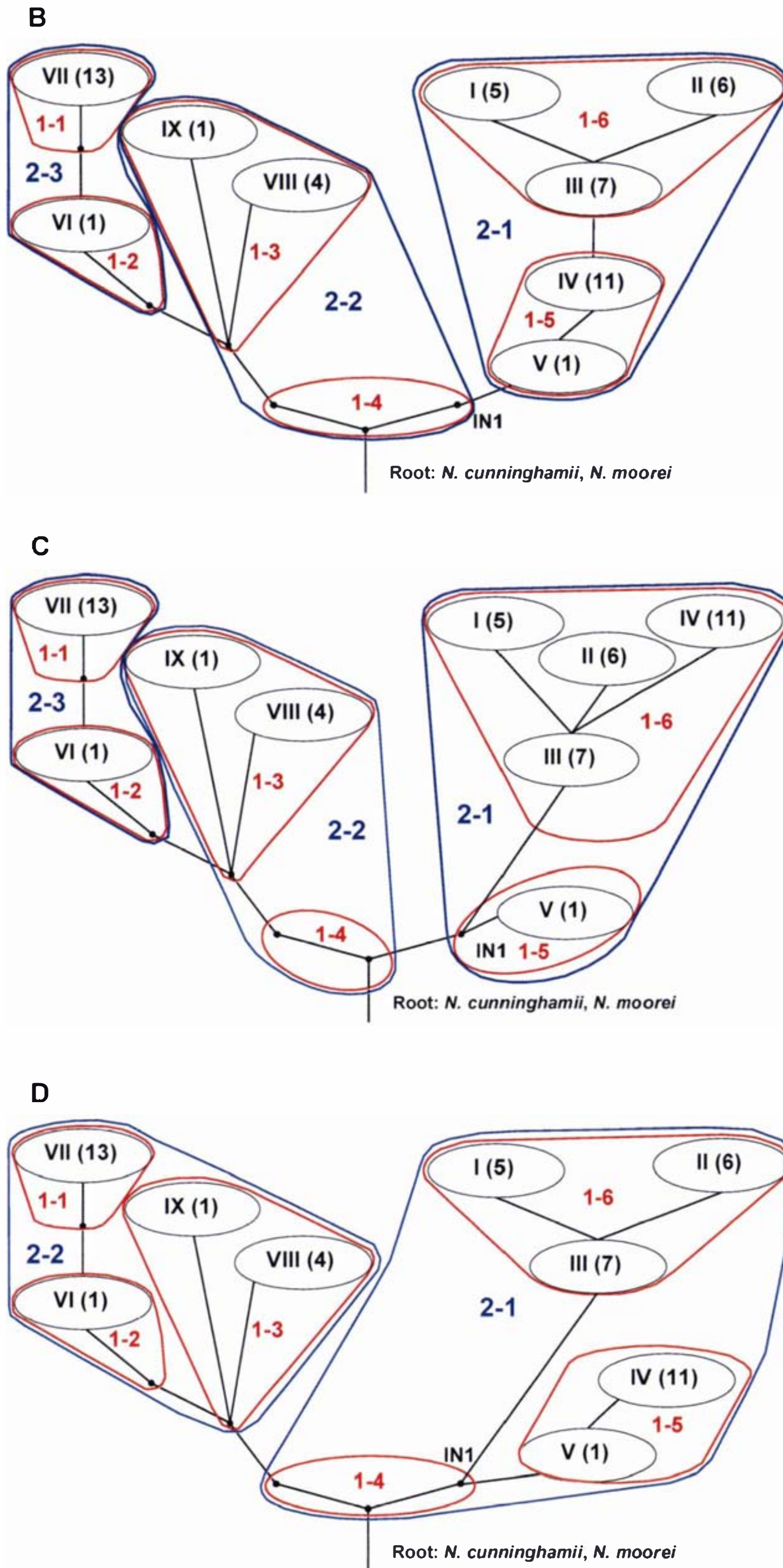

Figure 5.9: Alternative haplotype tree topologies B-D and respective nesting designs. Topologies represent alternative, equally parsimonious, resolutions of the ambiguity in the TCS network built from the recoded dataset (Figure 5.6 A).

Table 5.6: Interpretation of results of the NCAs for three alternative resolutions (B-D, Figure 5.9) of the TCS network ambiguity (Figure 5.6 A) using the inference key of Templeton (2005).

CLADE	CHAIN OF INFERENCE	DEMOGRAPHIC EVENT INFERRED
Topology B		
1-6	1-19-No	Allopatric fragmentation
2-1(V2)	1-2-11-12-No	Contiguous range expansion
Total Cladogram	1-2-11-12-No	Contiguous range expansion
Topology C		
1-6	1-2-11-12-No	Contiguous range expansion
Total Cladogram	1-2-11-12-No	Contiguous range expansion
Topology D		
1-6	1-19-No	Allopatric fragmentation
2-2	1-19-20-2-3-4-No	Restricted gene flow with isolation by distance
Total Cladogram		tip/interior status could not be determined

5.4 Discussion

Table 5.4 provides estimates for the divergence times of the different haplotypes identified in New Zealand *N. menziesii*. When interpreting these divergence times limitations of the data in this study should be considered. To calibrate the molecular clock it was assumed that *N. moorei* and *N. cunninghamii* diverged 20 mya. This divergence time estimate is based on fossil calibrations and, although Knapp *et al.* (2005) were able to show that the age of this divergence was unlikely to be much older than 20 million years, it still has to be regarded as a minimum age (Heads, 2006). Furthermore the age estimates provided here are age estimates for the divergence of haplotypes, not populations. It is unknown how long after the haplotypes formed the populations diverged (Knowles and Maddison, 2002). Thus the date estimates can provide only a tentative temporal framework for discussion of population divergence times (Graur and Martin, 2004).

5.4.1 *Lophozonia* population and haplotype history

The oldest divergence among extant New Zealand *N. menziesii* haplotypes is of Late Miocene or Early Pliocene age, marking the root of the phylogenetic tree of *N. menziesii* haplotypes (Figure 5.4). As the most basal haplotypes on both sides of the root can be found in the Nelson – Northern Westland region, it is conceivable that primary divergence of extant *N. menziesii* haplotypes began in that area. The NCA inferred contiguous range expansion as the cause of an early population divergence (but see below). The divergence date coincides with acceleration in the uplift of the Southern Alps (Walcott, 1979, 1998; McGlone *et al.*, 1996). *N. menziesii* is adapted to wet and cold, montane habitats (Ogden *et al.*, 1996). Thus it appears conceivable that newly available habitats of that type especially along the West Coast of the South Island, supported range expansion and possibly also the inner-specific differentiation of *N. menziesii*.

In the Mid Pliocene the populations with haplotypes that were to become the southern lineage of *N. menziesii* diverged again. Tectonic events in connection to the uplift of the Southern Alps might again have played a major role in this development and could have isolated populations as well as haplotypes. While one of these two new lineages can today be found in the Paparoa Ranges (VIII and IX), the other is now the sole extant lineage south of the Central Westland and Central Canterbury beech gaps (VII), being found in only one accession from the Southern Nelson region (VI).

Haplotype VI is basal to the very southern haplotype VII. Haplotype VI and VII are today separated by the Central South Island beech gaps. This divergence has been estimated to be of Early Pleistocene age. This period coincided with the onset of cooler annual temperatures and repeated heavy glaciation of the Central South Island and this event might have induced population and haplotype divergence.

Molecular clock estimates of divergence times within the lineage that today occupies the northern half of New Zealand (haplotypes I – V) suggest that haplotypes I, II, and III diverged in the Early Pleistocene. Divergence times for haplotypes III, IV, and V were not estimated. They only differ by the presence of direct sequence repeats which often evolve faster than nucleotide substitutions (Golenberg *et al.*, 1993). The divergence of populations with haplotypes IV and V from populations with haplotypes I, II, and III was inferred to have resulted from "restricted gene flow with isolation by distance". This finding is consistent with the generally low dispersal distances of *Nothofagus* seeds (Ogden *et al.*, 1996).

Populations bearing haplotypes I, II and III appear to have diverged through geographic isolation. Haplotype I (southern North Island) and haplotype II (northern North Island) are both the sole haplotypes in their respective populations. Today they are separated by the Manawatu beech gap. In the Late Pliocene these two regions would have been isolated from each other by a wide sea strait (Manawatu Strait) (McGlone, 1985; McGlone *et al.*, 2001; see also Chapter 2, Figure 2.10). The main southern North Island ranges began to form over the last 2 million years (King, 2000). However, the sea strait seems to have persisted until at c. 1 mya when the Ruahine and Kaweka Ranges began rapid uplift (Beu *et al.*, 1981). Furthermore the rapid growth of the southern North Island mountain ranges in the last 2 million years raises the possibility of isolation of populations of montane adapted *N. menziesii* in the taller southern and northern portions of the axial chain, with the relatively low-lying and late-forming central area acting as a partial barrier. With only one mutation separating both haplotypes, an accurate divergence time cannot be estimated. Geographic isolation through a Late Pliocene/Early Pleistocene sea strait or through Pleistocene glacial climates coupled with differential rates of mountain growth along the axial ranges, remain possible explanations for the origin of the Manawatu beech gap. Populations with haplotypes II and III are today separated by the Cook Strait which would have isolated North Island and South Island populations during interglacials from about the Mid Pleistocene on (McGlone, 1985). "Allopatric fragmentation" of populations with

haplotypes I, II and III was inferred from the NCA. This finding would also be consistent with population divergence through geographic isolation.

While cpDNA haplotypes follow a distinct geographic pattern, pollen mediated nuclear geneflow in *N. menziesii* appears to be much less restricted. Stöckler (2001) studied Amplified Fragment Length Polymorphism (AFLP) profiles of 220 accessions of *N. menziesii* from all over New Zealand. The fixation index (F_{st}) was calculated to test the assumption of random mating between individuals of all regions and measure geneflow ('migration') between populations. 5 different AFLP haplotypes were identified that showed local geographic structure. Migration rates were particularly high between adjacent regions, indicating that geneflow has been most frequent across Cook Strait and between the central and lower North Island populations. Geneflow decreased in correlation with the greater distances between the disjunct populations.

5.4.2 *Fuscospora* species, population and haplotype history

Only two distinct chloroplast haplotypes were identified in *Fuscospora* beeches. These differed as the result of 1 bp indel in the *trn*L-*trn*F intergenic spacer region. Although taxon sampling was limited (few accessions and for only two *Fuscospora* species) south of the Westland/Canterbury beech gap, our finding of a distinct southern haplotype in two *Fuscospora* species confirms earlier observations of Thomsen (2002). He sequenced the trnL region in 25 accessions of *Fuscospora* across its New Zealand distribution and found that haplotype B occured in all three *Fuscospora* species south of the Westland/Canterbury beech gap. This finding of distinct haplotypes shared by closely related tree species has been reported previously in other plant families (e.g. Ferris *et al*. 1993; Petit *et al*. 1997; McKinnon *et al*. 2004) and is suggestive of hybridisation/introgression.

The two haplotypes are geographically separated by the central South Island beech gaps. As mentioned above, divergence times cannot be estimated for haplotypes that only differ by sequence repeats. If both haplotypes would differ by one mutation their divergence time would be estimated at approximately 1.5 mya based on the substitution rate estimated for the *N. menziesii* dataset (0.00014 – 0.00015 substitutions per site per million years). As described above, sequence repeats are expected to occur more frequently than nucleotide substitution. Thus the real divergence time between both haplotypes is most likely even younger than this. Again

this would suggest that Pleistocene glaciation of the central South Island might have shaped the distribution pattern that can be observed in the South Island today.

No genetic differentiation of North Island populations was found. This could be due to the lack of polymorphism of the used markers in the *Fuscospora* beeches. Alternatively it could be explained by the fact that the Manawatu Gap which separates the two North Island haplotypes of *N. menziesii* is only a partial gap for *Fuscospora*. Isolated stands of *N. solandri* can be found near the Manawatu Gorge and in Pohangina Valley about 20 kilometres north of the Gorge. These stands could have been more extensive during earlier interglacials and interstadials and could have served as bridges between the main populations north and south of the Manawatu Gap. Also, the much lower level of divergence within New Zealand *Fuscospora* compared to *N. menziesii* could suggest that extant *Fuscospora* haplotypes are much younger than *N. menziesii* haplotypes, possibly due to a more recent population bottleneck (see Chapter 6). It is conceivable that the ancestor of present day haplotypes (which could easily be one of the two haplotypes observed in this study) only originated in the Mid to Late Pliocene. *N. solandri*, *N. fusca* and *N. truncata* appear to be genetically closely related (Knapp, unpublished results). Therefore it seems a plausible hypothesis that the ancestral haplotype comes from a single species that is ancestral to all three extant species and that existed some time in the Pliocene. This species would have spread from a centre of origin and speciated into the present day *Fuscospora* beeches of New Zealand. This would have probably occurred by occupying niches that became available through extinction of species that were less adapted to the harsher Pleistocene climate (see Chapter 2).

5.4.3 Test for influence of sample density on the NCA

A test for influence of sample density on the NCA was conducted to test for the robustness of NCA inferences. It had two interesting outcomes. First it shows that sample density can have an influence on the inferences of the NCA although the original sample size was not identified as inappropriate. Instead of inferring an inconclusive outcome for the smaller sample size, the inference key suggested a demographic process. A similar concern about incorrect inferences has been raised by Knowles and Maddison (2002).

Furthermore the demographic event inferred as cause for population differentiation on the "total cladogram" level changed from "contiguous range expansion" to "past fragmentation" of population fragments. The main difference between these two inferences is that under "past fragmentation" extant haplotypes of clades 2-1, 2-2, and 2-3 would have initially diverged, for example through historic isolation, and subsequently spread from three different centres of origin to reach their present day distribution. Under "contiguous range expansion" they would have diverged while spreading from a single centre of origin. Considering the biological and geographical context "past fragmentation" does not appear to be unlikely. The larger than average mutational difference between the northern and the southern haplotypes are more consistent with past fragmentation than contiguous range expansion. Furthermore the dynamic geological developments along the West Coast of the South Island that started at the end of the Miocene could have supported population fragmentation. Although the samples that were used to increase the sample density were fictional, they came from actual *N. menziesii* locations. All actual samples from the Southland and Otago region had haplotype VII. Hence it is a reasonable assumption that the fictional samples shared this haplotype. More samples from the less accessible regions of Southland would be needed to test this hypothesis.

5.4.4 Alternative TCS network resolutions and their impact on NCA inferences

As illustrated in the present study, inferences of a NCA are dependent on the topology of the TCS network used as input data. As also illustrated, a TCS network can sometimes contain internal ambiguities which represent equally parsimonious topologies. These different equally good topologies may lead to different NCA inferences. Hence it is desirable to select the "best" resolution of ambiguities observed in the TCS network. Several empirical criteria derived from coalescent theory have been suggested by Crandall and Templeton (1993) as being useful for identifying the optimal resolution. These criteria were used to identify the resolution shown in Figure 5.7. However, there were also features of the sequences that suggested other alternative resolutions as being more likely. Clearly this uncertainty should be considered when evaluating the robustness of NCA inferences.

6

Conclusion and future work

6.1 Goodbye Gondwana?

The extent to which the flora and fauna of New Zealand have a direct unbroken link with Gondwana is a question that has been controversially discussed for decades (i.e. Darlington, 1965; van Steenis, 1971; Mildenhall, 1980; Hill and Jordan, 1993; Pole, 1994; Linder and Crisp 1995; McGlone *et al.*, 1996; Macphail, 1997; Craw *et al.*, 1999; Crisci, 2001; Nelson and Ladiges, 2001; Stöckler *et al.*, 2002, McCarthy, 2003, Sanmartin and Ronquist, 2004; Knapp *et al.*, 2005; Cook and Crisp, 2005; McGlone, 2005, Renner, 2005; Heads, 2006, Waters and Craw, 2006 and many more). Is it time to say 'Goodbye Gondwana'?

This thesis reports the first rigorous molecular clock studies on the New Zealand forest that test hypotheses of transoceanic dispersal and vicariance. Results indicate that some but not all relationships between different Southern Hemisphere taxa can be explained by dispersal. That is, while multiple transoceanic dispersal events are the most likely explanation for relationships between Australian and New Zealand *Nothofagus* species, an hypothesis of vicariance (i.e. the divergence of populations caused by the breakup of Gondwana) cannot be rejected as an explanation for the relationships between Australian and South American *Nothofagus* species. Similarly, vicariance might also explain the relationship between Australian and New Zealand *Agathis* species.

These findings are significant in a number of ways. It has been regarded that *Nothofagus* is incapable of transoceanic dispersal (Preest, 1963). Thus if robust molecular clock analyses indicate dispersal, this finding in itself lends support to other inferences of transoceanic dispersal, suggested for other elements of the New Zealand flora (Winkworth *et al.*, 2002, Wagstaff *et al.*, 2002). It is not yet clear whether failure to reject vicariance as an explanation for the relationship between Australian and South American *Nothofagus* species, and for the relationship between Australian and New

Zealand *Agathis* species, is the result of a lack of resolution in the data, or whether it suggests possible complex biogeographic scenarios.

An interesting finding from molecular clock studies reported in this thesis is the apparent discrepancy between the fossil pollen record of New Zealand *Lophozonia* beeches and the divergence time estimates for New Zealand and Australian *Lophozonia* species. Both suggest transoceanic dispersal. However, if the clock estimates are accurate and the divergence of Australian and New Zealand Lophozonia species is indeed much younger than the first appearance of *Lophozonia* in the pollen fossil record of New Zealand, then the discrepancy might indicate successive dispersal events of *Lophozonia* beeches to New Zealand. Similar conclusions can be drawn from the comparison of divergence time estimates for New Zealand and Australian *Fuscospora* beeches. An inference of successive dispersal events is also strengthened by reports of the Oligocene Tasmanian macrofossil species *Nothofagus cethanica* (Hill, 1984), which shares unique features with extant New Zealand *Fuscospora* species. Divergence time estimates between Australian and New Zealand *Fuscospora* beeches are consistent with the age of *N. cethanica*. They are significantly younger than the first appearance of *Fuscospora* pollen in the New Zealand fossil record.

Assuming that dispersal is important for explaining Southern Hemisphere *Nothofagus* distributions, the likelihood that a particular taxon is on any given Southern Hemisphere landmass will presumably depend on a) time available for dispersal, b) dispersal ability, and c) opportunities for establishment (Nathan, 2006).

Regarding the time available for dispersal, it can be assumed that if long-distance dispersal is a highly stochastic process (Nathan, 2006), then in 80 million years (i.e. the time since New Zealand separated from Gondwana (Wilford and Brown, 1994; McLoughlin, 2001)) we might expect at least some events of long-distance dispersal between New Zealand and other landmasses.

The apparently poor dispersal abilities of *Nothofagus* have contributed greatly to the assumption that they are incapable of transoceanic dispersal (see Chapter 1). However, since the 1960s (Preest, 1963) little research has been published on putative modes of long distance dispersal for *Nothofagus* seeds. Considering that the nuts of *Nothofagus* are associated with scaly to spiny, almost hooked cupules (Figure 6.1), one possible hypothesis that is worthy of further investigation is that these cupules could in rare cases support epizoochory (i.e. animal related transport where the seeds

are attached to the body, hair or feathers of the vector). Birds have been implicated as possible vectors of epizoochoric dispersal in various biogeographic studies (Darwin, 1859; Carlquist and Pauly 1985; Lowrey, 1995; Carlquist, 1996).

Figure 6.1: *Nothofagus menziesii* nut (bottom) and cupule (top). The distance between two lines on the ruler is 1 mm.

As a result of tectonic and climatic changes throughout the Tertiary (Chapter 2) the opportunities for establishment of newly arrived *Nothofagus* species in New Zealand are likely to have changed greatly over time. Results from molecular clock analyses presented in Chapter 3 suggest that New Zealand and Australian *Nothofagus* species diverged in the Mid Oligocene to Mid Miocene. Whilst a complete drowning of New Zealand during the Oligocene appears unlikely (Chapter 4), extinction of species might have been widespread as a result of the severe reduction of land and habitats (Cooper and Cooper, 1995). Once New Zealand lands re-emerged following the Oligocene, unoccupied ecological niches would have been available for newly arrived species. The existence of these new niches would have increased the likelihood of establishment of *Nothofagus* propagules.

Similarly, the tectonic events and climate changes of the Late Pliocene and Pleistocene might also have opened up new habitats and supported the diversification of *Fuscospora* beeches in New Zealand. It remains an open question as to why the *Lophozonia* beech do not display the extant diversity of species seen with *Fuscospora*

beech. The high genetic diversity of *Nothofagus menziesii*, and the low genetic diversity of *Fuscospora* beech, but higher species number of the latter, suggest that *N. menziesii* was able to persist in locations in which *Fuscospora* beech was eliminated as due to climate changes. This inference is consistent with the finding that, if only one species of *Nothofagus* is present in the Pleistocene fossil record of a given area, it is almost invariably *Nothofagus menziesii* (McGlone *et al*., 1996).

The combination of nested clade analyses and molecular clock analyses have provided a useful framework for reconstructing the phylogeographic history of *Nothofagus menziesii* and correlating different phases of range expansion and population divergence to contemporary environmental factors. Much of this history is correlated with the Late Pliocene/Pleistocene period and as such the findings are consistent with those from Europe and the Americas (Demesure *et al*., 1996; Comes and Kadereit, 1998; Ferris *et al*., 1998; Abbott *et al*., 2000; Petit *et al*., 2003; Hewitt, 2004; Soltis *et al*., 2006 and others). These studies implicate the importance of Pleistocene climate changes for understanding extant patterns of plant biodiversity.

6.2 Future Work

It is too soon to say 'Goodbye Gondwana'. Studies are still needed to better understand the origins of New Zealand's forest flora. As suggested from fossil evidence (Chapter 2) important taxa for which molecular clock studies are needed include the Podocarpaceae, *Knightia* (Proteaceae), and *Beilschmiedia* (Lauraceae). Given the variance in divergence times for different chloroplast loci, additional markers (nuclear, mitochondrial and chloroplast) and studies on *Agathis australis* would also contribute to the current debate.

The findings from molecular systematic studies over the last 10 years, identifying transoceanic dispersal as predominant feature explaining plant distributions (i.e. Carlquist, 1996; Renner *et al*., 2000; Winkworth *et al*., 2002; Zhang and Renner, 2003; Renner, 2004; Wagstaff, 2004; Cook and Crisp, 2005; Winkworth *et al*., 2005; Waters and Craw, 2006; Cowie and Holland, 2006) highlight the need to better understand mechanisms of dispersal. Little is still understood about this phenomenon. An integrative approach is needed that brings together a) an understanding of past climatic patterns (i.e. Sanmartín *et al*., in press), b) date estimates, and c) knowledge of dispersal abilities of selected taxa. Further studies are needed to address each of these aspects.

One of today's biggest questions in plant biogeography is the effect of climate change on potential forest distribution. New Zealand is currently a fertile testing ground for environmental modelling of forest patterns (Leathwick, 2001; Hall and McGlone, 2006). These models are usually based on the relationship between extant species and current environmental conditions. Often they cannot exactly predict the observed distribution of taxa because historic events like glaciation or disturbance due to tectonic events and volcanism are not included in the simulations (Hall and McGlone, 2006). The historical distribution of taxa can be inferred from the fossil record if enough data is available. As shown in this thesis historic phylogeography and population development can also be inferred from molecular data using various statistical approaches. Results from phylogeographic analyses of New Zealand *Nothofagus* species presented here can supplement information used to model past and future New Zealand forest distribution. Further research is needed to reconstruct the phylogeography and phylogeographic history of other important New Zealand forest taxa like the podocarps. This would create opportunities to more accurately model the present day forest pattern and interpolate its future development given predicted climate change.

Willerslev *et al.* (2003) and Haile *et al.*, (in press) have recently raised expectations that a better understanding of present day plant distributions can also be obtained through analyses of the plant remains in Pleistocene soils. These authors were able to amplify and sequence ancient DNA from Siberian permafrost cores as well as from temperate New Zealand cave sediments. If reproducible, these findings raise the prospect for assessment of studying the change in both the genetic diversity and distribution of plant species over time (Wayne *et al.*, 1999).

Appendix I

General Materials and Methods: DNA extraction, amplification and sequencing

A1.1 Materials

A1.1.1 Laboratory equipment

Article	Source
Microcentrifuge Biofuge Fresco or Picofuge	Heraeus
Transilluminator (wavelength: 302 nm)	UVP Incorporated
GelDOc2000 Gel Documentation System	BIO-RAD
AB3730 capillary sequencer	Applied Biosystems
PTC-200 DNA Engine	MJ Research, U.S.A.
PTC-150 Minicycler	MJ Research, U.S.A.
T1 Thermocycler or T Gradient	Whatman Biometra
Dry bath incubator	Boekel Scientific
Milli-Q Ultrapure Water System	Millipore

A1.1.2 Chemicals and reagents

Article	Source
Sybrsafe	Invitrogen, USA
Ethidium Bromide	Sigma, USA
bromophenol blue	Serva, Germany
cetyl trimethyl ammonium bromide (CTAB)	Sigma, U.S.A.
chloroform	BDH, England
deoxynucleotide 5'-triphosphate [dNTP]	Boehringer Mannheim, Germany
ethanol p.A. 99.8-100%	BDH, England
Ethylenediaminetetra-acetic acid [EDTA]	BDH, England
formaldehyde (37%)	Sigma, U.S.A.
isopropanol	BDH, England
LE agarose	Boehringer Mannheim, Germany
polyvinyl-pyrrolidone (PVP)	Sigma, U.S.A.
silica gel (6-8 mesh)	BDH, England
sodium acetate	BDH, England
sodium carbonate (Na_2CO_3 anhydrous, AnalaR)	BDH, England
sodium chloride (NaCl)	BDH, England
sodium hydroxide (NaOH)	Sigma, U.S.A.
sodium thiosulphate ($Na_2S_2O_3$,).	BDH, England
Tris-acetate	Invitrogen U.S.A.

A1.1.3 Kits and ready-to-use products

Article	Source
Dye Terminator Sequencing Mix (Big Dye 3.1)	Applied BioSystems, U.S.A.
QiaQuick Gel Extraction Kit	Qiagen, Germany
QiaQuick PCR Purification Kit	Qiagen, Germany
CleanSeq	Agencourt, USA
Qiagen DNeasy Plant kit	Qiagen, Germany

A1.1.4 Enzymes

Enzyme	Source
Shrimp alkaline phosphatase (SAP)	USB Corporation USA
E. coli exonuclease I (Exo I)	USB Corporation USA
Taq polymerase	Roche, AB gene
RNase A	Sigma, U.S.A.

A1.1.5 DNA markers

Article	Source
100 bp DNA Ladder	Invitrogen, U.S.A.
1kb DNA Ladder	Invitrogen, U.S.A.
1kb plus DNA ladder	Invitrogen, U.S.A.
Low DNA Mass™ Ladder	Invitrogen, U.S.A.

A1.1.6 Software

Software program	Source
ClustalX 1.81	ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/
T3 package	http://abacus.gene.ucl.ac.uk/
Phylip 3.66 package	http://evolution.genetics.washington.edu/phylip.html
r8s 1.60	http://ginger.ucdavis.edu/r8s/
Paup* 4.0b10	Sinauer Associates, Inc. Publishers, U.S.A (no freeware)
BioEdit 5.0.9	http://www.mbio.ncsu.edu/BioEdit/bioedit.html
Oligo4	http://www.oligo.net/ (no freeware)
PAML	http://abacus.gene.ucl.ac.uk/software/paml.html
TCS 1.21	http://darwin.uvigo.es/software/tcs.html
GeoDis 2.5	http://darwin.uvigo.es/software/geodis.html
ArcGIS	http://www.esri.com/software/arcgis/ (no freeware)
Network 4.200	http://www.fluxus-engineering.com/sharenet.htm
Modeltest 3.7	http://darwin.uvigo.es/software/modeltest.html
Treeview 1.6.6	http://taxonomy.zoology.gla.ac.uk/rod/treeview.html

A1.1.7 Oligonucleotides

Oligonucleotides, used throughout this study as Polymerase Chain Reaction (PCR) primers, were obtained from either Sigma Aldrich or Invitrogen. Each primer was rehydrated to a final concentration of 1 nmol/µl and stored at –80°C. A working stock, with concentration of 10 pmol/µl, was prepared from this stock and stored at -20°C.

A1.1.7.1 Oligonucleotides taken from literature

Table A1.1: Primers taken from literature.

name	orientation	sequence (5' - 3')	reference
***atp*BFI**	*atp*B 3'	TAT GAG AAT CAA TCC TAC TAC TTC T	Savolainen *et al*., 2000
***atp*BRI**	*atp*B 5'	TCA GTA CAC AAA GAT TTA AGG TCA T	Savolainen *et al*., 2000
tabC	*trn*L intron 3'	CGA AAT CGG TAG ACG CTA CG	Taberlet *et al*., 1991
tabD	*trn*L intron 5'	GGG GAT AGA GGG ACTT GAA C	Taberlet *et al*., 1991
tabE	*trn*L - *trn*F spacer 3'	GGT TCA AGT CCC TCT ATC CC	Taberlet *et al*., 1991
tabF	*trn*L - *trn*F spacer 5'	ATT CGA ACT GGT GAC ACG AG	Taberlet *et al*., 1991
trnK5F	*trn*K 5'	GGG TTG CTA ACT CAA CGG TAG AG	Gadek *et al*. 2000 (modified from primer *trn*K3914F)
trnK3AR	*trn*K 3'	CGT ACA STA CTT TTG TGT TTM CG	Gadek *et al*. 2000 (modified from primer *515-2150R*)
trnD	*trn*D gene	ACC AAT TGA ACT ACA ATC CC	Demesure *et al*., 1995
trnT	*trn*T gene	CTA CCA CTG AGT TAA AAG GG	Demesure *et al*., 1995
trnE	*trn*E gene	AAC CGC TAG ACG ATG GGG GC	Thomsen, 2002

A1.1.7.2 Oligonucleotides specifically designed for this study

Primers that had to be designed for this study include all primers used to amplify the *atp*B - *psa*I region of the chloroplast genome of *Nothofagus* (Chapter 3) except for *atp*BFI and *atp*BRI (Table A1.1) which were taken from Savolainen *et al.* (2000). The location of these primers relative to the *atp*B - *psa*I fragment is shown in Figure A1.1. To amplify the *mat*K gene of the Araucariaceae chloroplast genome (Chapter 4) the primer combination *mat*K5F - *mat*K3AR (modified by Lockhart (pers. com) from Gadek *et al.*, 2000; see Table A1.5) was used. However, the sequences generated from the *mat*K5F and *mat*K3AR primers did not cover the whole fragment amplified with this primer combination. Therefore, the nested primers *mat*K5FintF and *mat*K3ARintR were designed to complete the sequence data for the Araucariaceae *mat*K gene.

Table A1.2: Primers designed specifically for this study.

name	orientation and location	sequence (5' end to 3' end)	specificity
Nothofagus			
***atp*BSII**	*atp*B internal 5'	CCA TTT CNG TRC YAA GRG TNG GYT GAT ARC	Angiosperms
***atp*BAI**	*atp*B internal 3'	GAY AAT ATY TTY CGN TTY GTY CAA GCR GGA TC	Angiosperms
***atp*BgapSI**	*atp*B – *rbc*L spacer 5'	GAA ATC MAA AAT AAA TGT TCG ATA GCA AAKC	*Nothofagus*
***rbc*LSI**	*rbc*L 5'	GAA GAA GGT TCT GTT ACT AAC ATG	*Nothofagus*
***rbc*LAI**	*rbc*L 3'	GGA CCT TGR AAA GTT TTA ACA TAA GC	*Nothofagus*
***rbc*LSIII**	*rbc*L 5'	GCT CTA GAA GCA TGT GTA CAA GC	*Nothofagus*
***rbc*LAIII**	*rbc*L 3'	CAT TTR CSA GCC TCA CGT ATA ATT TCA	*Nothofagus*
***rbc*LgapvV**	*rbc*L - *acc*D spacer 5'	ATT CCA TCA TAT TAA GCG GTA CT	*Nothofagus*
***acc*DSI**	*acc*D 5'	AAR CTC TAT GRA AAR ATS GTG GTT HAA TTY DAT RT	Angiosperms
***acc*DAI**	*acc*D 3'	GTA TTK TCA ATA GGA YCA AGA CTK YYG ATT G	Angiosperms
***acc*DintF**	*acc*D internal 5'	CTT TTT TAG TTA GCG ATA ATA ATA GG	*Nothofagus*
***rbc*LgapvR**	*acc*D internal 3'	TAT TCC AAC TAT ATT TAG TAT CAT AC	*Nothofagus*
***acc*DvV**	*acc*D internal 5'	AGR GGA ACC TTA CAA AGA TCG TAT	*Nothofagus*
***acc*DintR**	*acc*D internal 3'	AAG CGG AAG AGA TTT TAG CCA TT	Angiosperms
***acc*DSII**	*acc*D 5'	GCW TTT GCR GGT AAA AGR GTR ATT GAAC	Angiosperms
***acc*DAII**	*acc*D 3'	GTT CAA TYA CYC TTT TAC CYG CAA ASGC	Angiosperms
***acc*DvR**	*acc*D – *psa*I spacer 3'	TAT CCG CAC GAT TCT TTC TAC AAT	*Nothofagus*
***psa*IAI**	*acc*D – *psa*I spacer 3'	ATR AAG AAA TAA RGA AGY CAT TGC	Angiosperms
Araucariaceae			
***mat*K5FintF**	*mat*K internal 5'	TTT GTT CTT CCT TAT GAC GAA TGA	Araucariaceae
***mat*K3ARintR**	*mat*K internal 3'	ATG GAT TCG CAT TCA TAA ACA TAA GA	Araucariaceae

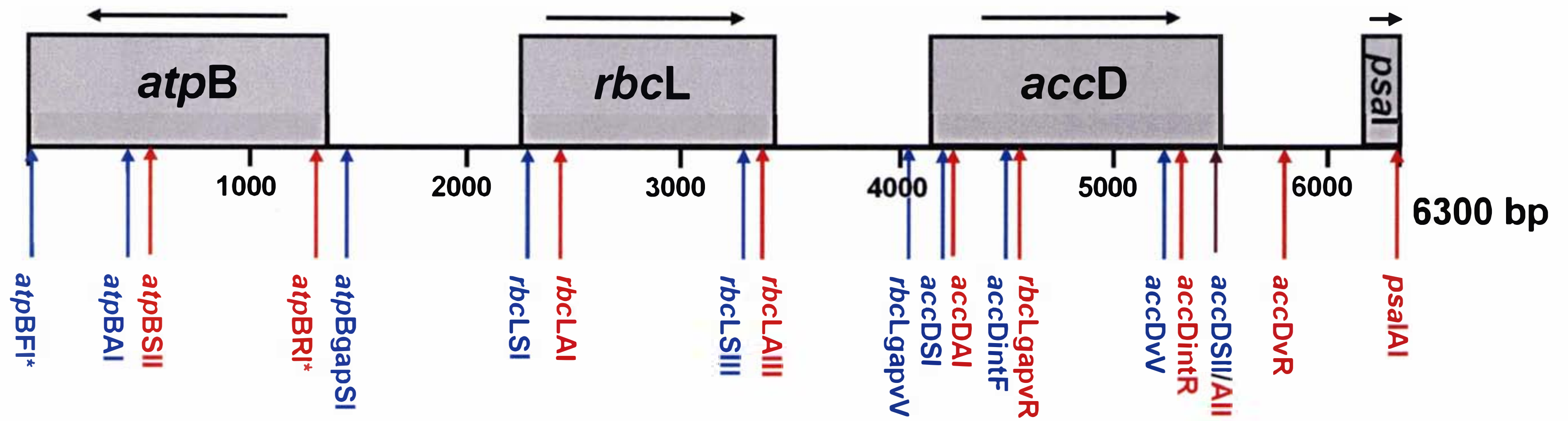

Figure A1.1: Location of primers specifically designed for this study.
Blue primers = forward with respect to the whole fragment. Red primers = reverse with respect to the whole fragment. Black arrows = direction of gene (5' end to 3'end). * These primer sequences were taken from literature (see Table A1.1)

A1.2 Methods

A1.2.1 Development of primers

A1.2.1.1 Primers for the study: "The trans-oceanic relationships of the genus Nothofagus"

An assemblage of sequence data covering the *atp*B – *psa*I region of a range of angiosperm species was obtained from GenBank (see Appendix III/ data CD). These were aligned either manually (for sequences that slightly overlapped with neighbouring sequences) or by using the program ClustalX 1.81 (Thompson *et al.*, 1997) (for different sequences of the same gene or intergene region).

The reference sequences were chosen based on their phylogenetic proximity to the taxa under investigation. The alignment was edited using BioEdit 5.0.9 (Hall, 1999) and manually searched for conserved regions. Provisional primers were designed to anneal to these conserved regions. A combination of forward and reverse primers were placed approximately every 1000 base pairs. The reverse primer was designed to anneal behind the forward primer of the next PCR fragment (approximately 70 – 100 base pairs 3' of the forward primer) to achieve a small overlap of adjacent sequences (Figure A1.1).

The provisional primers were then tested for false priming sites, stability of secondary structures and melting temperature using Oligo 4.0 (National BioSciences USA) and edited if necessary.

A1.2.1.2 Primers for the study: "The drowning of New Zealand and the problem of Agathis"

The primers *mat*K5FintF and *mat*K3ARintR for the Oligocene drowning study were designed using the same approach as described above. As reference an alignment of sequences from the *mat*K5F and *mat*K3AR primers were used.

A1.2.2 DNA extraction from plant material

DNA was extracted from fresh and dry plant tissue, using two different methods depending on age and character of the sample. For fresh tissue a protocol from Doyle and Doyle (1987) was used, while for dry plant tissue; herbarium samples, and multiple

samples, the Qiagen DNeasy Plant kit for DNA extraction was chosen to save time and obtain a better yield.

The method of Doyle and Doyle (1987) uses cetyl trimethyl ammonium bromide (CTAB) to bind DNA and chloroform to extract the DNA-CTAB complex from cell debris.

Polyvinyl-pyrrolidone (PVP, Mw=25 000) was used in CTAB extraction buffer for DNA extractions from plant material. CTAB was added to a sterile buffer stock solution immediately before use and allowed to dissolve at 60°C.

A1.2.2.1 Procedure for plant DNA extraction using CTAB based buffer for DNA extraction

Table A1.3: Composition of CTAB-based buffer used for DNA extraction
CTAB buffer:
1% [w/v] PVP 25 000
1.4 M NaCl
100 mM Tris-HCl [pH 8]
20 mM EDTA
1% [w/v] CTAB (added immediately before use)

1. Plant tissue was cut into small pieces and 20 – 30 mg was put into a 1.6 ml microcentrifuge tube using clean tweezers.
2. Plant tissue fragments were then snap frozen in liquid nitrogen by simply dispensing some liquid nitrogen into the microcentrifuge tube.
3. Liquid nitrogen was left to evaporate and frozen samples were grinded using a clean grinder. If necessary the sample was refrozen by suspending the tube into liquid nitrogen.
4. 600 µl of freshly prepared CTAB extraction buffer was added to the tube and mixed with the finely ground sample tissue.
5. The microcentrifuge tube was placed in a dry bath incubator at 65°C, incubated for 40 – 60 minutes and occasionally mixed by inversion.
6. The tube was then allowed to cool down for c. 5 minutes.
7. 600 µl of chloroform was added and mixed by repeated inversion.
8. This mixture was then left to incubate for 2-3 minutes at room temperature to allow the separation of aqueous and organic phase.

9. The tube was then centrifuged at 10 000 × g in a microcentrifuge at room temperature for 1 minute, to completely separate the phases and compact the interface.
10. The upper aqueous phase of the extraction was transferred to a new 1.6 ml microcentrifuge tube (pipette tip was cut off to increase the size of the opening of the pipette and minimise possible mechanical shearing of the DNA due to pipetting).
11. The DNA was precipitated with an equal volume of isopropanol and mixed by gently inverting once
12. The DNA was collected and transferred into a new tube.
13. The DNA pellet was washed twice with 1 ml 80% (v/v) ethanol to remove salt residues.
14. The DNA pellet was air dried and then resuspend in 25 – 40 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) or MilliQ H_2O.

A similar protocol was followed when the Qiagen DNeasy Plant kit for DNA extraction was used. To improve the quality of the DNA obtained it is filtered through a column after tissue lysis to more effectively remove cell debris and then precipitated with isopropanol, bound to a membrane and washed with Ethanol.

A1.2.2.2 Procedure for plant DNA extraction using Qiagen DNeasy Plant kit

Steps 1 – 3 were followed as described above. Further steps were performed as described in the protocol provided in the kit. The last step of the protocol (Elution of DNA from the membrane) was modified to increase the yield. Two elution steps using 50 µl elution buffer were performed, each into separate 1.6 ml microcentrifuge tubes. For the first elution, the buffer (at 65°C) was poured onto the membrane and left to incubate for 10 minutes at room temperature before DNA and buffer were collected by centrifugation at 12 000 × g for 5 minutes. For the second elution this procedure was repeated with the same membrane with an incubation time of 30 minutes.

For both methods horizontal agarose gel electrophoresis was used to determine size and mass of the obtained DNA fragments. A 3 µl aliquot of each elution was run on a 1% agarose gel following standard protocols as described in Sambrook *et al.* (1989)

DNA samples were mixed with 1 µl of 10× loading buffer (27.5% [w/v] Ficoll Ty 400, 0.44% [w/v] bromophenol blue and 0.44% [w/v] xylene cyanol) and the mixture

loaded onto the agarose gel. The 1kb plus™ DNA ladder was routinely included on electrophoretic gels as size standards. Samples were electrophoresed at 3-5 V/cm in 1× TAE buffer for approximately 60 min.

Following electrophoresis the DNA samples were visualised by ethidium bromide or SyBrSafe fluorescence on a UV transilluminator (wavelength 302 nm). A digital photograph of the illuminated gel was taken using the BioRad Gel documentation System

A1.2.3 Amplification of DNA regions using a one-step PCR protocol

PCR reactions were carried out in a total volume of 20 µl containing: 1 U *Taq* polymerase, 1 x PCR buffer (Tris-HCl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, (pH 8.7); supplied by the manufacturer), 10 pmol of each primer, 250 µM of each dNTP and 10-100 ng DNA template. Taq polymerase from Roche was supplemented by 1M Betaine. When Red Hot Taq (from ABGene) was used, 25mM $MgCl_2$ was added to the reaction to a final concentration of 1.5mM (1.2ul in a 20ul reaction). The thermocycling protocol for each set of primers is provided in Table A1.4. Thermocycling was carried out using a heated lid to prevent evaporation of the reaction mixture.

Following thermocycling, 3µl aliquots of each amplification were electrophoresed as described in Section 2.2 to determine the efficacy of individual reactions by comparison to the DNA mass and size ladder. For this purpose a Low DNA Mass™ Ladder was added in addition to the 1kb plus™ DNA ladder.

Table A1.4: PCR mix with different types of Taq

PCR reaction mix with Roche Taq		PCR reaction mix with Invitrogen Red Hot Taq	
MilliQ H_2O	8.3 µl	MilliQ H_2O	10.8 µl
Betaine	4.0 µl	$MgCl_2$	1.2 µl
10 x reaction buffer	2.0 µl	10 x reaction buffer	2.0 µl
dNTPs (total)	2.5 µl	dNTPs (total)	2.5 µl
5' end primer	1.0 µl	5' end primer	1.0 µl
3' end primer	1.0 µl	3' end primer	1.0 µl
DNA (in appropriate dilution/ 10 – 100 ng)	1.0 µl	DNA (in appropriate dilution/ 10 – 100 ng)	1.0 µl
Taq	0.2 µl	Taq	0.2 µl
Total volume	20 µl		20 µl

Table A1.5: Thermocycling protocols for amplification of chloroplast gene regions

amplified region	primer pair (5' – 3')	PCR program
The trans-oceanic relationships of the genus *Nothofagus*		If not described otherwise the PCR program was: 94 °C for 2 min [(94°C for 1 min; AT °C for 1 min; 72 °C for 3 min) 35 cycles] 72 °C for 5 min; hold at 4 °C; AT: annealing temperature; Ramping rate: 1°C/second
*atp*B gene partial sequence	*atp*BRI - *atp*BAI	AT: 46.6°C
*atp*B gene partial sequence	*atp*BSII - *atp*BFI	AT: 44.6°C
*atp*B – rbcL intergene region	*atp*BgapSI - *rbc*LAI	AT: 50.0°C
*rbc*L gene	*rbc*LSI - *rbc*LAIII	AT: 46.6°C
*rbc*L – *acc*D intergene region	*rbc*LSIII - *acc*DAI	AT: 45.2°C
*acc*D gene partial sequence	*acc*DSI - *acc*DAII	AT: 46.6°C
*acc*D – *psa*I intergene region partial sequence	*acc*DSII - *psa*IAI	AT: 50.0°C
5' end of *acc*D gene	*rbc*LgapvV - *rbc*LgapvR	AT: 45.2°C
3' end of *acc*D gene	*acc*DvV - *acc*DvR	AT: 44.6°C
*trn*L intron	tabC - tabD	96 °C for 2 min [(94°C for 1 min; 50,0 °C for 50 sec; 72 °C for 3 min) 35 cycles] 72 °C for 5 min; hold at 4 °C
*trn*L - *trn*F intergenic spacer	tabE - tabF	96 °C for 2 min [(94°C for 1 min; 50,0 °C for 50 sec; 72 °C for 3 min) 35 cycles] 72 °C for 5 min; hold at 4 °C
The drowning of New Zealand and the problem of *Agathis*		
*mat*K gene partial sequence	trnK5F – trnK3AR	AT: 50.0°C
*mat*K gene partial sequence (nested within trnK5F – trnK3AR)	matK5FintF – matK3ARintR	AT: 45.0°C
*trn*D - *trn*E intergene region	*trn*D - *trn*T	AT: 45.0°C
*trn*E gene		
*trn*E – *trn*T intergene region		
Riddle of the beech gap		
*atp*B – rbcL intergene region	*atp*BgapSI - *rbc*LAI	AT: 50.0°C
*acc*D – *psa*I intergene region partial sequence	*acc*DSII - *psa*IAI	AT: 50.0°C
*trn*E – *trn*T intergene region	*trn*E - *trn*T	94 °C for 2 min [(94°C for 1 min; 48.0 °C for 1 min; 72 °C for 2:30 min) 35 cycles] 72 °C for 5 min; hold at 4 °C
*trn*L intron	tabC - tabD	96 °C for 2 min [(94°C for 1 min; 50,0 °C for 50 sec; 72 °C for 3 min) 35 cycles] 72 °C for 5 min; hold at 4 °C
*trn*L - *trn*F intergenic spacer	tabE - tabF	96 °C for 2 min [(94°C for 1 min; 50,0 °C for 50 sec; 72 °C for 3 min) 35 cycles] 72 °C for 5 min; hold at 4 °C

A1.2.4 Purification of PCR products

Prior to sequencing, PCR products were treated with 2U shrimp alkaline phosphatase (SAP) and 10U E. *coli* exonuclease I (Exo I) to remove primers and dNTPs. 2 µl of SAP and 1 µl of Exo I were added to each amplification product. The mixture was then incubated at 37 °C for 30 minutes followed by 80 °C for 15 minutes to heat kill the enzymes, then cooled to 10 °C.

A1.2.5 DNA sequencing

Purified PCR fragments were sequenced using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit. The protocol for which, is based on a method introduced by Sanger and Coulson (1975) which uses polymerisation of DNA under the presence of dideoxynucleotide triphosphates (ddGTP, ddATP, ddTTP, or ddCTP). When a ddNTP is incorporated into an extending fragment, the chain is terminated. The different sized products can be separated by polyacrylamide gel electrophoresis and visualised. In the chemistry used by the ABI PRISM™ each ddNTP is labelled with a different and specific fluorescent dye which can be detected by the AB 3730 capillary sequencer

Procedure for setting up an automatic sequencing reaction

1. The mass of PCR fragment needed for the automatic sequencing reaction was determined following the formula:

$$\frac{\text{Length of PCR fragment [bp]}}{20} = \text{mass of PCR fragment needed [ng]}$$

bp: base pairs
ng: nanograms

2. The results of agarose gel electrophoresis (see above) were used to determine the volume of PCR product [µl] required to provide the mass of PCR fragment calculated above.
3. A sequencing reaction was set up. For each reaction a 4.5 µl aliquot of the sequencing mix containing: 1 µl Big Dye Terminator 3.1, 3.5 µl of 5 x Sequencing Buffer (ABI) and 1 µl (10 pmol) of the appropriate primer, was dispensed into a 0.2 ml reaction tube. The correct volume of PCR product (see 1+2) was added to this mix and brought up to a final volume of 20 µl with Milli-Q H_2O.

4. The reactions were thermocycled following the protocol [(96°C for 10 sec; 50,0 °C for 5 sec; 60 °C for 4 min) 27 cycles; hold at 10 °C]. Ramping between temperature steps was limited to 1°C/second.
5. The extension products were purified by ethanol precipitation. To do this the entire 20 µl reaction volume was dispensed into a 1.6 ml microcentrifuge tube and mixed with 2 µl of 3 M NaOAc (pH 5.2) and 2 µl of 125 mM EDTA. To precipitate the extension products, 50 µl of 100% ethanol was added to the reaction, incubated for 15 minutes at room temperature and then centrifuged for 30 minutes at 13 000 × g at 4°C in a microcentrifuge. After centrifugation, the supernatant was carefully removed from each tube. 700 µl of 70% (v/v) ethanol was added to each tube and inverted repeatedly to rinse the pellet. The tube was then centrifuged as before for 15 minutes. The ethanol was discarded and the DNA pellet dried at 37°C – 60°C on a dry bath incubator until the remaining ethanol had completely evaporated.

Alternatively sequencing reactions were cleaned using magnetic beads as provided with the "CleanSeq" kit following the protocol included in the kit.

The reactions were resuspended and run by the Allan Wilson Centre Genome Service, (Massey University, Palmerston North, New Zealand) on an AB3730.capillary sequencer.

Appendix II

Herbarium voucher numbers

A2.1 Accessions for Chapter 3

Species	Source	Country	Herbarium voucher
N. menziesii	Te Aroha	New Zealand	MPN 27272
N. solandri	Waikaremoana	New Zealand	MPN 27273
N. truncata	Taranaki	New Zealand	MPN 24995
N. fusca	Ruahine Range	New Zealand	MPN 27275
*N. moorei**	Barrington Tops National Park	Australia	MPN 27271
*N. cunninghamii**	Cultivated Tasmania	Australia	MPN 25020
*N. gunnii**	Cultivated Tasmania	Australia	MPN 27274
*N. alessandri**	Botanical Gardens, Valdiva	Chile	MPN 27277
*N. obliqua**	Botanical Gardens, Valdiva	Chile	MPN 27278
N. glauca	Botanical Gardens, Valdiva	Chile	MPN 27279
*N. nitida**	Botanical Gardens, Valdiva	Chile	MPN 28699
Castanea sativa	Cultivated variety Tuscany	Italy	/

*: These accessions were also used as outgroups in Chapter 5

A2.2 Accessions for Chapter 4

A2.2.1 Accessions collected for this study

Species	Source	Country	Herbarium voucher
Araucaria bidwillii	Royal Botanical Gardens, Sydney	Australia	NSW 611836
Araucaria angustifolia	Botanical Gardens, Valdivia	Chile	/
Araucaria cunninghamii	Royal Botanical Gardens, Sydney	Australia	NSW 4027099
Agathis microstachya	Royal Botanical Gardens, Sydney	Australia	NSW 494482
Agathis atropurpurea	Royal Botanical Gardens, Sydney	Australia	NSW 4155284
Agathis robusta	Royal Botanical Gardens, Sydney	Australia	NSW 4091071
Agathis macrophylla	University of the South Pacific	Fiji	/
Agathis australis	Esplanade Gardens, Palmerston North	New Zealand	ES-AA1*
Wollemia nobilis	Royal Botanical Gardens, Sydney	Australia	NSW 4167438
Prumnopitys ferruginea	Massey University, Palmerston North	New Zealand	MA-PF1*
Dacrydium cupressinum	Rangiwahia Track, Ruahine Range	New Zealand	RW-DC1*

*: Herbarium numbers are currently being assigned to these vouchers.

A2.2.2 Accessions numbers for sequences obtained from GenBank

Species	GenBank accession number	Species	GenBank accession number
	rbcL		***rbcL***
Agathis atropurpurea	AF502087	*Araucaria heterophylla*	U96462
Agathis australis	AF362993	*Araucaria humboldtensis*	U96471
Agathis borneensis	U96476	*Araucaria hunsteinii*	U96468
Agathis dammara	U96477	*Araucaria laubenfelsii*	U96463
Agathis lanceolata	U96481	*Araucaria luxurians*	U96464
Agathis macrophylla	U96485	*Araucaria montana*	U96457
Agathis microstachya	AF508921	*Araucaria muelleri*	U96465
Agathis montana	U96478	*Araucaria nemorosa*	U96458
Agathis moorei	U96480	*Araucaria rulei*	U96466
Agathis obtusa	U96482	*Araucaria schmidii*	U96473
Agathis ovata	U96483	*Araucaria scropulorum*	U96459
Agathis palmerstonii	U96479	*Araucaria subulata*	U96474
Agathis robusta	U96484	*Wollemia nobilis*	AF030419
Araucaria angustifolia	U96470	*Dacrydium cupressinum*	AF249634
Araucaria araucana	U96467	*Podocarpus elatus*	AF249606
Araucaria bernieri	U96460	*Prumnopitys ferruginea*	AF249656
Araucaria bidwillii	U96472	*Juniperus conferta*	L12573
Araucaria biramulata	U96475	*Pinus koraiensis*	NC 004677
Araucaria columnaris	U96461	*Taxodium distichum*	AY237152
Araucaria cunninghamii	U96469		
	***mat*K**		***trn*D-*trn*T**
Dacrydium cupressinum	AF457112	*Pinus koraiensis*	NC 004677
Prumnopitys ferruginea	AF457115		
Pinus koraiensis	NC 004677		

A2.3 Accessions for Chapter 5

A2.3.1 *Lophozonia* accessions

Species	Sample name and location	Herbarium Voucher
Nothofagus menziesii	Abel Tasman	MPN 24862
Nothofagus menziesii	Aorangi	MPN 24861
Nothofagus menziesii	Blackball	BB-SB2*
Nothofagus menziesii	Buller Gorge1	BG-SB1*
Nothofagus menziesii	Buller Gorge2	BG-SB2*
Nothofagus menziesii	Buller Gorge3	BG-SB3*
Nothofagus menziesii	Buller Gorge4	MPN 24899
Nothofagus menziesii	Bullock Creek1	BC-SB1*
Nothofagus menziesii	Bullock Creek2	BC-SB3*
Nothofagus menziesii	Catlins	MPN 24959
Nothofagus menziesii	Dora Hutt	MPN 24833
Nothofagus menziesii	Dusky Sound1	MPN 24939
Nothofagus menziesii	Dusky Sound2	MPN 24941
Nothofagus menziesii	East Cape	MPN 24787
Nothofagus menziesii	Haast Pass	MPN 24915
Nothofagus menziesii	Haast River	MPN 24911
Nothofagus menziesii	Haast Summit	MPN 24916
Nothofagus menziesii	Holdsworth	MPN 24848
Nothofagus menziesii	Homer Tunnel	MPN 24929
Nothofagus menziesii	Kaimanawa	MPN 24820
Nothofagus menziesii	Lewis Pass1	LP-SB1*
Nothofagus menziesii	Lewis Pass2	LP-SB2*
Nothofagus menziesii	Lewis Pass3	LP-SB3*
Nothofagus menziesii	Lewis Pass4	LP-SB4*
Nothofagus menziesii	Lewis Pass5	LP-SB5*
Nothofagus menziesii	Lewis Pass6	LP-SB6*
Nothofagus menziesii	Lewis Pass7	LP-SB7*
Nothofagus menziesii	Lower Haast	MPN 24914
Nothofagus menziesii	Marble Hill	MPN 24893
Nothofagus menziesii	Maruia River	MPN 24891
Nothofagus menziesii	Mt Arthur	MPN 24868
Nothofagus menziesii	Mt Brewter	MPN 24912
Nothofagus menziesii	Mt Cook	MPN 24909
Nothofagus menziesii	Mt Duppa	MPN 24874
Nothofagus menziesii	Murchison	MPN 24886
Nothofagus menziesii	Ohakune	MPN 24819
Nothofagus menziesii	Paenga	MPN 24888
Nothofagus menziesii	Rahu_Saddle	Ra-SB1*
Nothofagus menziesii	Reefton1	MPN 24895
Nothofagus menziesii	Reefton2	MPN 24898
Nothofagus menziesii	Roa	R-SB1*
Nothofagus menziesii	South Coast	MPN 24954
Nothofagus menziesii	Takitimu Range	MPN 26838
Nothofagus menziesii	Te Aroha	MPN 24780
Nothofagus menziesii	Tuatapere	MPN 24955

*: Herbarium numbers are currently being assigned to these vouchers.

Species	Sample name and location	Herbarium Voucher
Nothofagus menziesii	Waihaha	MPN 24817
Nothofagus menziesii	Waikanae	MPN 24837
Nothofagus menziesii	Waikaremoana	MPN 24800
Nothofagus menziesii	Wainuiomata	MPN 24853

*: Herbarium numbers are currently being assigned to these vouchers.

A2.3.2 *Fuscospora* accessions

Species	Sample name and location	Herbarium Voucher
Nothofagus truncata	Cobb Dam Road NT1	HB-CR1*
Nothofagus truncata	Cobb Dam Road NT2	HB-CR3*
Nothofagus truncata	Havelock Lookout NT	Hav-HB 1*
Nothofagus truncata	Little Barrier Island NT	LB-HBS2*
Nothofagus truncata	Thames NT	Co-HB1*
Nothofagus solandri	Craigieburn NS	CSI BB8*
Nothofagus solandri	Hinewai NS	BP BB1*
Nothofagus solandri	Jackson Bay NS	SWSI BB5*
Nothofagus solandri	Kaitoke NS1	BB10*
Nothofagus solandri	Kaitoke NS2	Ka BB2*
Nothofagus solandri	Kepler Track entrance	KT BB1*
Nothofagus solandri	Nelson Creek NS	NC BB1*
Nothofagus solandri	Sunrise Road NS	SNI-BB9*
Nothofagus solandri	Te Anau	Fj BB1*
Nothofagus solandri	Urewera NS	MPN 24990
Nothofagus fusca	Buller Gorge	BG RB1*
Nothofagus fusca	Bullock Creek	BC RB1*
Nothofagus fusca	Cobb Dam Road	RB CR3*
Nothofagus fusca	Hope Valley	SWSI RB4*
Nothofagus fusca	Kepler Track	KT RB1*
Nothofagus fusca	Otira River	TR RB1*
Nothofagus fusca	Otira River	TR RB2*
Nothofagus fusca	Queenstown	QT RB1*
Nothofagus fusca	Te Aroha	NWNI RB1*
Nothofagus fusca	Wangapeka	TD RB1*

*: Herbarium numbers are currently being assigned to these vouchers.

Appendix III

Data CD

See last page of thesis

Literature cited

Abbott, R.J.; Smith, L.C.; Milne, R.I.; Crawford, R.M.M.; Wolff, K.; Balfour J. (2000): Molecular analysis of plant migration and refugia in the Arctic. *Science* 289, 1343-1346.

Anderson, H.M.; Hiller, N.; Gess, R.W. (1995): Archmopteris (Progymnospermopsida) from the Devonian of southern Africa. *Botanical Journal Linnean Society* 117, 305-320.

Anderson, J.M.; Anderson, H.M.; Archangelsky, S.; Bamford, M.; Chandra, S.; Dettmann, M.; Hill, R.; McLoughlin, S.; Rösler, O. (1999): Patterns of Gondwana plant colonisation and diversification. *Journal of African Earth Sciences* 28, 145-167.

Avise, J.C; Arnold, J.; Ball, R.M. Jr.; Bermingham, E.; Lamb, T.; Neigel, J.E.; Reeb, C.A.; Saunders, N.C. (1987): Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* 18, 489-522.

Ballance, P.F. (1993): The paleo-Pacific, post-subduction, passive margin thermal relaxation sequence (Late Cretaceous– Palaeogene) of the drifting New Zealand continent. *In*: Ballance, P.F., ed., Sedimentary basins of the world. South Pacific sedimentary basins 2. pp. 93-110.

Banarescu, P.; Boscaiu, N. (1978): Biogeographie: Fauna und Flora der Erde und ihre geschichtliche Entwicklung. VEB Gustav Fischer, Jena.

Batt, G.E.; Braun, J.; Kohn, B.P.; McDougall, I. (2000): Thermochronological analysis of the dynamics of the Southern Alps, New Zealand. *Geological Society of America Bulletin* 112, 250-266.

Bellamy, D.; Springett, B.; Hayden, P. (1990): Moa's Ark: The voyage of New Zealand. Viking/Penguin, Auckland.

Beu, A.G.; Browne, G.H.; Grant-Taylor, T.L. (1981): New *Chlamys delicatula* localities in the central North Island and uplift of the Ruahine Range. *New Zealand Journal of Geology and Geophysics* 24, 127-132.

Brenner, G.J. (1984): Late Hauterivian angiosperm pollen from the Helez Formation, Israel. *In*: Sixth international palynological conference, Calgary 1984, Abstracts. p. 15.

Brundin, L. (1966): Transantarctic relationships and their significance, as evidenced by chironomid midges with a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagyiae. *Kungliga Svenska Vetenskapsakademiens Handlingar*, *Fjärde Serien*, 11, 1-472.

Brundin, L. (1972): Phylogenetics and Biogeography. *Systematic Zoology* 21, 69-79.

Burrows, C.J. (1965): Some discontinuous distributions of plants within New Zealand and their ecological significance II: disjunctions between Otago-Southland and Nelson-Marlborough and related distribution patterns. *Tuatara* 12, 9-29.

Campbell, J.D. (1985): Casuarinaceae, Fagaceae, and other plant megafossils from Kaikorai leaf beds (Miocene) Kaikorai Valley, Dunedin, New-Zealand. *New Zealand Journal of Botany* 23, 311-320.

Campbell, J.D. (2002): Angiosperm fruit and leaf fossils from Miocene silcrete, Landslip Hill, northern Southland, New Zealand. *Journal of the Royal Society of New Zealand* 32, 149-154.

Campbell, H. J.; Landis, C. A. (2003): New Zealand awash. *N. Z. Geographic 2003*, 6-7.

Carlquist, S.J. (1996): Plant dispersal and the origin of Pacific island floras. *In*: Keast, A.; Miller, S.E., eds., The origin and evolution of Pacific island biotas, New Guinea to Eastern Polynesia: patterns and processes. Academic Publishing, Amsterdam. pp. 153-164.

Carlquist, S.; Pauly, Q. (1985): Experimental studies on epizoochorous dispersal in Californian plants. *Aliso* 11, 167-177.

Clement, M.; Posada, D.; Crandall, K.A. (2000): TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9, 1657-1659.

Close, R.C.; Moar, N.T.; Tomlinson, A.I.; Lowe, A.D. (1978): Aerial dispersal of biological material from Australia to New Zealand. *Int. J. Biometeor.* 22, 1-19.

Cockayne, L. (1926): Monograph on the New Zealand beech forests. Part 1. The ecology of the forests and the taxonomy of the beeches. New Zealand Forest Service bulletin 4.

Cockayne, L. (1928): The Vegetation of New Zealand. 2nd ed. Engleman, Leipzig.

Comes, H.P.; Kadereit, J.W. (1998): The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* 3, 432-438.

Cook, L.G.; Crisp, M.D. (2005): Not so ancient: the extant crown group of *Nothofagus* represents a post-Gondwanan radiation. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 272, 2535–2544.

Cooper, A.; Cooper, R.A. (1995): The Oligocene bottleneck and New-Zealand biota - genetic record of a past environmental crisis. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 261, 293-302.

Cooper, A.; Lalueza-Fox, C.; Anderson, S.; Rambaut, A.; Austin, J.; Ward, R. (2001): Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature* 409, 704-707.

Cowie, R.H.; Holland, B.S. (2006): Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. *Journal of Biogeography* 33, 193-198.

Cracraft, J. (1974): Phylogeny and evolution of ratite birds. *Ibis* 116, 494-521.

Crandall, K.A.; Templeton, A.R. (1993): Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134, 959–969.

Crane, P.R.; Lidgard, S. (1989): Angiosperm diversification and paleolatitudinal gradients in Cretaceous floristic diversity. *Science* 246, 675--678.

Craw, R.C.; Gehan, J.R.; Heads, M.J. (1999): Panbiogeography, Tracking the history of life. Oxford University Press, Oxford.

Crisci, J.V. (2001): The voice of historical biogeography. *Journal of Biogeography* 28, 157-168.

Croizat, L. (1958): Panbiogeography or an introductory synthesis of zoogeography, phytogeography, and geology; with notes on evolution, systematics, ecology, anthropology, etc. Vol. 1 - The New World, Vol. 2a - The Old World (Chapters IX–XIII), Vol. 2b - The Old World (Continuation; Chapter XIV to General Addenda and Indices). (L. Croizat: Caracas).

Crook, K.A.W.; Belbin, L. (1978): The Southwest Pacific Area during the last 90 Million Years. *Journal of the Geological Society of Australia* 25, 23-40.

Daniel, I.L. (1989): Taxonomic investigation of elements from the Early Cretaceous megaflora from the middle Clarence Valley, New Zealand. Unpublished PhD thesis. University of Canterbury, Christchurch.

Darlington, P.J. Jr. (1957): Zoogeography: the geographical distribution of animals. John Wiley & Sons, New York.

Darlington, P.J. Jr. (1965): Biogeography of the southern end of the world: distribution and history of far-southern life and land, with an assessment of continental drift. Harvard University Press: Cambridge. Reprint 1968, McGraw-Hill: New York.

Darwin, C. (1859): The origin of species. Original text with preface and introduction. Random House, New York.

De Jersey, N. (1968): Triassic spores and pollen grains from the Clematis Sandstone. Geol. Surv. Queensland Publ. No. 338, Palaeontol. Pap. No. 14. Brisbane, Aust.: Geol. Surv. Queensland. 44 pp.

Demesure, B.; Comps, B.; Petit, R.J. (1996): Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. *Evolution* 50, 2515-2520.

Demesure, B.; Sodzi, N.; Petit, R.J. (1995): A set of universal primers for amplification of polymorphic noncoding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* 4, 129-131.

Dettmann, M.E.; Pocknall, D.; Romero, E.J.; Zamboloa, M. (1990): Nothofagidites Erdman ex Potonie 1960; a cataloque of species with notes on palaeogeographic distribution of *Nothofagus* Bl. (southern beech). *New Zealand Geological Survey Palaeontological Bulletin* 60, 1-79.

Dettmann, M.E.; Jarzen, D.M. (2000): Pollen of extant *Wollemia* (wollemi pine) and comparisons with pollen of other extant and fossil Araucariaceae. *In*: Harley, M.M.; Morton, C.M.; Blackmore, S., eds., Pollen and Spores: Morphology and Biology. Kew: Royal Botanic Gardens. pp. 197-203.

Devereux, I. (1967): Oxygen isotope palaeotemperature measurements on New Zealand Tertiary fossils. *N.Z.J. Sci.*, 10, 988-1011.

Diversity and Distributions (2005) volume 11, number 2.

Dodson, J.R. (1978): Vegetation history from northeast Nelson, New-Zealand. *New Zealand Journal of Botany* 16, 371-378.

Donald, K.M.; Kennedy, M.; Spencer, H.G. (2005): The phylogeny and taxonomy of austral monodontine topshells (Mollusca : Gastropoda : Trochidae), inferred from DNA sequences. *Molecular phylogenetics and evolution* 37, 474-483.

Doyle, J. J.; Doyle, J. L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19, 11-15.

Du Toit A.L. (1937): Our wandering continents. Oliver and Boyd, Edinburgh.

Edwards D.; Fanning U. (1985): Evolution and environment in the Late Silurian Early Devonian - the rise of the Pteridophytes. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 309, 147-165.

Ericson, P.G.P.; Christidis, L.; Cooper, A.; Irestedt, M.; Jackson, J.; Johansson, U. S.; Norman, J.A. (2002): A Gondwanan origin of passerine birds supported by DNA sequences of the endemic New Zealand wrens. *Proceedings of the Royal Society of London Series B, Biological Sciences* 269, 235-241.

Felsenstein, J. (2004): PHYLIP (Phylogeny Inference Package) version 3.6b. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Ferris C.; Oliver, R.P.; Davy, A.J.; Hewitt, G.M. (1993): Native oak chloroplasts reveal an ancient divide across Europe. *Mol. Ecol.*, 2, 337–344.

Ferris, C.; King, R.A.; Väinölä, R.; Hewitt, G.M. (1998): Chloroplast DNA recognizes three refugial sources of European oaks and suggest independent eastern and western immigrations to Finland. *Heredity* 80, 584-593.

Fleming, C.A. (1979): The Geological History of New Zealand and its Life. Auckland University Press, Auckland.

Foster, C.B.; Balme, B.E.; Helby, R. (1994): First record of Tethyan palynomorphs from the Late Triassic of Antarctica. *AGSO Journal of Australian Geology and Geophysics* 15, 239–246.

Gadek, P.A.; Alpers, D. L.; Heslewood, M.M.; Quinn, C.J. (2000): Relationships within Cupressaceae *sensu lato*: a combined morphological and molecular approach. *American Journal of Botany* 87, 1044–1057.

Graur, D.; Martin, W. (2004): Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *TRENDS in Genetics* 20, 80-86.

Gess, R.W.; Hiller, N.; (1995): A preliminary catalogue of fossil algal, plant, arthropod and fish remains from a Late Devonian black shale near Grahamstown, South Africa. *Annals Cape Provincial Museums (Natural History)* 19, 225-304.

Gould, R.E. (1975): The succession of Australian pre-Tertiary megafossil floras. *Botanical Review* 41, 453-481.

Golenberg, E.M.; Clegg, M.T.; Durbin, M.L.; Doebley, J.; Ma, D.P. (1993): Evolution of a noncoding region of the chloroplast genome. *Molecular Phylogenetics and Evolution* 2, 52-64.

Grunow, A.M. (1999): Gondwana events and palaeogeography: a palaeomagnetic review. *Journal of African Earth Sciences* 28, 53-69.

Haase, P. (1990): Environmental and floristic gradients in Westland, New Zealand, and the discontinuous distribution of Nothofagus. *New Zealand Journal of Botany* 28, 25-40.

Haile, J.; Holdaway, R.; Oliver, K.; Bunce, M.; Gilbert, M.T.P.; Nielsen, R.; Munch, K.; Ho, S.Y.W.; Shapiro, B.; Willerslev, E. (in press): Ancient DNA chronology within sediment deposits: Are paleobiological reconstructions possible and is DNA leaching a factor? *Molecular Biology and Evolution.*

Hall, G.M.J.; McGlone, M.S. (2006): Potential forest cover of New Zealand as determined by an ecosystem process model. *New Zealand Journal of Botany* 44, 211-232.

Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41, 95-98.

Heads, M. (1989): Integrating earth and life sciences in New Zealand natural history: the parallel arcs model. *New Zealand Journal of Zoology* 16, 549-585.

Heads, M. (1998): Biogeographic disjunction along the Alpine fault, New Zealand. *Biological Journal of the Linnean Society* 63, 161-176.

Heads, M. (2006): Panbiogeography of *Nothofagus* (Nothofagaceae): analysis of the main species massings. Journal of Biogeography 33, 1066-1075.

Hewitt, G.M. (2004): Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 359, 183-195.

Hickson, R.E.; Slack, K.E.; Lockhart, P.J. (2000): Phylogeny recapitulates geography: Why New Zealand has so many species of lizards. *Biological Journal of the Linnean Society* 70, 415-433.

Hill, R.S. (1984): Tertiary *Nothofagus* macrofossils from Cethana, Tasmania. *Alcheringia* 8, 81-86.

Hill, R.S. (1991): Tertiary *Nothofagus* (Fagaceae) macrofossils from Tasmania and Antarctica and their bearing on the evolution of the genus. *Botanical Journal of the Linnean Society* 105, 73-112.

Hill, R. S. (1995): Conifer origin, evolution and diversification in the Southern Hemisphere. *In*: Enright, N. J.; Hill, R. S., eds., Ecology of the Southern Conifers. Melbourne University Press: Melbourne. pp. 10-29.

Hill, R.S. (2001): Biogeography, evolution and palaeoecology of *Nothofagus* (Nothofagaceae): the contribution of the fossil record. *Aust J Bot* 49, 321-332.

Hill, R.S.; Brodribb, T.J. (1999): Southern conifers in time and space. *Aust. J. Bot.* 47, 639–696.

Hill, R.S.; Jordan, G.J. (1993): The evolutionary history of *Nothofagus* (Nothofagaceae). *Aust J Bot* 6, 111-126.

Hill, R.S.; Jordan, G.J.; Macphail, M.K. (1996): History and palaeoecology of Australian *Nothofagus* forests. *In*: Veblen, T.T.; Hill, R.S.; Read, J., eds., The Ecology and biogeography of *Nothofagus* forests. Yale University Press, New Haven. pp. 182-199.

Hill, R.S.; Truswell, E.M. (1993): *Nothofagus* fossils in the Sirius Group, Transantarctic Mountains: Leaves and pollen and their climatic implications. *In*: Kennett, J.P.; Warnke, D.A., eds., Antarctic Research Series Volume 60. The Antarctic Palaeoenvironment: A Perspective on Global Change. American Geophysical Union. Washington, D.C. pp. 67-73.

Hill, R.S.; Truswell, E.M.; McLoughlin, S.; Dettmann, M.E. (1999): The evolution of the Australian flora: fossil evidence. *In*: Orchard, A.E. ed., Flora of Australia, Vol. 1 (Introduction) 2nd edn. CSIRO Publishing: Melbourne. pp. 251–320.

Holland, B.R.; Huber, K.T.; Moulton, V.; Lockhart, P.J. (2004): Using consensus networks to visualize contradictory evidence for species phylogeny. *Mol. Biol. Evol.* 21, 1459-1461.

Hooker, J. D. (1853): Flora Novae Zelandiae. Part 1. Introductory essay. *In*: Botany of the Antarctic voyage of H.M. Discovery ships 'Erebus' and 'Terror' in the years 1831-1843., Vol. 2. Reeve, London. pp. 209-223.

Hornibrook, N.; Hornibrook, B.D. (1971): New Zealand Tertiary Climate. *New Zealand Geological Survey Report* 47, 19.

Hughes, N.F.; McDougall, A.B. (1987): Records of angiospermid pollen entry into the English Early Cretaceous succession. *Review of Palaeobotany and Palynology* 50, 255–272.

Hurr, K., Lockhart, P.J.; Heenan, P.B.; Penny, D. (1999): Dispersal of the *Edwardsia* Section of *Sophora* (Leguminosae) around the southern oceans: Molecular Evidence. *Journal of Biogeography* 26, 565-577.

Kamp, P.J. (1986): Late Cretaceous-Cenozoic development of the southwest Pacific region. *Tectonophysics* 121, 225-251.

Kemp, E.M. (1975): Palynology of Leg 28 Drill Sites. *Initial Rep. Deep Sea Drilling Project* 28, 599-623.

Kershaw, P; Wagstaff, B. (2001): The southern conifer family Araucariaceae: History, status, and value for palaeoenvironmental reconstruction. *Annu. Rev. Ecol. Syst:* 32, 397-414.

King, P.R. (2000): Tectonic reconstructions of New Zealand: 40 Ma to the present. *New Zealand Journal of Geology and Geophysics* 43, 611-638.

Kishino, H; Thorne, J.L.; Bruno, W.J. (2001): Performance of a divergence time estimation method under a probabilistic model of rate evolution. *Mol. Biol. Evol.* 18, 352-361.

Knapp, M.; Stöckler. K.; Havell, D.; Delsuc, F.; Sebastiani, F.; Lockhart, P.J. (2005): Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (southern beech). *PLoS Biology* 3, 38-43.

Knowles, L.L.; Maddison, W.P. (2002): Statistical phylogeography. *Molecular Ecology*, 11, 2623–2635.

Leathwick, J.R. (1998): Are New Zealand's *Nothofagus* species in equilibrium with their environment? *Journal of Vegetation Science* 9, 719-732.

Leathwick, J.R. (2001): New Zealand's potential forest pattern as predicted from current species-environment relationships. *New Zealand Journal of Botany* 39, 447-464

Linder, H.P.; Crisp, M.D. (1995): *Nothofagus* and Pacific Biogeography. *Cladistics* 11, 5-32.

Lowrey, T.E. (1995): Phylogeny, adaptive radiation, and biogeography of Hawaiian Tetramolopium. *In*: Wagner, W.L.; Funk, V.A. eds., Hawaiian biogeography. Smithsonian Inst. Press, Washington, D.C. pp. 195-220.

Lyell, C. (1831): Principles of Geology: Being an attempt to explain the former changes of the Earths surface by reference to causes now in operation: reprint 1970, Cramer.

Macphail, M.K. (1997): Comment on M. Pole (1994) The New Zealand Flora - entirely long distance dispersal? *Journal of Biogeography* 24,113-114.

Macphail, M.K.; Hill, K.; Partridge A.D.; Truswell, E. M.; Foster, C. (1995): Wollemi Pine – old pollen records for a newly discovered genus of gymnosperm. *Geology Today*, March-April, 48-50.

Manos, P. (1997): Systematics of *Nothofagus* (Nothofagaceae) based on rDNA spacer sequences (ITS): taxonomic congruence with morphology and plastid sequences. *American Journal of Botany* 84, 1137-1150.

Markgraf, V.; Romero, E.; Villagrán, C. (1996): History and Palaeoecology of South American *Nothofagus* Forests. *In*: Veblen, T.T.; Hill, R.S.; Read, J., eds., The Ecology and biogeography of *Nothofagus* forests. Yale University Press, New Haven. pp. 354-386.

Martin, P.G.; Dowd, J.M. (1993): Using Sequences of *rbc*L to study phylogeny and biogeography of *Nothofagus* species. *Australian Journal of Botany* 6, 441-447.

Matthew, W.D. (1915): Climate and evolution. *Annals of the New York Academy of Sciences* 24, 171-416.

McCarthy, D. (2003): The trans-Pacific zipper effect: disjunct sister taxa and matching geological outlines that link the Pacific margins. Journal of Biogeography 30, 1545-1561.

McGlone, M.S. (1983): History of New Zealand lowland forest since the last glaciation. *In* Thompson, K.; Hodder, A.P.H.; Edmonts, A.S., eds, Proceedings of a Symposium held at the University of Waikato, Hamilton, 27 – 28 May, 1980. University of Waikato, Hamilton, pp 1-17.

McGlone, M.S. (1985): Plant Biogeography and the Late Cenozoic history of New Zealand. *New Zealand Journal of Botany* 23, 723-749.

McGlone, M.S. (1989): Pollen analyses - Bidwill Hill. Appendix 1. *New Zealand Journal of Geology and Geophysics* 32, 499-513.

McGlone, M. S. (2005): Goodbye Gondwana. *Journal of Biogeography* 32, 739-740.

McGlone, M.S.; Duncan, R.P.; Heenan, P.B. (2001): Endemism, species selection and the origin and distribution of the vascular plant Flora of New Zealand. *Journal of Biogeography* 28, 199-216.

McGlone, M.S.; Howorth, R.; Pullar, W.A. (1984): Late Pleistocene stratigraphy, vegetation and climate of the Bay of Plenty and Gisborne regions, New Zealand. *New Zealand Journal of Geology and Geophysics* 27, 327-350.

McGlone, M.S.; Mark, A.F.; Bell, D. (1995): Late Pleistocene and Holocene vegetation history, Central Otago, South Island, *New Zealand. Journal of the Royal Society of New Zealand* 25, 1-22.

McGlone, M.S.; Mildenhall, D.C.; Pole, M.S. (1996): History and Palaeoecology of New Zealand *Nothofagus* Forests. *In*: Veblen, T.T.; Hill, R.S.; Read, J., eds., The Ecology and biogeography of *Nothofagus* forests. Yale University Press, New Haven. pp 83-130.

McGlone, M.S.; Topping, W.W. (1983): Late Quaternary Vegetation, Tongariro Region, Central North Island, New Zealand. *New Zealand Journal of Botany* 21, 53-76.

McKinnon, G.E.; Jordan, G.J.; Vaillancourt, R.E.; Steane, D.a.; Potts, B.M. (2004): Glacial refugia and reticulate evolution: the case of the Tasmanian eucalypts. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 359, 275-284.

McLoughlin, S. (2001): The breakup history of Gondwana and its impact on pre Cenozoic floristic provincialism. *Aust J Bot* 49, 271-300.

McQueen, D.R. (1954): Palaeozoic plants from New Zealand. *Nature* 173, 88.

Mildenhall, D.C. (1972): Fossil pollen of Acacia type from New Zealand. *New Zealand Journal of Botany* 10, 485-494.

Mildenhall, D.C. (1980): New Zealand late Cretaceous and Cenozoic plant biogeography: a contribution. *Paleogeography, Paleoclimatology, Paleoecology* 31, 197-233.

Mildenhall, D.C. (1994): Palynology of predominantly last glaciation sediments from the Mangaroa-Drillhole, Hutt Valley, New Zealand. *New Zealand Joumal of Geology and Geophysics* 37, 1-9.

Mildenhall, D.C. (1995): Pleistocene palynology of the Petone and Seaview drillholes, Petone, Lower-Hutt Valley, North-Island, New Zealand. *Journal of the Royal Society of New Zealand* 25, 207-262.

Miller, C.N. (1977): Mesozoic conifers. *Botanical review* 43, 217-280.

Moar, N.T. (1971): Contributions to the Quaternary history of the New Zealand flora - 6: Aranuian pollen diagrams from Canterbury, Nelson and north Westland, South Island. *New Zealand Journal of Botany* 9, 80-145.

Moar, N.T., Suggate, R.P. (1979): Contributions to the Quaternary history of the New Zealand flora. 8. Interglacial and glacial vegetation in the Westport District, South Island. *New Zealand Journal of Botany* 17, 361-387.

Moar, N.T.; Suggate R.P. (1996): Vegetation history from the Kaihinu (last) interglacial to the present, west coast, South Island, New Zealand. *Quaternary Science Reviews* 15, 521-547.

Morel, E.; Edwards, D.; Rodriguez, M.I. (1995): The first record of Cooksonia from South America in Silurian rocks of Bolivia. *Geological Magazine* 132, 449-452.

Muñoz, J.; Felicísimo, Á.M.; Cabezas, F.; Burgaz, A.R.; Martínez, I. (2004): Wind as a long-distance dispersal vehicle in the Southern Hemisphere. *Science* 304, 1144-1147.

Nathan, R. (2006): Long-Distance Dispersal of plants. *Science* 313, 786-788.

Nelson, G. (1975): Biogeography, the vicariance paradigm, and continental drift. *Syst. Zool.* 24, 490-504.

Nelson, G.; Ladiges, P.Y. (2001): Gondwana, vicariance biogeography and the New York School revisited. *Aust. J. Bot.* 49, 389-409.

Nelson, G.; Platnick, N. (1981): Systematics and biogeography: cladistics and vicariance. Columbia University Press, New York.

Newnham, R.M. (1992): A 30,000 year pollen, vegetation and climate record from Otakairangi (Hikurangi), Northland, New Zealand. *Journal of Biogeography* 19, 541-554.

Newnham R.M.; Lowe D.J.; Green J.D. (1989): Palynology, vegetation and climate of the Waikato Lowlands, North Island, New Zealand, since c 18,000 years ago. *Journal of the Royal Society of New Zealand* 19, 127-150.

Ogden, J. (1989): On the coenospecies concept and tree migrations during the oscillations of the Pleistocene climate. *Journal of the Royal Society of New Zealand* 19, 249-262.

Ogden, J.; Basher, L.; McGlone, M. (1998) Fire, forest regeneration and links with early human habitation: Evidence from New Zealand. *Annals of Botany* 81, 687-696.

Ogden, J.; Stewart, G.H.; Allen R.B. (1996): Ecology of New Zealand *Nothofagus* forests. *In*: Veblen, T.T.; Hill, R.S.; Read, J., eds., The Ecology and biogeography of *Nothofagus* forests. Yale University Press, New Haven. pp. 25-82.

Oliver, W.R.B. (1950): The Fossil Flora of New Zealand. *Tuatara* 3, 1-11.

Parrish, J.T.; Daniel, I.L.; Kennedy, E.M.; Spicer, R.A. (1998): Paleoclimatic significance of mid-Cretaceous floras from the middle Clarence Valley, New Zealand. *Palaios* 13, 149-159.

Pérez-Losada, M.; Høeg, J.T.; Crandall, K.A. (2004): Unraveling the evolutionary radiation of the Thoracian Barnacles using molecular and morphological evidence: A comparison of several divergence time estimation approaches. *Syst Biol* 53, 244-264.

Petit, R.J.; Pineau, E.; Demesure, B.; Bacilieri, R.; Ducousso, A.; Kremer, A. (1997): Chloroplast DNA footprints of postglacial recolonization by oaks. *PNAS*, 94, 9996-10001.

Petit, R.J.; Aguinagalde, I.; de Beaulieu, J-L.; Bittkau, C.; Brewer, S.; Cheddadi, R.; Ennos, R.; Fineschi, S.; Grivet, D.; Lascoux, M.; Mohanty, A.; Müller-Starck, G.; Demesure-Musch, B.; Palmé, A.; Martín, J.P.; Rendell, S.; Vendramin, G.G. (2003): Glacial refugia: Hotspots but not melting pots of genetic diversity. *Science* 300, 1563-1565.

Philippe, M.; Bamford, M.; McLoughlin, S.; Alves, L.S.R.; Falcon-Lang, H.J.; Gnaedinger, S.; Ottone, E.G.; Pole, M.; Rajanikanth, A.; Shoemaker, R.E.; Torres, T.; Zamuner, A. (2004): Biogeographic analysis of Jurassic-Early Cretaceous wood assemblages from Gondwana. *Review of Paleobotany and Palynology* 129, 141-173

Pocknall, D.T. (1989): Late Eocene to Early Miocene vegetation and climate history of New Zealand. *Journal of the Royal Society of New Zealand* 19, 1-18.

Pole, M. (1989): Early Miocene floras from Central Otago, New Zealand. *Journal of the Royal Society of New Zealand* 19, 121-125.

Pole, M. (1992): Cretaceous macrofloras of eastern Otago, New Zealand - Angiosperms. *Aust. J. Bot.* 40, 169-206.

Pole, M. (1994): The New-Zealand flora - entirely Long-Distance Dispersal. Journal of Biogeography 21, 625-635.

Pole, M. (1996): Plant macrofossils from the Foulden Hills Diatomite (Miocene), Central Otago, New Zealand. *Journal of the Royal Society of New Zealand* 26, 1-39.

Pole, M. (1998): Paleocene gymnosperms from Mount Somers, New Zealand. *Journal of the Royal Society of New Zealand* 28, 375-403.

Pole, M. (1999): Structure of a near-polar latitude forest from the New Zealand Jurassic. *Palaeogeography Palaeoclimatology Palaeoecology* 147, 121-139.

Pole, M.S. (2001): Can long-distance dispersal be inferred from the New Zealand plant fossil record? *Aust. J. Bot.* 49, 357-366.

Posada, D.; Crandall, K. (1998): MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817-818.

Posada, D.; Crandall, K.A.; Templeton, A.R. (2000): GeoDis: A program for the Cladistic Nested Analysis of the Geographical Distribution of Genetic Haplotypes. *Molecular Ecology* 9, 487-488.

Preest, D.S. (1963): A note on the dispersal characteristics of the seed of the New Zealand podocarps and beeches, and their biogeographical significance. *In*: Gressit, J.L., ed., Pacific Basin Biogeography 10th Pacific Science Congress. Bishop Museum Press. Honolulu. pp. 415-424.

Prothero, D.R. (1994): The Late Eocene-Oligocene extinctions. *Annual Review of Earth and Planetary Sciences* 22, 145-165.

Ree, R. (unknown date): Gap Recoder, available from http://maen.huh.harvard.edu:8080/services/gap_recoder

Renner, S.S. (2004): Plant dispersal across the tropical Atlantic by wind and sea currents. *International Journal of Plant Sciences* 165, S23-S33.

Renner, S.S. (2005): Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science* 10, 550-558.

Renner, S.S.; Foreman, D.B.; Murray, D. (2000): Timing Transantarctic disjunctions in the Atherospermataceae (Laurales): Evidence from coding and noncoding chloroplast sequences. *Syst Biol* 49, 579-591.

Retallack, G.J. (1980): Late Carboniferous to Middle Triassic megafossil floras from Sydney Basin. *Geological Survey of New South Wales Bulletin* 26, 384-430.

Rogers, G.M. (1989): The nature of the lower North Island floristic gap. *New Zealand Journal of Botany* 27, 221-241.

Ronquist, F. (1997): Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst Biol* 46, 195-203.

Rothwell, G.W.; Scheckler, S.E. (1988): Biology of ancestral gymnosperms. *In*: Beck, C.B., ed., Origin and evolution of Gymnosperms. Columbia University Press, New York. pp. 85-134.

Sambrook, J.; Fritsch, E. F.; Maniatis, T. (1989): Molecular cloning: a laboratory manual. 2 edn, 1-3, Cold Spring Harbor Laboratory Press, New York.

Sanderson, M.J. (2002): Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Mol. Biol. Evol.* 19, 101-109.

Sanderson, M.J. (2003): r8s: inferring absolute rates of molecular evolution and divergence dates in the absence of a molecular clock. *Bioinformatics* 19, 301-302.

Sanderson, M.J.; Doyle, J.A. (2001): Sources of error and confidence intervals in estimating the age of angiosperms from rbcL and 18S rDNA data. American Journal of Botany 88, 1499-1516.

Sanger, F.; Coulson, A. R. (1975): A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology* 94, 441-448.

Sanmartín, I.; Ronquist, F. (2004): Southern Hemisphere biogeography inferred by event-based models: Plant versus animal patterns. *Syst Biol* 53, 216-243.

Sanmartín, I.; Wanntorp, L.; Winkworth, R.C. (in press): West Wind Drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. *Journal of Biogeography*.

Savolainen, V.; Chase, M. W.; Morton, C. M.; Hoot, S. B.; Soltis, D. E.; Bayer, C.; Fay, M. F.; de Bruijn, A.; Sullivan, S.; Qiu, Y. L. (2000): Phylogenetics of flowering plants based upon a combined analysis of plastid *atp*B and *rbc*L gene sequences. *Systematic Botany* 49, 306-362.

Schäfer, A. (1997): Biogeographie der Binnengewässer; eine Einführung in die biogeographische Areal- und Raumanalyse in limnischen Ökosystemen. Teubner, Stuttgart.

Scotese, C.R.; Boucot, A.J.; Mckerrow, W.S. (1999): Gondwanan palaeogeography and palaeoclimatology. *Journal African Earth Sciences* 28, 99-114.

Setoguchi, H.; Osawa, T.A.; Pintaud, J.C.; Jaffré, T.; Veillon J.M. (1998): Phylogenetic relationships within Araucariaceae based on *rbc*L gene sequences. *American Journal of Botany* 85, 1507-1516.

Simmons, M.P.; Ochoterena H. (2000): Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* 49, 369-381.

Soltis, D.E.; Morris, A.B.; McLachlan, J.S.; Manos, P.S.; Soltis, P.E. (2006): Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* 15, 4261-4293.

Stockey, R.A. (1982): The Araucariaceae: an evolutionary perspective. *Rev. palaeobot. palynol* 37, 133-154.

Stöckler, K. (2001): Origin and Evolution of the New Zealand Forest Flora - a Molecular Phylogenetic Approach. Unpublished PhD thesis. Massey University, Palmerston North.

Stöckler K.; Daniel I.L.; Lockhart P.J. (2002): New Zealand Kauri (*Agathis australis* (D.Don) Lindl., Araucariaceae) survives Oligocene drowning. *Syst. Biol.* 51, 827-832.

Suggate, R.P. (1990): Late Pliocene and Quaternary glaciations of New Zealand. *Quaternary Science Reviews* 9, 175-197.

Suggate, R. P.; Stevens, G. R.; Te Punga, M. T. (1978): Geology of New Zealand. 2nd volume, Government Printer, Wellington.

Swenson, U.; Backlund, A.; McLoughlin, S.; Hill, R.S. (2001): *Nothofagus* Biogeography revisited with special emphasis on the enigmatic distribution of subgenus *Brassospora* in New Caledonia. *Cladistics* 17, 28-47.

Swofford, D.L. (2003): PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

Taberlet, P.; Gielly, L.; Pautou, G.; Bouvet, J. (1991): Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17, 1105-1109.

Templeton, A.R. (1998): Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* 7, 381-397.

Templeton, A.R. (2001): Using phylogeographic analyses of gene trees to test species status and processes. *Molecular Ecology* 10, 779-791.

Templeton, A.R. (2005): Inference key for the nested haplotype tree analysis of geographical distances. Distributed by the author under http://darwin.uvigo.es/download/geodisKey_11Nov05.pdf.

Templeton, A.R.; Boerwinkle, E.; Sing, C.F. (1987): A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in Drosophila. *Genetics* 117, 343-351.

Templeton, A.R.; Singh, C.F. (1993): A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics* 134, 659-669.

Templeton, A.R.; Routman, E.; Phillips, C. A. (1995): Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* 140, 767-782.

Thompson, J.D.; Gibson, T.J.; Plewniak, F.; Jeanmougin, F.; Higgins, D.G. (1997): The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24, 4876- 4882. Program available from ftp-igbmc.u-strasbg.fr and ftp.emblheidelberg.de; ftp.ebi.ac.uk.

Thomsen, T.H. (2002): Biogeography of *Nothofagus* subgenus *Fuscospora* in the South Island of New Zealand inferred from chloroplast DNA. Unpublished Master thesis. University of Canterbury, Christchurch.

Thorne, J.L.; Kishino, H.; Painter, I.S. (1998): Estimating the rate of evolution of the rate of molecular evolution. *Mol Biol Evol* 15, 1647-1657.

Thorne, J.L.; Kishino, H. (2002): Divergence time and evolutionary rate estimation with multilocus data. *Syst Biol* 51, 689-702.

Trewick, S.A.; Wallis, G.P. (2001): Bridging the "beech-gap": New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution* 55, 2170-2180.

Trewick, S.A.; Paterson, A.M.; Campbell, H.J. (2007): Hello New Zealand. *Journal of Biogeography* 34, 1-6.

Van Steenis, C.G.G.J. (1971): *Nothofagus,* key genus of plant geography, in time and space, living and fossil, ecology and phylogeny. *Blumea* 19, 65–98.

Vandergoes, M.J.; Fitzsimons, S.J.; Newnham, R.M. (1997): Late glacial to Holocene vegetation and climate change in the eastern Takitimu Mountains, western Southland, New Zealand. *Journal of the Royal Society of New Zealand* 27, 53-66.

Veblen, T.T.; Hill R.S.; Read J. (1996): Themes and concepts in the study of *Nothofagus* forests. *In*: Veblen, T.T.; Hill, R.S.; Read, J., eds., The Ecology and biogeography of *Nothofagus* forests. Yale University Press, New Haven. pp 1-10.

Wagstaff, S.J. (2004): Evolution and biogeography of the austral genus Phyllocladus (Podocarpaceae). *Journal of Biogeography* 31, 1569-1577.

Wagstaff, S.J.; Bayly, M.J.; Garnock-Jones, P.J.; Albach, D.C. (2002): Classification, origin, and diversification of New Zealand hebes (Scrophulariaceae). *Annals of the Missouri Botanical Garden* 89, 39-63.

Walcott, R.I. (1979): Plate motion and shear strain rates in the vicinity of the Southern Alps. *In*: Walcott, R.I.; Cresswell, M.M., eds., The origin of the Southern Alps. The Royal Society of New Zealand, Wellington. pp. 5-77

Walcott, R.I. (1998): Modes of oblique compression: late Cenozoic tectonics of the South Island of New Zealand. *Reviews of Geophysics* 36, 1-26.

Wallis, G.P.; Trewick, S.A. (2001): Finding fault with vicariance: A critique of Heads (1998). *Syst. Biol.* 50, 602–609.

Wardle, J.A. (1984): The New Zealand Beeches – ecology, utilisation and management. New Zealand Forest Service, Wellington.

Wardle, P. (1963): Evolution and distribution of the New Zealand flora, as affected by Quaternary climates. *New Zealand Journal of Botany* 1, 3-17.

Wardle P. (1988): Effects of glacial climates on floristic distribution in New Zealand.1. A review of the evidence. *New Zealand Journal of Botany* 26, 541-555.

Waters, J.M.; Craw D. (2006): Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. *Syst. Biol.* 55, 351-356.

Wayne, R.K.; Leonard, J.A.; Cooper A. (1999): Full of sound and fury: The recent history of ancient DNA. *Annual Review of Ecology and Systematics* 30, 457-477.

Wegener, A. (1912): Die Entstehung der Kontinente. *Geologische Rundschau* 3, 276-292.

Wilford, G.E.; Brown, P.J. (1994): Maps of late Mesozoic-Cenozoic Gondwana breakup: some palaeogeographical implications. *In*: Hill, R.S., ed., History of the Australian vegetation: Cretaceous to recent. Cambridge University Press. Cambridge. pp. 5-13.

Willerslev, E.; Hansen, A.J.; Binladen, J.; Brand, T.B.; Gilbert, M.T.P.; Shapiro, B.; Bunce, M.; Wiuf, C.; Gilichinsky, D.A.; Cooper, A. (2003): Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300, 791-795.

Winkworth, R.C. (2000): Evolution of the New Zealand alpine flora: origins, diversification and dispersal. Unpublished PhD thesis. Massey University, Palmerston North.

Winkworth, R.C.; Robertson, A.W.; Ehrendorfer, F.; Lockhart, P.J. (1999): The importance of dispersal and recent speciation in the flora of New Zealand. *Journal of Biogeography* 26, 1323-1325.

Winkworth, R.C.; Wagstaff, S.J.; Glenny, D.; Lockhart, P.J. (2002): Plant dispersal N.E.W.S from New Zealand. *TRENDS in Ecology & Evolution* 17, 514-520.

Winkworth, R.C.; Wagstaff, S.J.; Glenny, D.; Lockhart, P.J. (2005): Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. *Organism diversity and Evolution* 5, 237-247.

Yang Z (1997): PAML: A program package for phylogenetic analysis by maximum likelihood. *Comput Appl Biosci* 13, 555-556.

Yang, Z (2003): T3 "Thornian Time Traveller" available at http://abacus.gene.ucl.ac.uk/.

Zhang, L-B.; Renner. S. (2003): The deepest splits in Chloranthaceae as resolved by chloroplast sequences. *International Journal of Plant Science* 164, 383-392.

Ziegler, A.M.; Hutver, M.L.; Rowley, D.B.; (1997): Permian world topography and climate. *In*: Martini, I.P., ed., Late Glacial and Postglacial environmental changes - Quaternary, Carboniferous-Permian and Proterozoic. Oxford University Press, New York, pp.111-146.

Supplemental bibliography:

http://www.palaeos.com
http://en.wikipedia.org

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Relaxed Molecular Clock Provides Evidence for Long-Distance Dispersal of *Nothofagus* (Southern Beech)

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***Nothofagus* (southern beech), with an 80-million-year-old fossil record, has become iconic as a plant genus whose ancient Gondwanan relationships reach back into the Cretaceous era. Closely associated with Wegener's theory of "Kontinentaldrift", *Nothofagus* has been regarded as the "key genus in plant biogeography". This paradigm has the New Zealand species as passengers on a Moa's Ark that rafted away from other landmasses following the breakup of Gondwana. An alternative explanation for the current transoceanic distribution of species seems almost inconceivable given that *Nothofagus* seeds are generally thought to be poorly suited for dispersal across large distances or oceans. Here we test the Moa's Ark hypothesis using relaxed molecular clock methods in the analysis of a 7.2-kb fragment of the chloroplast genome. Our analyses provide the first unequivocal molecular clock evidence that, whilst some *Nothofagus* transoceanic distributions are consistent with vicariance, trans-Tasman Sea distributions can only be explained by long-distance dispersal. Thus, our analyses support the interpretation of an absence of *Lophozonia* and *Fuscospora* pollen types in the New Zealand Cretaceous fossil record as evidence for Tertiary dispersals of *Nothofagus* to New Zealand. Our findings contradict those from recent cladistic analyses of biogeographic data that have concluded transoceanic *Nothofagus* distributions can only be explained by vicariance events and subsequent extinction. They indicate that the biogeographic history of *Nothofagus* is more complex than envisaged under opposing polarised views expressed in the ongoing controversy over the relevance of dispersal and vicariance for explaining plant biodiversity. They provide motivation and justification for developing more complex hypotheses that seek to explain the origins of Southern Hemisphere biota.**

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Introduction

An important principle of evolutionary inference is that explanations for the past require an understanding of mechanisms and processes applicable in the present [1]. It is perhaps this sticking point more than any other that has polarised views over the relative importance of vicariance and dispersal for explaining extant plant biodiversity. In 1915, Alfred Wegener put forward a testable hypothesis and mechanism that could explain the transoceanic distribution of animal and plant species. In the 21st century, with many DNA studies now implicating the importance of long-distance dispersal for explaining plant biodiversity [2,3,4,5], it is disconcerting that there is currently a very poor understanding of the mechanisms of transoceanic dispersal (but see [6,7,8,9,10]). Indeed, the inference that the seeds of extant *Nothofagus* species are not suited for dispersal across large distances has played a major role in motivating the hypothesis that transoceanic distributions of *Nothofagus* (Figure 1) can only be explained by vicariance [11,12,13,14,15]. This hypothesis posits that following the Cretaceous breakup of Gondwana, *Nothofagus* rafted and evolved in situ upon different Southern Hemisphere lands. Whilst very attractive, this hypothesis fits somewhat uncomfortably with the findings from analyses of morphological and molecular data. In particular, whilst earlier molecular data have been insufficient for rigorous molecular clock analyses, their interpretation has favoured hypotheses of transoceanic dispersal [16,17,18].

Based on the sequence of Gondwana breakup, a hypothesis of vicariance most parsimoniously predicts that Australian *Nothofagus* species should be most closely related to South American species. This follows since South America and Australia were connected via Antarctica until approximately 35 million years (Myr) ago (Figure 1). In contrast, New Zealand is thought to have separated from Australia 80 Myr ago [19,20]. Thus to explain the close relationship between Australian and New Zealand species by vicariance, it is necessary to argue that extinction of Australian and/or closely related South American species has occurred [12]. Whilst this explanation is ad hoc, the fossil record does provide evidence

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Abbreviations: BRMC, Bayesian relaxed molecular clock; ML, maximum likelihood; Myr, million years; PL, penalized likelihood

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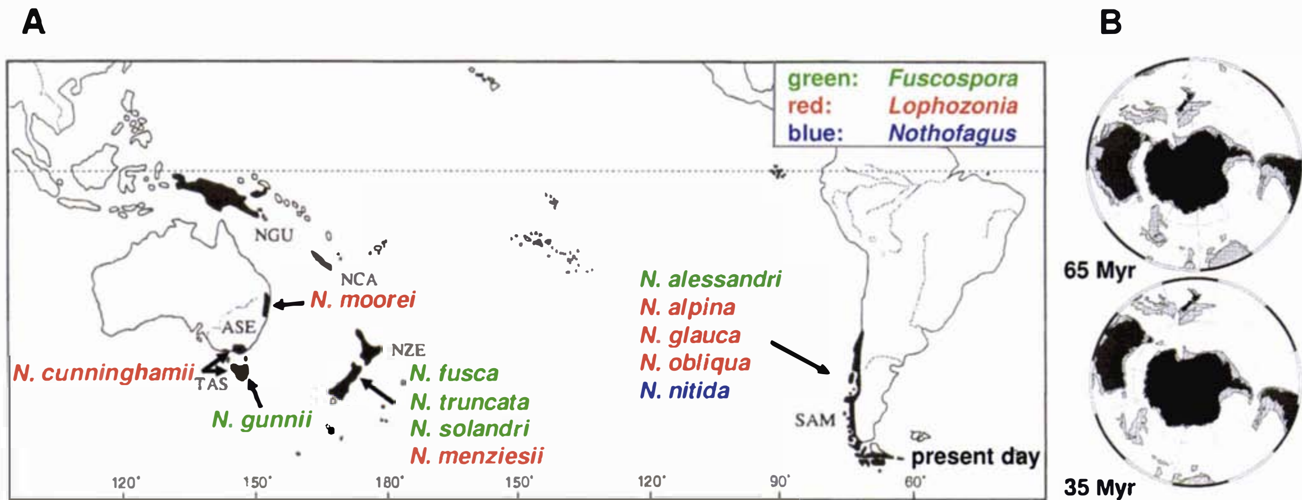

Figure 1. Southern Hemisphere Maps and Present-Day *Nothofagus* Distribution

(A) Transoceanic distribution of *Nothofagus* subspecies *Lophozonia* and *Fuscospora* and South American species *N. nitida* (subgenus *Nothofagus*). Map adapted from Swenson et al. [43]. ASE, Australia; NCA, New Caledonia; NGU, New Guinea; NZE, New Zealand; SAM, South America; TAS, Tasmania.

(B) Relationship of Australia, New Zealand, and South America 65 Myr and 35 Myr before present, reconstructed from http://www.odsn.de/ (link "Plate Tectonic Reconstructions").

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for numerous *Nothofagus* extinctions in Australia, South America, and New Zealand [21,22,23].

However, the fossil record has also been interpreted as indicating multiple events of transoceanic dispersal of *Nothofagus* from Australia to New Zealand. Whilst the extinct "ancestral" *Nothofagus* pollen type occurred in New Zealand prior to the breakup of Gondwana, *Fuscospora* pollen first appeared in New Zealand during the Palaeocene (65 Myr ago) and *Lophozonia* pollen first appeared during the late Eocene (50 Myr ago; [24]). Sixty-five Myr ago the Tasman Sea had already reached its present-day size [19,20]. Hence it is possible that extant New Zealand *Nothofagus* subgenera did not have the opportunity to reach New Zealand via overland migration. Hill [25] has also described the species *Nothofagus cethanica*, which first appeared in Oligocene macrofossils from Tasmania. This species shares unique features with extant *N. fusca* and *N. truncata* from New Zealand and may share a sister relationship with these species explained by trans-Tasman Sea dispersal [26].

A contribution to the debate over the relative importance of vicariance and dispersal can be made by estimating the divergence times of extant species. However, DNA sequences of insufficient length have prevented robust molecular clock analyses from being undertaken. For this reason, we report the sequencing of a 7.2-kb chloroplast genome fragment covering the gene regions (*trnL–trnF* and *atpB–psaI*; see Table 1 for accession numbers) for 11 species of three *Nothofagus* subgenera (*Lophozonia, Fuscospora,* and *Nothofagus*). Our aim has been to date divergence of extant species in the subgenera *Lophozonia* and *Fuscospora*. We have carried out relaxed molecular clock analyses using the methods of Sanderson [27,28] and Thorne et al. [29]. Our findings are that, whilst vicariance is likely to explain some transoceanic relationships amongst *Nothofagus* species, phylogenetic relationships between trans-Tasman species in both *Lophozonia* and *Fuscospora* can only be explained by mid- to late-Tertiary transoceanic dispersal.

Results

Figure 2 shows an optimal maximum-likelihood reconstruction of phylogenetic relationships for chloroplast DNA sequences (7.2-kb comprising the *atpB–psaI* region and the *trnL–trnF* region; 7,269 nucleotide sites) for *Nothofagus* (subgenera or pollen groups (a) *Lophozonia*, (b) *Fuscospora*, and (c) *Nothofagus*) and outgroup *Castanea sativa* (not shown). In a sensitivity analysis of 60 substitution models, the tree shown in Figure 2 was always recovered with very little difference in branch lengths regardless of the substitution model used. Of all substitution models evaluated, K81uf+G was identified as the best fitting one for the data based on hierarchical likelihood ratio tests and the Akaike Information Criterion. This substitution model and also the F84+ Γ_8 model were used for further analyses. The latter was included because the Bayesian relaxed molecular clock (BRMC) approach as implemented in the program MULTIDIVTIME (see Materials and Methods) only allows the use of the JC and the F84 models. Thus analysis with the F84+ Γ_8 model allowed a comparison of date estimates to be obtained using different relaxed molecular clock methods. All nodes of the optimal ML tree recovered in the sensitivity analysis received nonparametric bootstrap support greater than 97%, with the only exception being the grouping of *N. cunninghamii* with *N. moorei*, which received 72% support.

Divergence times for the nodes in this tree (Figure 2) were estimated using the penalized likelihood (PL) method [27] and BRMC method [29,30,31]. For these analyses, a period of 70–80 Myr was used to calibrate the divergence between the three fossil pollen groups representing subgenera *Lophozonia*, *Nothofagus*, and *Fuscospora*. These four pollen groups all first appeared in the fossil record approximately 75 Myr ago [32]. A second constraint of a minimum of 20 Myr for the divergence of *N. cunninghamii* and *N. moorei* was also used. This constraint was based on observations reported by Hill [26] that 20-Myr-old fossils intermediate between *N. moorei* and *N.*

Table 1. Origin of *Nothofagus* Samples and Sequence Accession Numbers

Species	Source	Country	Herbarium Voucher	Accession Number
N. menziesii	Te Aroha	New Zealand	MPN 27272	AY605494 AY605514 AY605504 AY605524
N. solandri	Waikaremoana	New Zealand	MPN 27273	AY605497 AY605517 AY605507 AY605527
N. truncata	Taranaki	New Zealand	MPN 24995	AY605498 AY605510 AY605500 AY605520
N. fusca	Ruahine Range	New Zealand	MPN 27275	AY605491 AY605511 AY605501 AY605521
N. moorei	Barrington Tops National Park	Australia	MPN 27271	AY605495 AY605515 AY605505 AY605525
N. cunninghamii	Cultivated Tasmania	Australia	MPN 25020	AY605490 AY605518 AY605508 AY605528
N. gunnii	Cultivated Tasmania	Australia	MPN 27274	AY605493 AY605513 AY605503 AY605523
N. alessandri	Cultivated Valdivia	Chile	MPN 27277	AY605489 AY605509 AY605499 AY605519
N. obliqua	Cultivated Valdivia	Chile	MPN 27278	AY605496 AY605516 AY605506 AY605526
N. glauca	Cultivated Valdivia	Chile	MPN 27279	AY605492 AY605512 AY605502 AY605522
N. nitida	Cultivated Valdivia	Chile	MPN 28699	AY745879 AY745880 AY745881 AY745882
C. sativa	Cultivated variety Tuscany	Italy	–	AY548965 AY548966

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cunninghamii were recorded from Tasmania and that fossils closely resembling *N. moorei* were also present at that time. The inferred ages for the remaining nodes of the tree, obtained under the F84+ Γ_8 model of substitution are given in Table 2 and graphically illustrated on Figure 2. The variance on these estimates was low and the values were little influenced by the choice of substitution model (Table 3). The robustness of the estimates to calibration error was tested by constraining the divergence of Australian and New Zealand sister taxa to 65 Myr (the time before present when the Tasman Sea reached its present position; thus this date provided us with a lower bound for divergence times of trans-Tasman *Nothofagus* disjunctions that might be explained by vicariance). Constraining these two nodes in this way produced unrealistic age estimates for all basal nodes. For

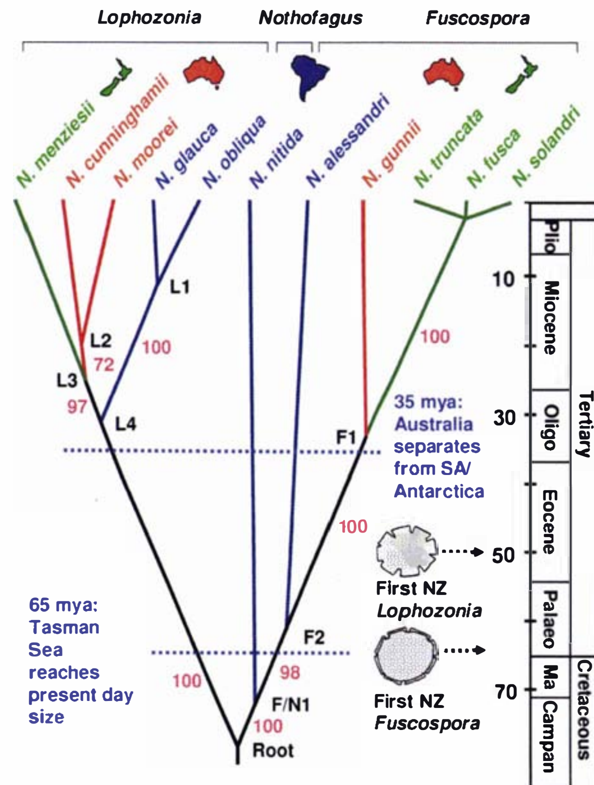

Figure 2. ML Tree Indicating Evolutionary Relationships for *Nothofagus* Species Based on the *atpB-psaI* and *trnL-trnF* Region of the Chloroplast Genome (7,269 bp)

Divergence dates (in Myr) were obtained with an F84+ Γ_8 substitution model using the BRMC approach of Thorne et al. [29]. For the dates indicated, the age of the root node and that of F/N1 were constrained to 70–80 My ; L2 was also constrained in accordance with fossil data [26] at 20 Myr. Violet numbers show bootstrap values. The pollen grains represent the first appearance of the respective pollen type in the New Zealand fossil record. Plio, Pliocene; Oligo, Oligocene; Palaeo, Palaeocene; Ma, Maastrichian; Campan, Campanian. L1–L4, *Lophozonia* 1–4; F1–F2, *Fuscospora* 1–2; F/N1, *Fuscospora/Nothofagus* 1.
DOI: 10.1371/journal.pbio.0030014.g002

Table 2. Estimated Divergence Dates and Standard Deviations (in Brackets) of Different *Nothofagus* Clades

Node Number/ Constraint	BRMC Date Estimate		PL Date Estimate	
	Root: 70–80 Myr F/N1: 70–80 Myr L2: 20 Myr	F1, L3: 65 Myr	Root: 70–80 Myr F/N1: 70–80 Myr L2: 20 Myr	F1, L3: 65 Myr
L1	**15** (± 6)	**37** (± 14)	**0.6** (± 0.6)	–
L2	23[b] (± 3)	**47** (± 9)	20[b]	**40** (± 16)
L3	**27** (± 4)	65[a]	**20** (± 4)	65[a]
L4	**34** (± 4)	**83** (± 7)	**25** (± 4)	**118** (± 20)
F1	**33** (± 8)	65[a]	**13** (± 4)	65[a]
F2	**61** (± 4)	**116** (± 18)	**42** (± 6)	**213** (± 40)
F/N1	72[b] (± 1)	**138** (± 22)	70[b]	**316** (± 64)
Root	78[b] (± 1)	**191** (± 31)	80[b]	**634** (± 113)

The numbers in bold are all the nodes that were estimated without constraints.
Dates are based on different calibration dates and estimation approaches and are given in Myr before present.
[a] Node fixed.
[b] Node constrained.
DOI: 10.1371/journal.pbio.0030014.t002

Table 3. Variation of Estimated Divergence Times (in Myr) under 60 Symmetrical Models of DNA Substitution

Node Number/Constraint	L1		L2		L3		L4		F1		F2		F/N1		Root	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Root: 70–80 Myr F/N1: 70–80 Myr L2: 20 Myr	0.7	0.7	20[a]	20[a]	21.7	21.8	26.6	27.0	12.7	13.3	41.1	42.4	70[a]	70[a]	80[a]	80[a]

Dates estimated using PL approach.
[a]Node constrained.
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example, using the BRMC method, which additionally required a prior expectation to be specified for the age of the root node (which we specified at 75 Myr—the time of appearance of all four extant pollen types), we estimated a more likely age for the root node at 191 Myr. For the PL approach, which does not require specification of a prior, we estimated the age of the root node at 634 Myr. Other basal nodes in both the *Fuscospora* and *Lophozonia* lineages were also much older than reasonably expected (see Table 2).

Discussion

Our findings from molecular clock analyses using five independent calibrations (for four nodes), suggest that the sister relationships of the Australasian (Australia and New Zealand) species within both *Lophozonia* and *Fuscospora* lineages are too young to be explained by continental drift (as indicated by the inferred ages of nodes F1 and L3). Transoceanic dispersal appears the most likely explanation for the trans-Tasman sister relationships indicated in Figures 1 and 2. In contrast, the age inferred for node F2, using both relaxed clock methods is compatible with a hypothesis of continental drift as an explanation for the sister relationship between South American and Australasian *Fuscospora* lineages. The age for node L4, which separates Australasian and South American *Lophozonia,* may also be consistent with vicariance. The BRMC method dates it at 34 Myr before present. However, the PL method estimates this node to be only 25 Myr old, an age too recent to be consistent with vicariance. Thus we regard our results for node L4 as equivocal. Nevertheless, southern beeches are likely to have been present in Antarctica 25 myr ago [33], and thus long-distance dispersal across the young southern ocean between South America and Australia via Antarctica may be conceivable.

The robustness of our phylogenetic inferences has been investigated by varying the substitution model (60 symmetric models were used), estimating the variance of age estimates, and evaluating the influence of calibrations on divergence times. With the exception of the root node, the PL method consistently gave more recent age estimates than did the BRMC method. Both methods showed sensitivity to the number of calibration points used, a finding consistent with recent observations on the performance of relaxed molecular clock methods [34]. In general, the date estimates produced by the BRMC approach were more consistent with the fossil record [26]. A relevant question is whether or not additional calibration points could make date estimates older and thus change our conclusion of trans-Tasman dispersal. We suggest that this may be unlikely, given the observation that constraining a minimum age for trans-Tasman sister species to 65 Myr leads to greatly inflated and unrealistic age estimates for all basal nodes. Hence to explain this finding we would need to invoke a further hypothesis of a dramatic and independent slowing in the rate of evolution in *Lophozonia, Fuscospora,* and *Nothofagus* lineages.

Thus the hypothesis that present-day distribution patterns of *Nothofagus* can be explained by continental drift following the breakup of Gondwana and subsequent extinction of some species [24] can be rejected on the basis of the divergence dates that we have estimated. These dates also indicate that present-day *Nothofagus* species in New Zealand are not the direct descendants of the *Fuscospora* and *Lophozonia* southern beeches that reached New Zealand in the Palaeocene and Eocene eras, respectively [24]. This finding highlights the caution that needs to be taken when interpreting fossil evidence for the apparent first appearance of extant taxa. Fossils that identify specific evolutionary lineages may not necessarily indicate the origins for extant taxa or suggest a continuous presence for these taxa. Similar concerns follow from the findings of molecular analyses for *Ascarina* and *Laurelia* in New Zealand [2,4].

The strength of our molecular analyses highlights the importance of future research into potential mechanisms of long-distance dispersal, and in particular reinvestigation of the transoceanic dispersal properties of *Nothofagus* seeds. For the reasons that we outline in our introduction, it seems likely that only once the mechanisms of long-distance dispersal are understood will hypotheses based on DNA divergence time estimates be truly convincing. DNA sequence analyses have also suggested that long-distance dispersal and continental drift are both important for explaining distributions of the conifer *Agathis* (Araucariaceae) in the South Pacific [35]. Although the molecular evidence for *Agathis* is not as strong as it is for *Nothofagus,* the findings from the molecular studies on these genera highlight the importance of considering more complex hypotheses of relationship in debates concerning the relative importance of dispersal and vicariance.

Materials and Methods

Sequence data. Chloroplast DNA sequences (7.2 kb comprising the *atpB–psaI* region and the *trnL–trnF* region) were determined for each of 11 accessions of *Nothofagus* (subgenera or pollen groups *Lophozonia, Fuscospora,* and *Nothofagus*) sampled in South America, Australia, and New Zealand (see Table 1). These genome regions were also determined for *C. sativa* (an outgroup taxon from Fagaceae) and aligned using progressive multiple-sequence alignment: ClustalX version 1.81 [36]. This resulted in an unambiguous alignment of 7,269 nucleotide sites. Data are missing for approximately 250 bp of the *atpB* gene and *atpB–rbcL* intergene region of *Nothofagus.*

Tree building. Phylogenetic analyses were conducted with PAUP* version 4.0b10 [37] under the ML criterion. A model sensitivity test was conducted, investigating a range of 60 symmetrical models of DNA substitution corresponding to the 56 implemented in MODELTEST version 3.06 [38] (http://darwin.uvigo.es/software/modeltest.html) plus F84, F84+I, F84+Γ_8, and F84+I+Γ_8. ML parameters of these models were estimated by PAUP* following the approach used in MODELTEST. These parameters were then used to conduct 60 individual ML heuristic searches in PAUP* with tree bisection-reconnection branch swapping and a neighbour-joining starting tree. ML bootstrap proportions were obtained after 100 replications, using the same search strategy and ML parameters as for the analysis of the original dataset.

Molecular dating: The PL method. Divergence dates were obtained using the PL method of Sanderson [27] as implemented in the program r8s, version 1.60 [28] (http://ginger.ucdavis.edu/r8s/) with the TN algorithm. The outgroup was excluded using the "prune" command. The degree of autocorrelation within lineages was estimated using cross-validation as suggested by Sanderson [27], and the correcting smoothing parameter λ defined accordingly. Divergence dates were estimated on the 60 ML phylograms recovered in the phylogenetic model sensitivity analysis. Ages for each node across the 60-ML trees were summarized using the "profile" command. Confidence limits on dating estimates were computed by using nonparametric bootstrapping of the original dataset as suggested by Sanderson and Doyle [39]. The program SEQBOOT of the PHYLIP 3.6 package [40] was used to generate 100 bootstrap resampled datasets of 7,269 sites in length. ML branch lengths of the optimal topology were then estimated under the F84+ Γ_8 model for each of the bootstrap resampled datasets using PAUP*. Divergence estimates were then calculated for each of the 100 bootstrap replicates using r8s to obtain standard deviations on each node by the "profile" command and the settings described above.

Molecular dating: The BRMC method. The BRMC approach was applied using the program MULTIDIVTIME as implemented in the Thornian Time Traveller (T3) package [41]. First, the program BASEML of the PAML package version 3.13 [42] (http://abacus.gene.ucl.ac.uk/software/paml.html) was used to estimate the ML parameters of the F84+ Γ_8 substitution model, using the ML topology previously identified. Second, the program ESTBNEW (ftp://abacus.gene.ucl.ac.uk/pub/T3/) was used to estimate branch lengths of the ML topology and the corresponding variance–covariance matrix. Finally, the program MULTIDIVTIME was used to run a Markov chain Monte Carlo for estimating mean posterior divergence times on nodes with associated standard deviations from the variance–covariance matrix produced by ESTBNEW. The Markov chain was sampled 10,000 times every 100 cycles after a burn-in stage of 100,000 cycles. We used a 75-Myr (SD = 37.5 Myr) prior [32] for the expected number of time units between tip and root and a prior of 200 Myr for the highest possible number of time units between tip and root. Other priors for gamma distribution of the rate at root node and the Brownian motion constant describing the rate variation (i.e., the degree of rate autocorrelation along the descending branches of the tree) were derived from the median branch length. As for the PL method, the outgroup was not included in this analysis.

Supporting Information

Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/) accession numbers for the sequences discussed in this paper are given in Table 1.

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Author contributions. MK, KS, DH, FD, and PJL conceived and designed the experiments. MK and KS performed the experiments. MK, KS, FD, and PJL analyzed the data. DH and FS contributed reagents/materials/analysis tools. MK, DH, and PJL wrote the paper. ■

References

1. Lyell C (1970) Principles of geology: Being an attempt to explain the former changes of the earth's surface by reference to causes now in operation. New York: S-H Service Agency. 3 v.
2. Renner SS, Foreman DB, Murray D (2000) Timing transantarctic disjunctions in the Atherospermataceae (Laurales): Evidence from coding and noncoding chloroplast sequences. Syst Biol 49: 579–591.
3. Winkworth RC, Wagstaff SJ, Glenny D, Lockhart PJ (2002) Plant dispersal N.E.W.S from New Zealand. Trends Ecol Evol 17: 514–520.
4. Zhang L-B, Renner S (2003) The deepest splits in Chloranthaceae as resolved by chloroplast sequences. Int J Plant Sci 164: 383–392.
5. Givnish TJ, Millam KC, Evans TM, Hall JC, Pires JC, et al. (2004) Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American–African disjunctions in Rapateaceae and Bromeliaceae based on *ndhF* sequence data. Int J Plant Sci 165: S35–S54.
6. Close RC, Moar NT, Tomlinson AI, Lowe AD (1978) Aerial dispersal of biological material from Australia to New Zealand. Int J Biometeorol 22: 1–19.
7. Carlquist SJ (1996) Plant dispersal and the origin of Pacific island floras. In: Keast A, Miller SE, editors. The origin and evolution of Pacific island biotas, New Guinea to Eastern Polynesia: Patterns and processes. Amsterdam: Academic. pp. 153–164.
8. Hurr K, Lockhart PJ, Heenan PB, Penny D (1999) Dispersal of the Edwardsia section of *Sophora* (Leguminosae) around the southern oceans: Molecular evidence. J Biogeogr 26: 565–577.
9. Muñoz J, Felicisimo AM, Cabezas F, Burgaz AR, Martinez I (2004) Wind as a long-distance dispersal vehicle in the Southern Hemisphere. Science 304: 1144–1147.
10. Renner S (2004) Plant dispersal across the tropical Atlantic by wind and sea currents. Int J Plant Sci 165: S23–S33.
11. Preest DS (1963) A note on the dispersal characteristics of the seed of the New Zealand podocarps and beeches, and their biogeographical significance. In: Gressit JL, editor. Pacific Basin biogeography, a symposium. Honolulu: Bishop Museum Press. pp. 415–424.
12. Linder HP, Crisp MD (1995) *Nothofagus* and Pacific biogeography. Cladistics 11: 5–32.
13. Craw RC, Gehan JR, Heads MJ (1999) Panbiogeography: Tracking the history of life. Oxford: Oxford University Press.
14. McCarthy D (2003) The trans-Pacific zipper effect: Disjunct sister taxa and matching geological outlines that link the Pacific margins. J Biogeogr 30: 1545–1561.
15. Sanmartin I, Ronquist F (2004) Southern Hemisphere biogeography inferred by event-based models: Plant versus animal patterns. Syst Biol 53: 216–243.
16. Martin PG, Dowd JM (1993) Using sequences of *rbcL* to study phylogeny and biogeography of *Nothofagus* species. Aust J Bot 6: 441–447.
17. Hill RS, Jordan GJ (1993) The evolutionary history of *Nothofagus* (Nothofagaceae). Aust J Bot 6: 111–126.
18. Manos P (1997) Systematics of *Nothofagus* (Nothofagaceae) based on rDNA spacer sequences (ITS): Taxonomic congruence with morphology and plastid sequences. Am J Bot 84: 1137–1150.
19. Wilford GE, Brown PJ (1994) Maps of late Mesozoic–Cenozoic Gondwana breakup: Some palaeogeographical implications. In: Hill RS, editor. History of the Australian vegetation: Cretaceous to recent. Cambridge: Cambridge University Press. pp. 5–13.
20. McLoughlin S (2001) The breakup history of Gondwana and its impact on pre Cenozoic floristic provincialism. Aust J Bot 49: 271–300.
21. Hill RS, Jordan GJ, Macphail MK (1996) History and paleoecology of Australian *Nothofagus* forests. In: Veblen TT, Hill RS, Read J, editors. The ecology and biogeography of *Nothofagus* forests. New Haven (Connecticut): Yale University Press. pp. 182–199.
22. Markgraf V, Romero E, Villagrán C (1996) History and palaeoecology of South American *Nothofagus* forests. In: Veblen TT, Hill RS, Read J, editors. The ecology and biogeography of *Nothofagus* forests. New Haven (Connecticut): Yale University Press. pp. 354–386.
23. McGlone MS, Mildenhall DC, Pole MS (1996) History and palaeoecology of New Zealand *Nothofagus* forests. In: Veblen TT, Hill RS, Read J, editors. The ecology and biogeography of *Nothofagus* forests. New Haven (Connecticut): Yale University Press. pp. 83–130.
24. Hill RS (2001) Biogeography, evolution and palaeoecology of *Nothofagus* (Nothofagaceae): The contribution of the fossil record. Aust J Bot 49: 321–332.
25. Hill RS (1984) Tertiary *Nothofagus* macrofossils from Cethana, Tasmania. Alcheringia 8: 81–86.
26. Hill RS (1991) Tertiary *Nothofagus* (Fagaceae) macrofossils from Tasmania and Antarctica and their bearing on the evolution of the genus. Bot J Linn Soc 105: 73–112.

27. Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. Mol Biol Evol 19: 101–109.
28. Sanderson MJ (2003) r8s: Inferring absolute rates of molecular evolution and divergence dates in the absence of a molecular clock. Bioinformatics 19: 301–302.
29. Thorne JL, Kishino H, Painter IS (1998) Estimating the rate of evolution of the rate of molecular evolution. Mol Biol Evol 15: 1647–1657.
30. Kishino H, Thorne JL, Bruno WJ (2001) Performance of a divergence time estimation method under a probabilistic model of rate evolution. Mol Biol Evol 18: 352–361.
31. Thorne JL, Kishino H (2002) Divergence time and evolutionary rate estimation with multilocus data. Syst Biol 51: 689–702.
32. Dettmann ME, Pocknall D, Romero EJ, Zamboloa M (1990) *Nothofagidites* Erdman *ex* Potonie 1960: A catalogue of species with notes on palaeogeographic distribution of *Nothofagus* Bl. (southern beech). NZ Geol Surv Palaeontol Bull 60: 1–79.
33. Hill RS, Truswell EM (1993) *Nothofagus* fossils in the Sirius Group, Transantarctic Mountains: Leaves and pollen and their climatic implications. In: Kennett JP, Warnke DA, editors. Antarctic research series, Volume 60. The Antarctic palaeoenvironment: A perspective on global change. Washington, DC: American Geophysical Union. pp. 67–73.
34. Pérez-Losada M, Høeg JT, Crandall KA (2004) Unraveling the evolutionary radiation of the thoracian barnacles using molecular and morphological evidence: A comparison of several divergence time estimation approaches. Syst Biol 53: 244–264.
35. Stöckler K, Daniel IL, Lockhart PJ (2002) New Zealand Kauri (*Agathis australis* (D.Don) Lindl., Araucariaceae) survives Oligocene drowning. Syst Biol 51: 827–832.
36. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876–4882 (Program available: ftp-igbmc.u-strasbg.fr. Accessed 16 November 2004.)
37. Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4 [computer program]. Sunderland (Massachusetts): Sinauer.
38. Posada D, Crandall K (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics 14: 817–818.
39. Sanderson MJ, Doyle JA (2001) Sources of error and confidence intervals in estimating the age of angiosperms from *rbcL* and 18S rDNA data. Am J Bot 88: 1499–1516.
40. Felsenstein J (2002) PHYLIP (PHYLogeny inference package), version 3.6 [computer program]. Available: http://evolution.genetics.washington.edu/phylip.html. Accessed 18 November 2004.
41. Yang Z (2003) T3 "Thornian time traveller" [computer program]. Available: http://abacus.gene.ucl.ac.uk/. Accessed 16 November 2004.
42. Yang Z (1997) PAML: A program package for phylogenetic analysis by maximum likelihood. Comput Appl Biosci 13: 555–556.
43. Swenson U, Backlund A, McLoughlin S, Hill RS (2001) *Nothofagus* biogeography revisited with special emphasis on the enigmatic distribution of subgenus *Brassospora* in New Caledonia. Cladistics 17: 28–47.