Chapter 6: Conclusions

Outline of discussion

By investigating the genetic patterns of the chloroplast of three widespread plants of cool temperate rainforest in southeastern Australia, *Nothofagus cunninghamii*, *Tasmannia lanceolata* and *Atherosperma moschatum*, this thesis has enabled a new understanding of how these species have responded to past climatic changes during the Pleistocene. Because of their relatively high chloroplast diversity, it was possible to make stronger inferences concerning the history of *N. cunninghamii* and *T. lanceolata* than for *A. moschatum*, which showed low chloroplast diversity. As a result this discussion will focus mainly on *N. cunninghamii* and *T. lanceolata*. The major themes that will be discussed are the evidence for the importance of tolerance or adaptation of these species during past climatic changes, the limited mobility in *N. cunninghamii* and *T. lanceolata* inferred from the patterns of the seed-mediated chloroplast and how this limited dispersal contrasts with the northern hemisphere, and the depth of phylogeographic patterns in the species that reflect events that occurred long before the last glacial.

The roles of adaptation and tolerance in the history of southern cool temperate rainforests

A principal finding of this thesis is the strong genetic signal for multiple glacial refugia in both *Nothofagus cunninghamii* and *Tasmannia lanceolata*. Considering that survival within refugia is unlikely to always result in genetic patterns that signal the presence of refugia, the number of refugia is likely to be greater than can be inferred from the chloroplast patterns. For example, different refugial populations of a species may share the same haplotype. In addition, although chloroplast patterns may indicate survival of one species in an area the genetic signal may be ambiguous for other plants in the same area. For example, presence of an endemic and diverged haplotype of *N. cunninghamii* in northeast Tasmania contrasts with the occurrence of a single widespread haplotype in *T. lanceolata* in the same region.

Evidence for multiple glacial refugia fits well with expectations for the southern hemisphere that species were able to survive during glacials in numerous topographically protected sites (Macphail & Colhoun, 1985; McGlone, 1985; Markgraf et al., 1995). Importantly, this study provides strong evidence that locations of survival for forest species included areas that would be unexpected based on our
current understanding of paleoclimates and the climatic tolerances of species inferred from the current climatic ranges. The strongest cases for this are evidence for possible multiple refugia for *N. cunninghamii* in northeast Tasmania, the evidence for survival of this species above the LGM climate tree line in western Tasmania and genetic evidence that *T. lanceolata* withstood the predicted dry glacial climates in southeastern Tasmania and the Grampians in Victoria. Both southeastern Tasmania and the Grampians are currently at the lower end of rainfall for the species (i.e. 800-1000mm per annum), and there is evidence that, like northeast Tasmania (see Chapter 2), southeastern Tasmania was strongly affected by glacial aridity during the last glaciation (Colhoun, 1977, 1985b, 2002). Within each of these regions the presence of divergent, endemic chloroplast lineages are strong evidence for refugial status. However, each of these regions shows little diversity, a pattern entirely consistent with the strong bottlenecks that would be expected in dry climates for rainforest species. This inference is supported by the higher chloroplast diversity observed in western Tasmania where glacial climates are thought to have been much wetter than the east. These findings contribute to a growing understanding of the ability of populations of some woody plants to withstand extreme climates for thousands of years and the importance of the resilience of plants in shaping the current biota (Petit et al., 2008).

The genetic evidence for the survival of *N. cunninghamii* and *T. lanceolata* in apparently hostile climates raises some questions about how these species were able to adapt or tolerate past climatic change. One hypothesis is that both *N. cunninghamii* and *T. lanceolata* were able to tolerate glacial climates in non-analogue environments, that is, combinations of climate and environmental factors that no longer exist in the present interglacial. This proposal is based on the idea that the distribution of these species in the current interglacial may not reflect the full breadth of the ecological range of these species during different parts of the Pleistocene. Another alternative is adaptation where the species were able to survive glacial climates via changes in the functional traits of these species. Changes in functional traits, and therefore the potential climatic range of the species, may have been achieved through plasticity or the evolution of new gene variants. At least during the last glacial the acquisition of genes via hybridisation with other species was impossible in *N. cunninghamii, A. moschatum* and (within Tasmania at least) in *T. lanceolata* unless they co-existed with unknown extinct species at the time.
Limited mobility during the Holocene

A strong feature of the chloroplast phylogeographic patterns of both *N. cunninghamii* and *T. lanceolata* is the evidence for very limited mobility. The geographical extent of haplotypes and the low mixing of haplotypes, particularly in *T. lanceolata* indicates that migration of these species out of glacial refugia within their current ranges during the Holocene probably occurred over distances less than 100 km, and in many cases the distance can be inferred to be less than 50 km. The low chloroplast divergence observed between most haplotypes in *A. moschatum* and the widespread occurrence of an ancestral haplotype means that it is difficult to infer movements in this species. The low cpDNA diversity observed in *A. moschatum* compared to both *N. cunninghamii* and *T. lanceolata* may be due to stronger bottlenecks, and means that the current wide range of this species may be explained by the capacity for long-distance seed dispersal of this wind dispersed species. However, this assertion is tentative and awaits further investigation via faster evolving genetic markers (see Chapter 4).

The evidence for limited mobility in the Holocene of *N. cunninghamii* and *T. lanceolata* contrasts with the northern hemisphere where, despite the increasing recognition of small northern glacial refugia that occurred close to the ice sheets (McLachlan *et al.*, 2005; Anderson *et al.*, 2006; Petit *et al.*, 2008), migrations extending over 1000's of kilometres must be inferred to account for the spread of species into formerly glaciated landscapes. Some possible explanations can be forwarded here for the discrepancy between the low mobility that seems to characterise the cool temperate rainforest of southeastern Australia and the significant mobility inferred for many forest trees during the Holocene in the northern hemisphere. Firstly, unlike in the northern hemisphere, expansive areas of more or less continuous and suitable habitat did not become available in southeastern Australia at the end of the Holocene. The patchy distribution of available habitat in southeastern Australia broken by drier landscapes unsuitable for rainforest would have decreased the chance of long-distance dispersal events reaching suitable mesic sites. In addition within the mesic pockets that were made available during the Holocene for rainforest plants, species recovery from glacial climates was dominated by expansion from local refugia. As described by Hewitt (1996) initial founding populations increase exponentially and subsequent colonization events are less likely to be successful after the community dynamics changes to replacement dynamics in more closed vegetation at carrying
Another contributing factor may be the landscape differences between southeastern Australia and Europe and North America lead to selection for intrinsically higher dispersability in parts of the north compared to the south (i.e. selection of tolerant, 'stay at home' species in the south versus high mobility in parts of species ranges in the north). For example, phenotypic differences have been observed between populations of *Frangula alnus* in formerly glaciated northern Europe and Mediterranean populations (faster generation time and smaller fruits more suited to migratory birds in northern populations; Hampe & Bairlein, 2000). A similar case has been observed in northern European populations of *Pinus contorta* which reportedly have more mobile wind-dispersed seeds than southern European populations (Cwynar & MacDonald, 1987). The chloroplast phylogeographies of southeastern Australia most closely resemble the phylogeographies of temperate forest species at similar latitudes in the Mediterranean mountains of southern Europe with evidence for low mobility and deep divergence between current populations (Petit et al., 2005).

**Antiquity of haplotypes and geographic patterns of haplotypes**

The deep divergence of haplotypes observed between some populations across the ranges of *N. cunninghamii* and *T. lanceolata* provides strong evidence that the current chloroplast structure of these species reflects events that occurred long before the last glacial. This contributes to a growing number of studies of temperate plants that have observed deep phylogeographic patterns, for example, in the Mediterranean region (Lumaret et al., 2002; Petit et al., 2005; Magri et al., 2007), California (Grivet et al., 2006) and southeastern United States (Morris et al., 2008). The nesting of *N. moorei* within the chloroplast variation observed in *N. cunninghamii* provides evidence for great depth of divergence of lineages within *N. cunninghamii* (see Chapter 2). Though the complete relationship between postglacial populations and their modern genetic diversity cannot be fully understood without the analysis of DNA haplotypes from fossil plants (Magri et al., 2006), the chloroplast phylogeography of *N. cunninghamii* provides evidence of stasis of the species with a highly diverged lineage restricted to the northeast highlands of Tasmania and multiple lineages in western Tasmania. Though a temporal element (Morris et al., 2008) is absent in *T. lanceolata* and *A. moschatum* (e.g. molecular dating), apart from some evidence for the slow evolution of the chloroplast, the strong divergence of endemic haplotypes or lineages in some regions within the ranges of these species is
consistent with long term occupation of areas possibly for multiple glacial episodes, for example, in southeastern Tasmania, the Grampians, and western Tasmania for *T. lanceolata* and Monga and possibly the Blue Mountains in *A. moschatum*. Whether the morphological similarity that is observed between most of these populations that have probably been isolated for multiple glacial periods is a result of morphological stasis (e.g. Dick et al., 2003), consistent with the slow evolution of trees (Petit & Hampe, 2006), or pollen mediated gene flow between these regions needs to be examined.

Although some populations of *N. cunninghamii* and *T. lanceolata* may have remained isolated for long periods, the shallow divergence (i.e. one or two mutations) of haplotypes observed between populations of these species in parts of Tasmania and the Central Highlands of Victoria (and the Australian Alps for *T. lanceolata*) provides evidence that both species must have been able to migrate across the 250 km wide Bass Strait. Considering the fact that populations of both species in the Central Highlands of Victoria harbour endemic haplotype clades that are nested within the chloroplast diversity present in Tasmania, any migration is most likely to have occurred before the last glacial and the direction of movement is most easily explained as having occurred northwards from Tasmania. A similar argument could be made for *A. moschatum*, although because the single haplotype of this species in Victoria and southeastern NSW differs from Tasmania by only one mutation a Holocene dispersal cannot be ruled out. Both *N. cunninghamii* and *T. lanceolata* may have been able to migrate during a similar event such as the convergence of suitable climates and the opening of Bass Strait which has been exposed repeatedly throughout the Pleistocene, as has been postulated for *N. cunninghamii* (Howard & Hope, 1970; Bridgewater, 1976; Hope, 1994). The genetic evidence for pre-last glacial migration of both *N. cunninghamii* and *T. lanceolata* across Bass Strait fits well with chloroplast phylogeographic studies of *Eucalyptus* species that occur in both Tasmania and southern Victoria including *E. globulus* (Freeman et al., 2001), *E. viminalis, E. ovata* (Marthick, 2005) and *E. regnans* (Nevill et al., 2008).