

Glacial refugia and reticulate evolution: the case of the Tasmanian eucalypts

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Tasmania is a natural laboratory for investigating the evolutionary processes of the Quaternary. It is a large island lying 40–44° S, which was repeatedly glaciated and linked to southeastern continental Australia during the Quaternary. Climate change promoted both the isolation of species in glacial refugia, and an exchange between Tasmanian and mainland floras. *Eucalyptus* is a complex and diverse genus, which has increased in abundance in Australia over the past 100 kyr, probably in response to higher fire frequency. Morphological evidence suggests that gene flow may have occurred between many eucalypt species after changes in their distribution during the Quaternary. This paper summarizes recent genetic evidence for migration and introgressive hybridization in Tasmanian *Eucalyptus*. Maternally inherited chloroplast DNA reveals a long-term persistence of eucalypts in southeastern Tasmanian refugia, coupled with introgressive hybridization involving many species. Detailed analysis of the widespread species *Eucalyptus globulus* suggests that migration from mainland Australia was followed by introgression involving a rare Tasmanian endemic. The data support the hypothesis that changes in distribution of interfertile species during the Quaternary have promoted reticulate evolution in *Eucalyptus*.

Keywords: glacial refugia; *Eucalyptus*; chloroplast; phylogeography; hybrid; Tasmania

1. INTRODUCTION

This paper summarizes recent work on the evolution of *Eucalyptus* (family Myrtaceae) in the southeastern region of Australia, particularly in the island of Tasmania. Starting with the pioneering work of Barber & Jackson (1957), the Tasmanian eucalypts have been used to address a range of evolutionary questions, including natural selection and convergent evolution. These studies have revealed highly dynamic interactions among populations and within groups of related species (see, for example, Potts & Reid 1988). However, many questions remain unanswered. The use of new phylogeographical techniques (as summarized in Avise 2000) is today enabling closer analysis of the historical processes that shaped this dominant Australian tree genus.

Taxonomically, *Eucalyptus* is considered a difficult genus, presenting problems partly analogous to those of *Quercus* and *Pinus* (Pryor & Johnson 1971). Approximately 700 species are currently recognized (Brooker 2000). Clinal variation, morphological convergence and hybridization within subgenera are common. Taxonomic reclassification continues (Brooker 2000) and the generic status of major lineages within the eucalypt alliance, *Angophora* and *Corymbia*, is currently in dispute (Brooker

2000; Ladiges & Udovicic 2000; Steane *et al.* 2002). Molecular evidence has become increasingly important in elucidating relationships between and within lineages (see, for example, Steane *et al.* 1999, 2002; Udovicic & Ladiges 2000).

In particular, molecular techniques offer unprecedented opportunities for testing alternative hypotheses for patterns of morphological variation. Populations of intermediate morphology sometimes link pairs or groups of eucalypt species over a wide geographical range. On this evidence, Pryor & Johnson (1981) suggested that redistributions of eucalypts, in response to environmental changes since the Pliocene (*ca.* 5.3 Ma), might have enabled divergent populations or species to come into contact and exchange genes. The frequency of hybridism and 'species breakdown' appears higher in eastern Australia than in western Australia, a fact that might be attributable to greater redistribution of taxa in the former region (Pryor & Johnson 1981). Hybridization between species in *Eucalyptus* has been proposed as an important process contributing to intraspecific genetic variability, and as a means of habitat invasion independent of seed dispersal (Potts & Reid 1988). However, until recently, molecular evidence for widespread genetic exchange between species (reticulate evolution) was lacking. This paper summarizes our genetic evidence for spatial redistributions and gene flow between eucalypt species, and relates these to recent information on Quaternary climate change and glacial refugia in southeastern Australia.

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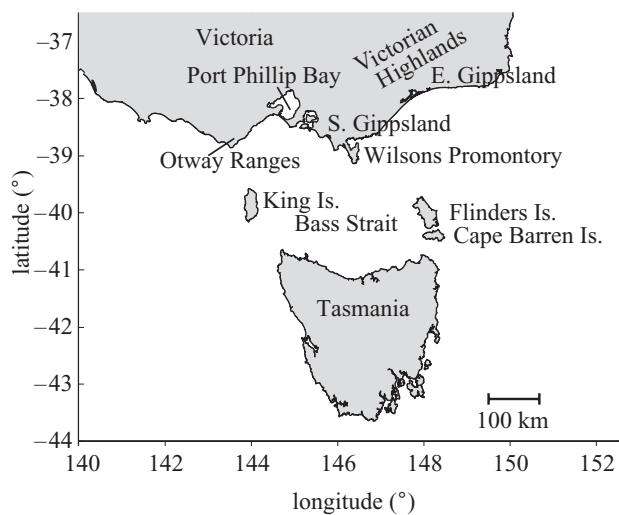


Figure 1. Present-day Tasmania and southeastern continental Australia.

2. THE SOUTHEAST AUSTRALIAN ENVIRONMENT

Tasmania is a large island (*ca.* 320 km × 320 km) situated between latitudes 40°44' and 43°38' S (figure 1). It is currently separated from Victoria, which covers the tip of mainland southeastern Australia, by the Bass Strait, which is 250 km wide and relatively shallow (mostly less than 100 m deep). The two regions have been repeatedly separated and rejoined by changes in sea level at least since the Early Miocene (17 Ma; Baillie 1989). Tasmania and Victoria show clear floristic links, and both have temperate climates with warm to hot summers and mostly mild and wet winters. However, Tasmania's southerly position, more maritime climate, mountainous topography and distinctive geology have created several environments that lack mainland analogues.

The natural vegetation of Victoria and Tasmania is a complex mosaic reflecting geology, fire history, rainfall, altitude and microsite effects. Most of the vegetation is dominated by *Eucalyptus* species (Jackson 1999; Australian Native Vegetation Assessment 2001). Dry open eucalypt forest (10–30 m tall) and open eucalypt woodlands are common in all areas of low to moderate rainfall (e.g. most of western and central Victoria, the east coast and midlands of Tasmania). Tall (greater than 30 m) wet eucalypt forests occur in most regions with high rainfall (notably the Otways region, Gippsland, the northeastern highland of Tasmania and western Tasmania). Such forests also occur in appropriate microsites in areas with moderate rainfall (e.g. in steep gullies throughout eastern Tasmania). Cool temperate rainforest grows in relatively fire-free sites throughout the range of tall, wet eucalypt forest. The southwestern third of Tasmania, which receives over 2000 mm of rain annually and has old, highly siliceous rocks that are very poor sources of soil nutrients, is dominated by a mosaic of moorland, low dense woody vegetation, eucalypt forest and cool temperate rainforest. Alpine vegetation occurs at high altitudes in eastern Victoria (greater than 1800 m above sea-level), across the central highlands of Tasmania, and in isolated areas (mostly greater than 1100 m above sea-level) elsewhere in Tasmania (Kirkpatrick 1997). Extant vegetation patterns have been strongly influenced by climate change during

the Quaternary, which caused both extinctions and redistributions within the regional flora.

3. CLIMATE CHANGE IN SOUTHEASTERN AUSTRALIA DURING THE QUATERNARY

In southeastern Australia, the cold phases of the climate cycles of the Quaternary resulted in glacial and periglacial activity, aridity, temperature depression and reduced atmospheric carbon dioxide concentrations. There is geological evidence for at least five Quaternary glaciations in Tasmania: during the Early Pleistocene (1.68–0.788 Ma); the Middle Pleistocene (788–120 ka); the Penultimate Glacial (*ca.* 135 ka); the early Last Glacial (*ca.* 70 ka); and the LGM (*ca.* 23–17 ka) (Colhoun *et al.* 1996; Augustinus *et al.* 1997; Kiernan *et al.* 2001). Many other undetected episodes of glaciation may have occurred. The maximum extent of ice occurred during the Early Pleistocene (Colhoun *et al.* 1996; Augustinus *et al.* 1997), when *ca.* 7000 km² of Tasmania was covered by ice compared with *ca.* 1100 km² during the LGM. The Kosciuszko region of New South Wales was lightly glaciated during the LGM and earlier in the Quaternary (Barrows *et al.* 2001), but convincing evidence for glaciation of the Victorian Highlands is lacking.

The vegetation of the Late Pliocene and the earliest part of the Quaternary appears to have been substantially different from that of subsequent periods. Diverse rainforests grew at one site in central Victoria (Stony Creek Basin) until *ca.* 1.4 Ma (J. M. K. Sniderman, personal communication) in a region that now supports only dry-climate vegetation. Significant plant extinction during the Early Pleistocene is shown by the fact that 45% of the species in the one diverse and well-documented plant macrofossil site from this period (Regatta Point, western Tasmania) are now extinct (Jordan 1997). The extinctions appear to have been stepwise, starting during the Late Pliocene (Macphail *et al.* 1993, 1995). Although plant extinctions occurred as late as the Late Pleistocene (*ca.* 120–12 ka) (Jordan & Hill 1991), fossil assemblages from the Middle and Late Pleistocene are essentially modern in floristics. This implies significant changes in vegetation during the early Quaternary (i.e. before 788 ka). These changes may have been driven by the climatic events that caused the most extensive glaciation in Tasmania.

Pollen-based reconstructions of mainland Australian climates suggest that variation in aridity was the crucial climatic feature during the second half of the Quaternary (Kershaw & Nanson 1993). The vegetation oscillated (presumably in accord with the Milankovitch cycles) between vegetation similar to that of the present and that of a much drier climate. Typically, rainforest was replaced by dry forests with isolated patches of wet forest, and dry forest was replaced by open woodlands and low open shrubland. In Tasmania, both temperature and aridity appear to have been important. For example, the Darwin Crater core in western Tasmania shows repeated vegetation cycles from wet forest to subalpine heaths and herbaceous vegetation (Colhoun & van der Geer 1998). The subalpine heaths included drought-sensitive taxa such as the conifer *Microstrobos*. Their increase in prominence

was, therefore, likely to have been caused by temperature depression rather than aridity.

During sea-level depressions of more than *ca.* 55 m, Tasmania was connected to Victoria by a low-lying land bridge (the Bassian Plain) that linked northeastern Tasmania to South Gippsland through Flinders Island, and at somewhat lower sea-levels linked northwestern Tasmania to the Port Phillip Bay region through King Island (Jackson 1999). People arrived in northern Australia at least 55 ka, and had spread to Tasmania by 34 ka (Cosgrove 1995). The exact timing and impact of their arrival remains controversial. However, there is no doubt that fire frequencies increased during the Late Pleistocene, resulting in dramatic changes in vegetation. Charcoal levels and the proportion of fire-adapted species (including eucalypts) in pollen cores clearly increase during this period (Kershaw *et al.* 2002).

Owing to their wide range of habitat preferences, different eucalypt species probably responded differently to Quaternary climate changes. In the cold periods, eucalypts currently adapted to mid-to-high altitude Tasmanian habitats may have been able to persist in local refugia through altitudinal migrations, while lowland species were forced to the edges of the island or were driven to extinction. Combined pollen records from Victoria suggest that xerophytic woods and scrub were more extensive in southeastern Australia than at present (Pickett *et al.* 2003). Pollen and charcoal from two sites suggest that the Victorian south-central highlands were virtually treeless during the LGM (McKenzie 1997). In Tasmania, the altitudes of cirques suggest terrestrial temperatures *ca.* 6–6.5 °C colder than present for the LGM (Colhoun *et al.* 1996). The Tasmanian climate was probably also more continental because of the greatly increased land area, and was certainly more arid in most areas. Most of western Tasmania was alpine grassland and herbland, with refugia for sclerophyll (eucalypt) woodland and rainforest below 100 m on valley floors near the coast (Colhoun 2000). Pollen profiles (Macphail & Jackson 1978; Sigleo & Colhoun 1981) suggest that the Tasmanian Midlands were completely deforested. The treeline was close to present sea-level in much of Tasmania (e.g. Kirkpatrick & Fowler 1998). Although Tasmania and Victoria were connected during the LGM, the Bassian Plain probably provided some sort of barrier to dispersal of forest species owing to aridity. Low open shrubland and grassy woodland, with only sparse eucalypts, are likely to have occupied most of the Bassian Plain (e.g. Hope 1978). The same situation may have prevailed during many (or possibly all) earlier glacials.

Kirkpatrick & Fowler (1998) modelled the distribution of major vegetation categories for Tasmania during the LGM, and proposed that (in addition to west coast refugia described above) small scattered refugia for eucalypt forest occurred in the southeast of the island, with more extensive stands covering the northern tips of the island and parts of the former Bassian Plain. Their model depended on the assumption that the dominant rainforest species, *Nothofagus cunninghamii*, survived in eastern Tasmania through the Late Pleistocene, implying that rainfall in eastern Tasmania was similar to current rainfall. *Nothofagus cunninghamii* is a poor disperser (Hickey *et al.* 1982), so its current presence in eastern Tasmania is

difficult to explain through dispersal from western Tasmania across a gap of *ca.* 60 km. As a result, many authors (e.g. Macphail & Colhoun 1985) have argued that this and other tree species in Australia may have recolonized from many small refugia, rather than advancing on major fronts. However, there is no fossil evidence for the survival of *N. cunninghamii* in eastern Tasmania, and some evidence suggests that eastern Tasmania was considerably more arid during the Last Glacial than at present (e.g. Sigleo & Colhoun 1982; Bowden 1983). Accordingly, conditions in eastern Tasmania may have been even more hostile for most *Eucalyptus* species than proposed by Kirkpatrick & Fowler (1998).

Pollen records from both western and eastern Tasmania show a rapid expansion of forest during the early Holocene (Macphail 1979), although the major phase of forest expansion in southern Victoria was somewhat later, at 5–7 ka (McKenzie & Kershaw 2000). This is entirely consistent with ocean core data, which suggest that sea surface temperatures near Tasmania continued to rise after 14 ka and by 10 ka were 1–2 °C warmer than they are now (Sikes *et al.* 2002; W. Howard, personal communication). By contrast, sea-levels rose slowly (e.g. Fairbanks 1989). In South Australia they were still *ca.* 60 m below current levels at 10 ka (Belperio *et al.* 2002). Thus, sea-levels were low enough during the period of forest expansion of the early Holocene to maintain continuous (or nearly continuous) land between Tasmania and Victoria. This may have provided corridors for dispersal of forest species during the early Holocene. Similar lags in sea-level rise may also have provided dispersal corridors after earlier deglaciations.

4. PATTERNS OF GENETIC VARIATION IN THE TASMANIAN EUCALYPTS

The Tasmanian eucalypts are a mixture of widespread and endemic species (12 and 17 species, respectively), suggesting periods of both isolation and biotic exchange. Many of the endemic species are restricted to eastern Tasmania, especially the southeastern corner, where species richness is highest (Williams & Potts 1996). The mosaic of species is on a fine spatial scale due to topographical complexity, because altitude and aspect play major roles in species' distributions (Wardell-Johnson *et al.* 1997). Relative to the rest of Australia, Tasmania has a limited eucalypt flora, with only 12 species of the subgenus *Eucalyptus* (*sensu* Brooker 2000; formerly *Monocalyptus*) and 17 species from one section (*Maidenaria*) of the subgenus *Symphyomyrtus*. The two subgenera are reproductively isolated, and only *Symphyomyrtus* section *Maidenaria* is considered in this paper.

Maidenaria, confined to southeastern Australia, comprises 74 species (Brooker 2000). So far, no solid phylogenetic reconstruction of this section has been possible. An analysis based on morphology was complicated by homoplasy, intergradation of taxa and problems of character coding (Chappill & Ladiges 1996), whereas analysis of sequences of the ITS region of the nuclear ribosomal RNA genes found little variation between species (Steane *et al.* 1999, 2002). However, morphological and ecological variation is considerable, and species occupy Tasmanian

habitats ranging from alpine plateaux (e.g. *E. vernicosa*) to coastal lowlands (e.g. *E. globulus*).

Our recent studies have shown that patterns of variation in the cp genome of Tasmanian *Eucalyptus* are consistent with widespread gene flow between species, possibly arising from spatial redistributions during the Quaternary. In eucalypts, two studies (Byrne *et al.* 1993; McKinnon *et al.* 2001*b*) have established that the cp genome is maternally inherited. The distribution of cpDNA variants (haplotypes) therefore tracks the movement of seed across the landscape, and can reveal regions in which individuals are related through the maternal line. This paper collates and extends cpDNA data from Steane *et al.* (1998), Jackson *et al.* (1999), McKinnon *et al.* (2001*a*), Freeman *et al.* (2001) and Whittock (2000). Initial studies (Steane *et al.* 1998, Jackson *et al.* 1999) used the RFLP technique with hybridization to cpDNA probes, whereas all subsequent studies used polymerase-chain-reaction-based techniques, which were correlated with earlier RFLP data (Vaillancourt & Jackson 2000). Our sampling of section *Maidenaria* now covers 40 species across southeastern Australia, although Tasmania has been the main focus.

All studies so far have provided consistent findings about patterns of cpDNA variation within *Maidenaria*. These may be summarized as follows.

- (i) Chloroplast DNA is variable within many species. Eighteen out of 23 species with replicate samples show intraspecific variation.
- (ii) Intraspecific cpDNA variation shows significant geographical structuring.
- (iii) Geographical patterns of cpDNA are identical for many Tasmanian species. Individuals that are geographically close tend to share cpDNA lineages or specific cpDNA haplotypes, despite being from different species.
- (iv) The distributions of some cpDNA haplotypes are correlated with former glacial refugia in Tasmania.

5. BROAD-SCALE PATTERNS OF cpDNA VARIATION ACROSS SPECIES OF MAIDENARIA

The initial studies of Steane *et al.* (1998) and Jackson *et al.* (1999) demonstrated that several species of *Maidenaria* sampled on the mainland carried cpDNA haplotypes from either or both of two highly divergent lineages (labelled 'northern' (N) and 'central' (C); Jackson 1999). The C lineage was also found in four endemic species from central Tasmania, whereas a third lineage (labelled 'southern' or S) was found in five endemic species from southeastern Tasmania. Intriguingly, individuals that were geographically close appeared to share local cpDNA lineages, regardless of species boundaries. For example, the widespread species *E. globulus* had C and N haplotypes in Victoria, C haplotypes in northern Tasmania, and S haplotypes in southeastern Tasmania. The Tasmanian endemics *E. urnigera* and *E. gunnii* had C haplotypes in central Tasmania and S haplotypes in southeastern Tasmania.

The pattern of geographically structured cpDNA lineage sharing indicated that introgressive hybridization

between eucalypt species might have occurred in the past. Because cpDNA is maternally inherited in eucalypts, first-generation hybrids would carry the cpDNA markers of the maternal species only. Over ensuing generations, pollination of hybrids and their progeny by the paternal species could produce individuals resembling the paternal species, but carrying cpDNA (and potentially some nuclear DNA) from the maternal species. Segregation of inherited characters could also result in later-generation hybrids reverting to one parental type or the other, but carrying foreign DNA markers. Introgressive hybridization is common in some plant genera, but can be difficult to distinguish from two other processes that may lead to intraspecific polymorphism and marker sharing between species: convergent evolution (species having multiple origins from different gene pools) and lineage sorting (retention of shared ancestral polymorphisms across species).

To clarify this question, McKinnon *et al.* (2001*a*) investigated the pattern of intraspecific cpDNA variation and interspecific cpDNA sharing within Tasmania in more detail. All 17 Tasmanian species of *Maidenaria* were sampled across their natural geographical ranges in Tasmania and the Bass Strait islands. An average of nine samples per species were analysed, and in all cases, sampled individuals were selected to be morphologically pure representatives of their species. Fourteen species showed intraspecific polymorphism within Tasmania, and in eight of these, a matching north-south disjunction in cpDNA was evident. Figure 2 shows recently expanded data from this study for selected species. The restricted endemics of southeastern Tasmania, *E. cordata* (80 samples) and *E. morrisbyi* (16 samples), appear fixed for S haplotypes, except for a single specimen of *E. morrisbyi*. More widespread Tasmanian endemics such as *E. urnigera* (not shown), *E. gunnii* (17 samples) and *E. rodwayi* (10 samples) exhibit a mixture of haplotypes. C haplotypes are found in all parts of the species' ranges, but these are mixed with S haplotypes in southeastern Tasmania. A similar pattern prevails in non-endemic species, such as *E. globulus* (approximately 250 samples, data from Freeman *et al.* 2001), *E. viminalis* (43 samples), *E. ovata* (33 samples) and *E. dabrympleana* (14 samples). An additional ('eastern Tasmanian' or ET) lineage, apparently most closely related to the C lineage (McKinnon *et al.* 2001*a*) is common in the east coast endemic *E. barberi* (30 samples) and is also found in *E. gunnii*, *E. globulus* and *E. viminalis* in the same region. Collated data across all samples of *Maidenaria* are very consistent for cpDNA distribution (figure 3; approximately 500 samples across 40 species; note that sample sizes vary widely between species).

Attempts to resolve the evolutionary relationships between the different cpDNA lineages have been only moderately successful. In the RFLP study of Jackson *et al.* (1999), the C, S and N lineages formed a polytomy. Nevertheless, some suppositions can be made. The extensive geographical distribution of the C lineage, which has now been identified in 23 different species, suggests that it is a major ancestral lineage within *Maidenaria*. By contrast, the S lineage is a minor lineage concentrated in southeastern Tasmania, where it is fixed in two endemic species. The time of divergence of the C and S lineages

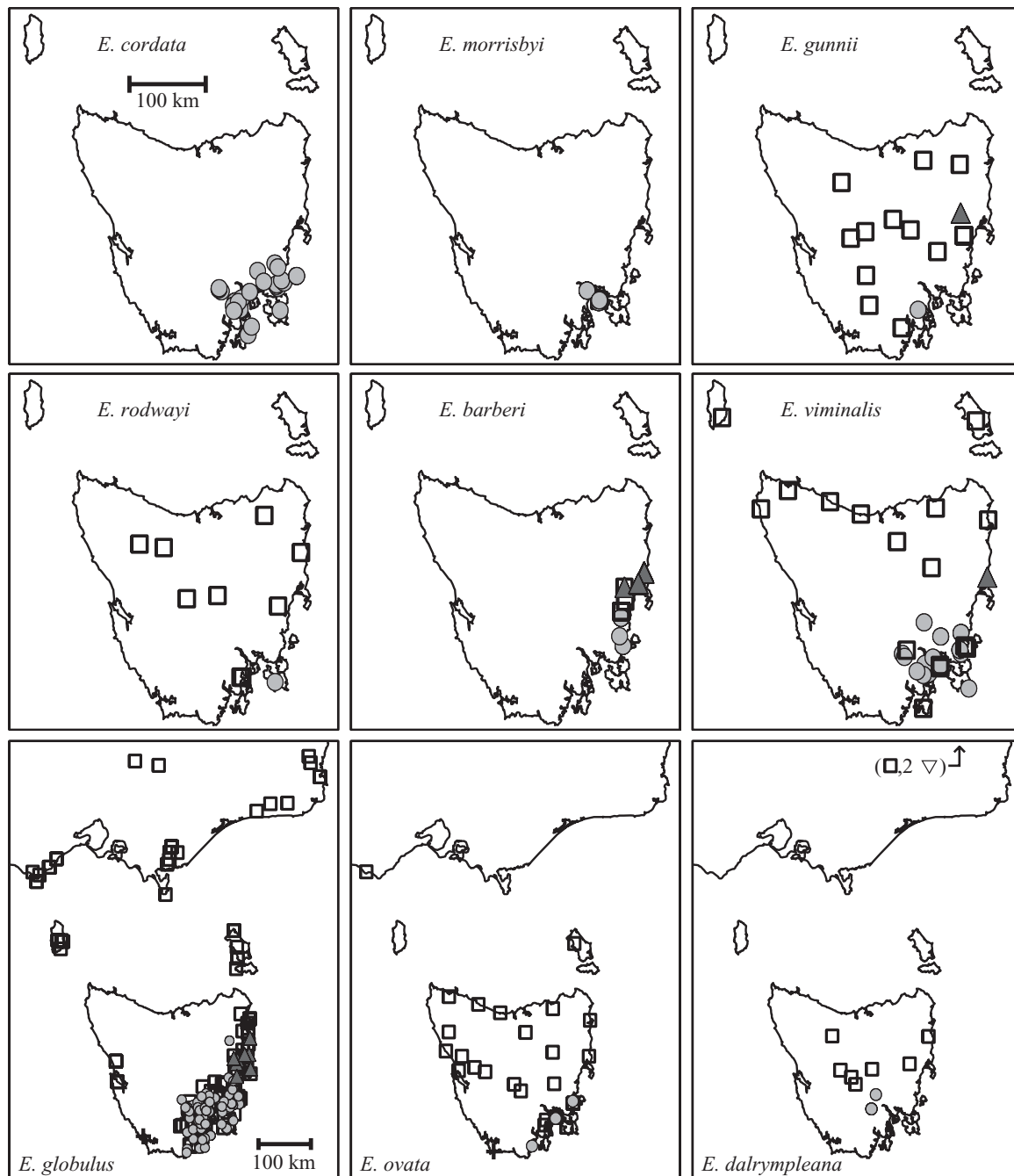


Figure 2. Distribution of cpDNA haplotypes in selected Tasmanian species of *Eucalyptus* subgenus *Symphyomyrtus* section *Maidenaria*. Open squares denote C haplotypes; shaded circles denote S; shaded triangles denote ET; crosses denote a minor lineage I; and open triangles denote N (see text, § 6). The three samples of *E. dalrympleana* shown in brackets are from New South Wales.

is difficult to determine, but RFLP and sequence data place it at *ca.* 3–0.8 Ma (McKinnon *et al.* 2001a). The divergence may, therefore, reflect a long-term isolation between gene pools correlating with the climatic events that caused significant extinctions during the Early Pleistocene.

Whereas C haplotype sharing may reflect common ancestry, another explanation must be sought for the localized sharing of minor restricted Tasmanian haplotypes (S, ET) among diverse species, most of which have C haplotypes elsewhere. Neither convergent evolution nor lineage sorting is a parsimonious explanation for the data. Under convergent evolution, many species would have

multiple origins. Under lineage sorting, many species with wide geographical ranges must have lost the same haplotypes independently in all areas except eastern Tasmania, after arising from the same polymorphic ancestor. This possibility is unlikely unless S haplotypes convey a selective advantage in eastern Tasmania. However, it is difficult to see what selective mechanism would act uniformly across all the species occupying different habitats within this region. For instance, habitat differences between the lowland species *E. globulus* and the subalpine species *E. gunnii* within eastern Tasmania greatly surpass habitat differences within *E. globulus* across its geographical range. The most likely explanation for localized S and ET

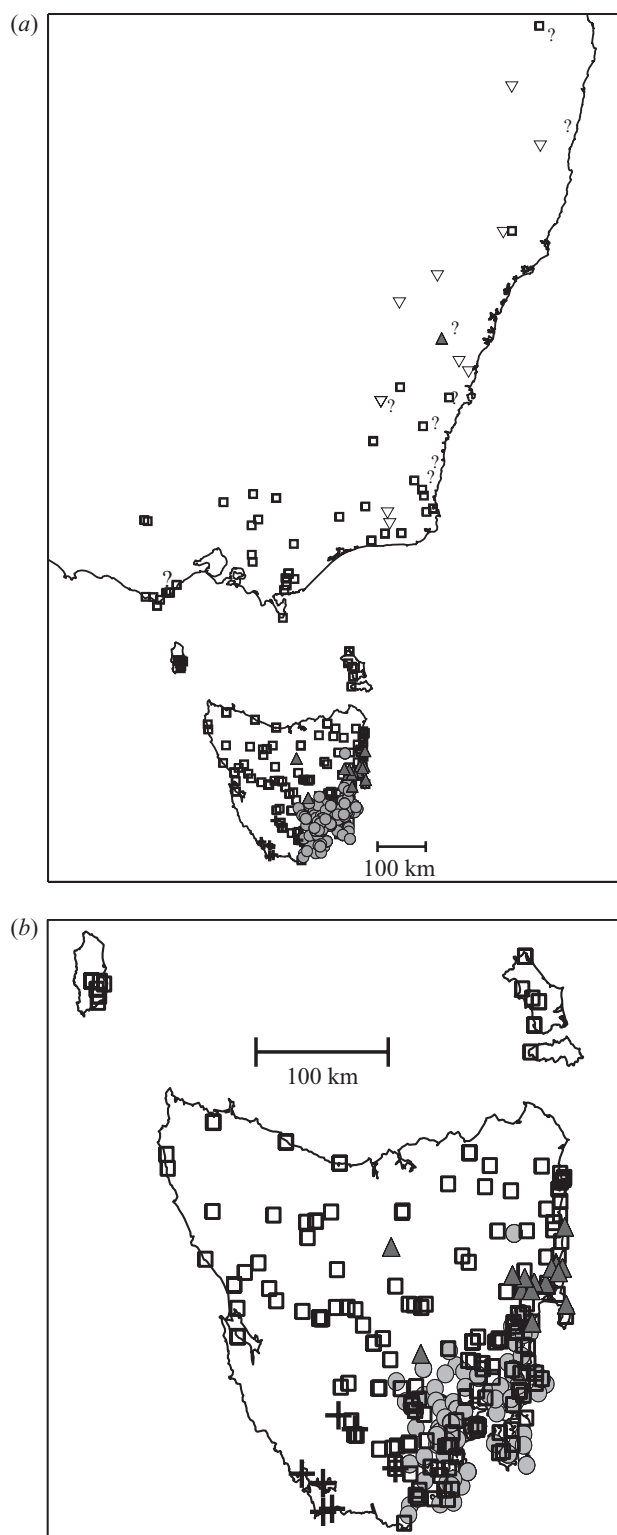


Figure 3. Collated cpDNA data across 40 species of *Eucalyptus* subgenus *Symphyomyrtus* section *Maidenaria*. Open squares denote C haplotypes; shaded circles denote S; shaded triangles denote ET; crosses denote I; open triangles denote N; and question marks denote unclassified haplotypes (see text, § 6). (a) Southeastern Australia; (b) Tasmania and the Bass Strait islands.

haplotype sharing is gene flow between species. This conclusion is in keeping with observations of the weak reproductive barriers between many Tasmanian species of *Maidenaria* (Williams & Potts 1996).

The demonstration of cpDNA introgression between sympatric forest tree species is not unusual. What is unusual is the remarkable number of species that appear to be involved in this example. Twelve eucalypt species share S haplotypes in southeastern Tasmania. Some of these species have not been recorded as forming natural hybrids directly with one another, for example *E. globulus* and *E. morrisbyi*. However, both of these species form natural hybrids with *E. ovata* and *E. viminalis*, which also hybridize naturally with each other. Haplotype sharing between *E. globulus* and *E. morrisbyi* could, therefore, be due to sequential hybridization events with *E. viminalis* or *E. ovata* as intermediary. Haplotype sharing between *E. ovata* and *E. viminalis* could arise from direct hybridization, or from both species hybridizing independently with *E. morrisbyi*. Sampling of one, or even a few species would clearly give an incomplete phylogeographical picture in *Eucalyptus*, and the same principle may apply to other genera in which reproductive barriers are weak.

6. RELATIONSHIP BETWEEN cpDNA VARIATION AND FORMER GLACIAL REFUGIA

The distribution of the S lineage is significantly correlated with the modelled location of a southeastern Tasmanian glacial refuge (McKinnon *et al.* 2001a), suggesting that S haplotypes survived the LGM in one or more species there, with only limited subsequent dispersal. The post-glacial expansion of species carrying S haplotypes may have been met by a wave of colonization from the north by species with C haplotypes. It is unclear how long C haplotypes have been present in eastern Tasmania, but their presence there could also pre-date the LGM, and some recolonization of central Tasmania by C haplotypes from the southeast cannot be ruled out.

The combined evidence supports the persistence in southeastern Tasmania of a cpDNA lineage (S) that diverged before the Middle Pleistocene. Endemics such as *E. cordata* and *E. morrisbyi*, which are fixed for S haplotypes, may have arisen from an isolated local gene pool carrying this lineage, whereas more widespread species such as *E. globulus* arose from northern gene pools predominantly carrying the C lineage. Contact between species from the different gene pools, enabled both by the exposure of land-bridge dispersal corridors and the opening to recolonization of former glacial terrain, has apparently resulted in subsequent widespread introgressive hybridization. Gene flow between species could have occurred at any time after contact, and extant patterns of cpDNA sharing may reflect both recent and ancient exchange between repeatedly separated and reconnected gene pools.

7. DETAILED PHYLOGEOGRAPHICAL ANALYSIS OF *EUCALYPTUS GLOBULUS* AND *EUCALYPTUS CORDATA*

Two single-species studies have investigated in more depth the pattern of cpDNA variability in Tasmanian eucalypts. Both studies used sequence data from a hypervariable region of the cp genome, enabling fine-scale discrimination of genetic variation. The study of Freeman *et al.* (2001) investigated cpDNA phylogeography in 270

samples of *E. globulus* from Victoria and Tasmania. In Victoria, four subspecies of *E. globulus* have traditionally been recognized (Kirkpatrick 1975). These were recently reinstated to specific status (Brooker 2000), but the former taxonomy is retained here for consistency with previous studies. In Tasmania, only subspecies *globulus* occurs. The large flower size of *E. globulus* appears to influence its crossing behaviour, such that it is unlikely to act as the female in crosses with other Tasmanian species (reviewed in Williams & Potts 1996). It is therefore more likely to acquire cpDNA from other species than to donate it.

The high morphological variability of Victorian *E. globulus*, and its apparently close taxonomic relationship to several other mainland species, suggest that the origin of *E. globulus* may have been in Victoria, with subsequent migration of subspecies *globulus* into Tasmania. An analysis of cpDNA, which included all subspecies in Victoria, gave results supporting this interpretation. Victoria showed a high diversity of C haplotypes, including haplotypes basal to and within this clade. Haplotype distribution patterns were consistent with seed migration of *E. globulus* ssp. *globulus* from Victoria into Tasmania along the western side of the Bassian Plain, down the west coast of Tasmania and around to the east coast. In eastern Tasmania, a patchy mosaic of haplotypes from the C, S and ET lineages was found. Individual haplotypes formed overlapping patches 50–100 km in diameter, with no sharp geographical boundary between haplotypes of different lineages. S haplotypes were present in high frequencies and showed considerable diversity, suggesting that the S lineage must have been present in Tasmanian *E. globulus* for some time. However, data from *E. globulus* alone could not distinguish between alternative hypotheses of: (i) an early acquisition of the S lineage, followed by dispersal and divergence; or (ii) more recent, repeated episodes of localized introgression.

A likely candidate for the role of cpDNA donor to *E. globulus* is *E. cordata*, a rare southeastern Tasmanian endemic that is fixed for the S lineage. This apparently relict species has about 35 small scattered populations, some of which are sympatric with *E. globulus* (Potts 1988), with which it has been observed to hybridize (Williams & Potts 1996). A survey of cpDNA variation in 80 samples of *E. cordata* and sympatric samples of *E. globulus* (G. McKinnon, unpublished data) shows numerous instances of localized haplotype sharing, consistent with frequent introgression of cpDNA from *E. cordata* into *E. globulus*. Although four shared haplotypes are confined to single sympatric populations, two others (S41 and S43) are found in *E. globulus* up to 27 km from the nearest extant *E. cordata* population, suggesting that some time has elapsed since introgression. Among *E. globulus* individuals with S haplotypes, 27% have haplotypes identical to those found in *E. cordata*, whereas a further 31% have haplotypes differing from S41 and S43 only at two rapidly evolving poly A/T regions. The data do not exclude involvement of other species, but strongly support a role for *E. cordata* as a haplotype donor to *E. globulus*. Progeny testing in *E. cordata* populations shows that this species may be under threat of genetic assimilation by co-occurring species of *Maidenaria* (Potts 1989).

8. EVIDENCE FROM THE NUCLEAR GENOME

In determining evolutionary history, it is desirable to use as many genetic markers as possible. The cpDNA data indicate that reticulate evolution has been an important feature in the history of Tasmanian *Eucalyptus*. Some independent evidence from the nuclear genome would help to support the conclusion that introgressive hybridization has significantly affected genetic variability (and potentially adaptability) in eucalypt species.

Several studies on other plant species have used sequence data from the ITS region of the nuclear ribosomal DNA to demonstrate reticulate evolution. For example, Aguilar *et al.* (1999) showed that ITS sequences in *Armeria* were more congruent with geographical distribution than with taxonomic classification. In *Maidenaria*, ITS sequences exhibit little variation (Steane *et al.* 1999) and other markers are needed. A study complementary to our cpDNA studies has now been completed on sequence variation in introns of the single copy nuclear gene for CCR in 208 samples of *E. globulus* and 50 samples of *E. cordata* (G. McKinnon, unpublished data). As expected, phylogeographic structure in this gene (which is dispersed by seed and pollen flow) is much more diffuse than in cpDNA (which is dispersed only by seed). For example, the distribution of CCR alleles strongly suggests pollen flow between *E. globulus* of Flinders Island and northeastern Tasmania, in agreement with microsatellite evidence (D. Steane, unpublished data), whereas cpDNA distribution indicates a barrier to seed dispersal between these regions. In addition, lineage sorting complicates patterns of CCR allele sharing between species and is difficult to distinguish from ancient introgression. Two findings are more consistent with introgression. First, *E. globulus* of southern Tasmania shows a greater allelic diversity for CCR than in Victoria, with higher levels of an allele closely related to the dominant allele in *E. cordata*. Secondly, within southern Tasmania, levels of this allele are highest in individuals carrying S haplotypes. The finding that both random amplified polymorphic DNA (Nesbitt *et al.* 1995) and microsatellites (Jones *et al.* 2002) are slightly more variable in Tasmanian than Victorian populations of *E. globulus* is also consistent with introgression in Tasmania.

9. CONCLUSIONS

Fossil pollen evidence shows that major redistributions of eucalypt forest occurred in southeastern Australia during the Quaternary, probably in response to both temperature and aridity changes. Patterns of morphological variation within *Eucalyptus*, in particular intergradation between species, suggest that these redistributions might have enabled divergent species to come into contact and exchange genes through hybridization (reticulate evolution). Our findings on molecular variation in Tasmanian *Eucalyptus* strongly support this hypothesis. Collocated cpDNA data from all Tasmanian species of *Eucalyptus* subgenus *Symphyomyrtus* section *Maidenaria* suggest that extensive introgressive hybridization has occurred, after the mixing of gene pools that probably diverged before the Middle Pleistocene. Detailed analysis supports migration of the widespread species, *E. globulus*, into Tasmania, followed by multiple occurrences of

introgressive hybridization with the rare Tasmanian endemic, *E. cordata*.

The main pre-mating barriers to hybridization within subgenera in natural eucalypt populations are geographical isolation and differences in flowering time (Williams & Potts 1996). Disruptions to these barriers during the Quaternary may have included major migrations between mainland Australia and Tasmania, enabling contact between formerly allopatric species, and more local (e.g. altitudinal) migrations affecting local species. Different present-day altitudinal preferences in the Tasmanian eucalypts range from near sea-level (e.g. *E. globulus*) to over 1000 m (e.g. *E. vernicosa*; Williams & Potts 1996), and complex altitudinal redistributions must have occurred in response to temperature depression. Disruptions of flowering time, coincident with climate change, are also likely and may have enabled intermittent gene flow between species that do not currently overlap in flowering time. The effect of Quaternary climate change on the ability of interspecific hybrids to persist and successfully backcross with parental species is a more complex question. Post-mating barriers to successful hybridization in eucalypts include poor F₁ hybrid vigour in some crosses (Lopez *et al.* 2000) and advanced-generation hybrid breakdown (Paton 1981). However, hybridization in many plant genera is promoted by habitat disturbance, which creates opportunities favouring new genetic combinations. In addition to breaking geographical isolation between species, Quaternary climate change may have favoured hybrid survival through the availability of new habitats. Hybrids may also have competed well against inbred progeny from pure species suffering reduced population sizes.

Our results for Australian *Eucalyptus* are concordant with studies of other genera from the Northern Hemisphere. The glaciations of the Quaternary resulted in cyclic expansion and contraction of many species' ranges in response to changes in aridity and temperature, leading to hybridization between divergent populations, subspecies and species brought into sympatry (reviewed in Hewitt 1999, 2000). Different spatial and temporal patterns of hybridization during the Quaternary are becoming apparent. In some cases, hybridization has occurred since the LGM in zones where subspecies or species have met during post-glacial expansion from different refugia. In others, earlier hybridization during glacial periods has been inferred. For instance, cpDNA diversity in northern populations of *Packera pseudaura* (Asteraceae) in Alberta suggests former hybridization with other *Packera* species that migrated southwards during periods of glaciation (Yates *et al.* 1999; Golden & Bain 2000). In southeast Spain, species of *Armeria* (Plumbaginaceae), which now occur at different altitudes, may have hybridized during Quaternary compressions of vegetation within massifs (Larena *et al.* 2002).

One of the best-studied examples of hybridization in a redistributed species complex is that of the oaks. A systematic sharing of local cpDNA markers has been demonstrated for seven species of *Quercus* throughout Europe (Dumolin-Lapègue *et al.* 1997). Initially, Ferris *et al.* (1993) suggested that sharing of the same cpDNA haplotype in *Quercus robur* and *Q. petraea* had probably occurred after hybridization at a time of low population size when

the two species were confined to a glacial refuge. Subsequent post-glacial expansion then led to the two species sharing haplotypes in recolonized terrain. A more recent fine-scale analysis by Petit *et al.* (1997) showed that introgressive hybridization could have played an important role in the recolonization process itself. *Quercus robur* may have acted as the pioneer species recolonizing new territories through seed dispersal, whereas *Q. petraea* followed by pollinating established populations of *Q. robur*. In the case of *E. globulus* and *E. cordata*, asymmetric crossing may be causing gradual replacement of *E. cordata* within the bounds of a former glacial refuge.

These findings emphasize the importance of studying intraspecific variation in context. Phylogeographical studies often focus on single species, an approach that may be justified when the possibility of introgression is low, but which may lead to erroneous conclusions when genetic markers have been transferred locally between species with different histories. The example of *Eucalyptus* demonstrates that phylogeographical patterns may reflect a very complex combination of processes. The adaptive significance of introgressive hybridization in *Eucalyptus* is still unknown, but the process is thought to play a major evolutionary role in plants (Arnold 1997; Rieseberg 1998). Its proposed consequences include increased intraspecific genetic diversity, the transfer or origin of adaptations and the release of novel gene combinations to be sifted by natural selection. Further work is needed to understand the importance of introgression for adaptation and innovation in *Eucalyptus* and other genera.

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Discussion

G. E. Hewitt (*School of Biological Sciences, University of East Anglia, Norwich, UK*). Could I be a bit 'heretical'? Could the haplotype that you say colonized the west of Tasmania from Victoria have gone the other way—from a refuge on Tasmania to Victoria?

G. E. McKinnon. Overall the biogeographical and chloroplast evidence are consistent with migration of *E. globulus* from Victoria into Tasmania, but there could certainly have been some backflow of haplotypes from western Tasmania to Victoria.

M. Lascoux (*Department of Evolutionary Biology, Uppsala University, Uppsala, Sweden*). We also observe important introgression in the CYTOFOR project, so I agree that species need to be considered together rather than in isolation when we believe that they can hybridize. Did you have any information on gene flow among species? Is there any asymmetry?

G. E. McKinnon. There is asymmetry in the crossing behaviour of some eucalypt species, apparently because the pollen tubes of small-flowered species cannot grow down the full length of the style of large-flowered species. *Eucalyptus globulus* has a large flower and can pollinate other Tasmanian species, but the reciprocal crosses are mostly unsuccessful. The genetic data are consistent with this, showing unidirectional introgression of cpDNA from *E. cordata* into *E. globulus*.

M. Lascoux. Eucalypts have been introduced in many countries, in South America, Africa, etc. Do you know if their crossing behaviour has been studied in these new environments?

G. E. McKinnon. There are many examples of hybridization in multi-species plantings of eucalypts outside Australia. Some species have formed successful stable hybrids which grow well in exotic environments—for instance the widely planted Mysore gum in India is thought to be a stabilized hybrid between *E. tereticornis* and *E. camaldulensis*. *Eucalyptus* × *algeriensis* is widespread in Algeria and is considered an example of acclimation by means of hybridization.

GLOSSARY

- CCR: cinnamoyl coA reductase
 cp: chloroplast
 ITS: internal transcribed spacer
 LGM: last glacial maximum
 RFLP: restriction fragment length polymorphism