

MANAGING AUSTRALIA'S EUCALYPT GENE POOLS: ASSESSING THE RISK OF EXOTIC GENE FLOW

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ABSTRACT: Most eucalypts are endemic to Australia but they have been introduced into more than 100 countries and there are now over 20 million hectares of eucalypt plantations globally. These plantations are grown mainly for pulpwood but there is expanding interest in their use as a renewable source of solid wood products and energy. In Australia, the eucalypt plantation estate is nearing one million hectares, located mainly in temperate regions and dominated by *Eucalyptus globulus* and *E. nitens* (subgenus *Symphyomyrtus*), which are grown mainly outside their natural ranges. While eucalypt species from different major subgenera do not hybridise, hybrids within subgenera are often reported, including hybrids with plantation species. Concerns were raised in the late 1990s that pollen-mediated gene flow from locally exotic plantation eucalypts may affect the integrity of adjacent native eucalypt gene pools. As Australia is the centre-of-origin of most eucalypt species used in plantations around the world, exotic gene flow is one of the many issues that require management for industry sustainability and certification purposes. We here summarise over a decade of research aimed at providing the framework and biological data to help assess and manage the risk of gene flow from these plantations into native gene pools in Australia.

Keywords: *Eucalyptus globulus*, *Eucalyptus nitens*, exotic gene flow, genetic risk, genetic pollution, hybridisation, sustainable forest management, gene pool conservation

There are over 800 species of eucalypts and, while most are endemic to Australia (Ladiges 1997; Slee et al. 2006), many species were rapidly translocated around the world during the nineteenth century (Doughty 2000; Potts et al. 2004). There are now over 20 million hectares of eucalypt plantations globally (Iglesias-Trabado et al. 2009). These plantations are dominated by just a few species and their hybrids from the subgenus *Symphyomyrtus* (Harwood 2011). They are grown mainly for pulpwood, but there is expanding interest in their use as a renewable source of solid wood products, biochemicals and energy (Nolan et al. 2005; Rockwood et al. 2008; Shepherd et al. 2011). In Australia, the last 25 years have seen a rapid expansion of eucalypt plantations, with the estate now nearing one million ha (Gavran 2014). These plantations are mainly located in temperate regions, are dominated by *Eucalyptus globulus* and *E. nitens* (both in the subgenus *Symphyomyrtus*, section *Maidenaria*), and are almost all grown outside their natural ranges. While hybridisation does not occur between the major eucalypt subgenera, hybrids between species within subgenera are often reported, including hybrids with plantation species (Griffin et al. 1988). Concerns were raised in the late 1990s that

pollen-mediated gene flow from locally exotic plantation eucalypts may threaten the integrity of adjacent native eucalypt gene pools (Potts et al. 2001; Potts et al. 2003). As Australia is the centre-of-origin of most eucalypt species, the management of exotic gene flow is one of the many issues required for industry sustainability and certification purposes. We thus conducted research to underpin risk assessment and management strategies to minimise the offsite genetic impacts of these plantations. Most work has focused on pollen-mediated gene flow from the *E. globulus* and *E. nitens* pulpwood plantations (Table 1). However, parallel work has been undertaken for *Corymbia* species and hybrids being used in plantations in subtropical areas of NSW and Queensland (Barbour et al. 2008a; Dickinson et al. 2012; Abasolo et al. 2014; Shepherd & Lee 2016), as well as other eucalypts being used in farm forestry and revegetation (Potts et al. 2001; Byrne & Stone 2011; Byrne et al. 2011; Stone et al. 2011).

HYBRIDISATION AS A RISK

Globally, risks associated with hybridisation have received most attention in relation to exotic gene flow from genetically modified (GM) sources. However,

gene flow from exotic non-GM sources can have similar consequences, particularly in compromising the genetic integrity of natural populations (Potts et al. 2003; Laikre et al. 2010; Byrne et al. 2011). This is the case in industries that involve the release or translocation of large numbers of individuals with different, restricted or manipulated genetic diversity into areas where they can interact with related natural populations. Such industries include agriculture, fisheries, wildlife management, forestry and revegetation. The number of individuals involved in these large scale biological releases is often staggering. In a review of the issue, Laikre et al. (2010) reported that over 10 billion hatchery-bred fish are released into northern hemisphere rivers and oceans annually, and in Sweden more than 30 billion Norway spruce trees were planted in the twentieth century alone. Exotic gene flow may arise through the release or translocation of locally exotic or genetically differentiated members of the same species (Schröder & Prasse 2013; Unger et al. 2014), cross-compatible species or hybrids (Potts et al. 2003; Talbot et al. 2012; Meirmans et al. 2014).

Laikre et al. (2010) identified four main ways in which the genetic integrity of natural populations can be affected by exotic gene flow from large scale biological releases or translocations, which can: 1) change population structure (reduce differentiation between populations); 2) change genetic composition (genetic replacement); 3) cause the breakdown of local genetic adaptation; and 4) reduce genetic diversity. Fisheries are the main industries with release histories and research programs of sufficient length and magnitude to detect these effects, and they have all been found in natural populations (see Box 2 in Laikre et al. 2010). Although fisheries is the best studied system, exotic gene flow has now been detected in other industries involving large-scale biological releases or translocations (Laikre et al. 2010). For example, in forestry, and the case of *Eucalyptus* in Australia, where exotic gene flow from plantations has been studied mainly for its effect on other native eucalypt species, because temperate plantation species are principally grown outside their natural range. Furthermore, the recent expansion of the plantation industry relative to the generation time of



Figure 1: The landscape context of hybridisation has been assessed in Victoria, where small remnant patches of native *E. ovata* forest are often surrounded by *E. globulus* plantations. Top left: collecting open pollinated seed from remnant *E. ovata* trees surrounded by mature *E. globulus* plantation. Top right: the seed was sown into boxes (at the HVP nursery near Traralgon) and visually screened for exotic hybrids. Bottom left: the distinctive morphology of the hybrids (red arrows) makes visual screening highly effective. Bottom right: paired hybrid (red arrow) and pure (green arrow) siblings from the same seedlot which have now been planted in a field trial to assess relative hybrid fitness. Over 24,000 seedlings were screened in this study (see top right) and a hybridisation rate of 1.6 % found (Larcombe et al. 2014). Hybridisation was highest on the edges of patches and declined very rapidly with distance.

the species means that detection of exotic hybrids in the wild has usually been restricted to early life cycle, first generation hybrids (F_1 s between exotic plantation species and a native eucalypt — Figure 1) (Barbour et al. 2002; Barbour et al. 2003; Barbour et al. 2008b; Larcombe et al. 2014), although progression to the second generation of hybridisation (F_2 and backcrossing) has been reported in *Corymbia* (Shepherd & Lee 2016).

Here we summarise more than a decade of research aimed at providing the framework and biological data to help assess and manage the risk of exotic gene flow from eucalypt plantations in Australia.

THE HISTORY OF EXOTIC GENE FLOW RESEARCH IN AUSTRALIA

The case of Eucalyptus nitens in Tasmania

Our research was initially focused on the island of Tasmania, where *E. nitens* was introduced in the late 1980s and, by 2013, had expanded to 208,000 ha of plantations (Gavran 2014), which represents about 3% of the land surface of the main island. *Eucalyptus nitens* is native to highland regions of Victoria and NSW, but most genetic material in Tasmanian breeding programs and plantations is from the Central Victorian race, particularly the Toorongro provenance (Hamilton et al. 2008; Hamilton et al. 2011). The species is grown widely in Tasmania and generally replaces *E. globulus* as the species of choice in plantations above 350 m of altitude or on sites prone to *Mycosphaerella* leaf disease (caused by *Teratosphaeria* species — Potts et al. 2011; Wardlaw 2012). While most of the plantation estate is grown for pulpwood, approximately 10% has been pruned, thinned and grown in longer rotations to target solid wood markets (Potts et al. 2011).

The potential for hybridisation between *E. nitens* and Tasmanian eucalypt species was first identified when abnormal plants (later confirmed to be hybrids) were detected in *E. nitens* plantations (Dr W. Tibbits, North Forest Products, pers. comm.). These hybrid seedlings originated from open pollinated seed from an *E. nitens* seed orchard that was surrounded by native forest containing *E. ovata* and *E. viminalis*. Subsequent research revealed that exotic *E. nitens* hybrids were also occurring in open pollinated seed collected from the adjacent *E. ovata* trees (hybridisation rate ranged from 0.04 to 16% per tree — Barbour et al. 2002), demonstrating a potential avenue for exotic gene flow. Furthermore, *E. ovata* × *nitens* hybrid seedlings were found soon after, growing in disturbed areas near plantations (Barbour et al. 2003). At that stage, most of the *E. nitens* plantation estate in Tasmania was immature and yet to flower. With 17 of the island's eucalypt species belonging to the same subgenus as *E.*

nitens (subgenus *Symphyomyrtus*; and the same section, *Maidenaria*) (Figure 2), and thus potentially at risk of hybridisation and introgression, research was initiated to identify and better understand barriers to hybridisation (Table 1). This research was aimed at developing a risk assessment framework for use by Forest Practices Officers (Roberts et al. 2009). The framework focused on identifying the probability of first-generation hybridisation occurring, based on flowering season overlap (Barbour et al. 2006a), crossability (Barbour et al. 2005a) and distance (Barbour et al. 2005b). The potential consequences of this hybridisation was assessed in terms of proportion of the geographic range of the native species that was likely to be impacted and the conservation value of the species, forest type (e.g. threatened forest community) or landscape (e.g. National Park).

To study crossability, our approach has been to quantify and rank native species based on the proportion of hybrid seedlings produced after applying *E. nitens* pollen to the receptive stigma of open flowers on native trees in the wild with no isolation (Barbour et al. 2005a; Larcombe et al. 2016b). This 'dabbing' technique (supplementary pollination — Delaporte et al. 2001, Patterson et al. 2004) allows the exotic pollen to compete with self, intraspecific, and interspecific pollen originating from surrounding compatible species, and was used specifically to mimic natural pollination. *Eucalyptus nitens* seedlings are morphologically distinct from all of the Tasmanian *Symphyomyrtus* species. The least differentiated native species is *E. globulus* but, even then, the fused apical buds of *E. nitens* can be used as a morphological marker (Tibbits 1988). The F_1 hybrid seedlings are, therefore, generally detectable through visual inspection of open pollinated progeny grown from seed collected from the native trees, whether this be following supplementary (Barbour et al. 2005a) or natural (Barbour et al. 2002) crossing. This methodology is very efficient and has been validated using: (i) controlled crossing (where flowers were emasculated and isolated — Barbour et al. 2005a); (ii) molecular markers (Barbour et al. 2002; Barbour et al. 2010); and (iii) near-infrared reflectance spectroscopy (Humphreys et al. 2008; O'Reilly-Wapstra et al. 2013). The morphological differences between exotic F_1 hybrids and the native species at the seedling stage have been documented to facilitate operational risk assessments (Barbour et al. 2005a; Roberts et al. 2009).

Hybrids have been produced using *E. nitens* as the pollen parent for all native Tasmanian *Symphyomyrtus* species, with the exception of *E. urnigera* and *E. globulus* (Larcombe et al. 2016b). *Eucalyptus nitens* has smaller flowers and shorter styles than any of the Tasmanian *Symphyomyrtus* species, and the pollen tubes of the small-

Table 1: The extent of pre- and post-mating barriers to hybridisation between plantation (*E. nitens* and *E. globulus*) and native eucalypts.

Barrier	<i>E. nitens</i>		<i>E. globulus</i>	
	Comment	Reference	Comment	Reference
Pollen quantity (flower production in plantations)	Flower abundance higher at lower altitude sites in Tasmania, and higher when tree densities are lower.	Moncur et al. 1994; Williams et al. 2006	Starts three years after plantation establishment but genetically variable; flowering mostly occurs on plantation edges.	Moncur et al. 1994; Chambers et al. 1997; Barbour et al. 2008b
Flowering season	Varies plastically with altitude; proportion of native species flowering which overlapped with <i>E. nitens</i> from the same altitude zone ranged from 0 to 0.58.	Moncur et al. 1994; Barbour et al. 2006b	Shown to be controlled by both genetic and environmental factors; genetic-based differences within and between races; Furneaux and Tasmanian races flower earlier than mainland races; year-to-year variation related to heat sum; usually inherited in an intermediate manner in F ₁ s but sometimes not (i.e. <i>E. ovata</i> x <i>globulus</i>).	Jones et al. 2011; López et al. 2000a
Pollen vectors	Insects only, possible transfer to all synchronously flowering co-occurring species	Hingston et al. 2004b	Birds and insects; birds more efficient pollinators of the large flowers; likely limited pollen transfer to small flowered species offering insufficient nectar rewards.	Hingston and Potts 1998; Hingston et al. 2004d; Hingston et al. 2004c; Hingston et al. 2004a
Pollen dispersal	<i>E. ovata</i> landscape, Tasmania: decays exponentially with distance, most hybrids found in open pollinated seed within 200 m of a plantation boundary, maximum observed dispersal distance was 1.6 km, may be greater in fragmented landscapes.	Barbour et al. 2005b	<i>E. ovata</i> landscape, Victoria: overall rate of exotic hybridisation was 1.62%, with small patches having higher rates than larger patches, as did trees on the edges of patches and paddock trees in open pasture 50–200 m from the plantation edge; intraspecific study shows dispersal was landscape dependent.	Larcombe et al. 2014; Mimura et al. 2009
Crossability	Incompatible with subgenus <i>Eucalyptus</i> , barriers identified, crossability decreases with increasing style length of the female, no relationship with genetic distance for the Tasmanian <i>Symphyomyrtus</i> species.	Gore et al. 1990; Barbour et al. 2005a; Larcombe et al. 2016b; Larcombe et al. 2016a	Incompatible with subgenus <i>Eucalyptus</i> , wide-ranging intra- and inter-sectional crossing shows no relationship with style length but crossability declines significantly with increasing genetic distance. Possible reduced cross success with small flowered species due to pollen tube ‘over shoot’.	Gore et al. 1990; Larcombe et al. 2015b; Larcombe et al. 2016a
Hybrid fitness (vegetative)	In the wild	Barbour et al. 2006a	78% reduction in F ₁ hybrid fitness compared to native <i>E. ovata</i> .	Larcombe et al. 2014
	Field trials	Costa e Silva et al. 2012; Larcombe et al. 2016a; Larcombe et al. 2016b;	Reduced fitness of <i>E. ovata</i> x <i>globulus</i> and <i>E. nitens</i> x <i>globulus</i> F ₁ hybrids compared to both parental species.	López et al. 2000a; Volker et al. 2008; Costa e Silva et al. 2012; Larcombe et al. 2016a
Hybrid fitness (reproductive)	Viable pollen from <i>E. nitens</i> x <i>cordata</i> F ₁ s successfully crossed with three species; reduced reproduction of surviving <i>E. nitens</i> x <i>globulus</i> hybrids	Barbour et al. 2007; Larcombe et al. 2016a	No evidence of decreased <i>E. nitens</i> x <i>globulus</i> hybrid pollen inviability.	Larcombe et al. 2016a

flowered *E. nitens* do not appear to be able to grow to the length required to fertilise the ovules of the larger-flowered species. Indeed, style length alone accounts for 46% of the variation in the rate of exotic hybridisation in our recent crossability studies, with crossability dropping markedly once the style length exceeds twice that of *E. nitens* (Larcombe et al. 2016b). This post-mating barrier has long been known in the case of the largest-flowered species, *E. globulus*, which is completely incompatible as a female in the cross with *E. nitens*, although the reciprocal cross is possible (Gore et al. 1990). As a measure of the probability of accumulated genetic incompatibilities, the genetic distance (calculated using molecular markers) between native species and *E. nitens* did not explain the variation in crossability, at least at the point of assessment which was shortly after germination and under glasshouse conditions (Larcombe et al. 2016b). This may reflect the fact that all the *Symphyomyrtus* species on the island belong to the same taxonomic section (*Maidenaria*) and, thus, there is not a great range in genetic divergence (Figure 2). In the case of *E. urnigera*, no hybrids were recovered following dabbing with *E. nitens* pollen. This absence of hybrids may be due, in part, to the relatively long style of *E. urnigera*. However, given the subalpine winter-flowering habit of *E. urnigera*, it is also possible that pollen from the summer-flowering *E. nitens* was maladapted to the temperature at which the controlled pollinations of *E. urnigera* took place (around 3° C — Larcombe et al. 2016b). After consideration of crossability, flowering time and the proportion of the species ranges which are proximal to *E. nitens* plantations, four native species stand out as being at high risk of exotic hybridisation. These are the widespread *E. ovata*, the two related endemics *E. gunnii* and *E. archeri*, and the rare *E. perriniana* (Figure 2).

The frequency of exotic F₁ hybrids in open pollinated seed collected from *E. ovata* along transects running away from the plantation edge out to 1600 m, showed that the vast majority of hybrids occur within 200 m of the plantations, although rare pollination events may extend to at least 1600 m (Barbour et al. 2005b). Risk assessment is now focused on the eucalypt species within 500 m of a plantation (Roberts et al. 2009). A similar plantation buffer distance is now being tested in what was predicted to be one of the highest risk situations in Tasmania. This case study will be used to inform an adaptive management strategy associated with the development of a *E. nitens* plantation estate of nearly 600 ha in the landscape surrounding a small reserve of one of the three populations of *E. perriniana* in Tasmania (Barbour et al. 2010; Larcombe et al. 2012). At the beginning, a small plantation of 60 ha of *E. nitens* was situated 630 m away from the *E. perriniana* population, but subsequent plantings were done up to a

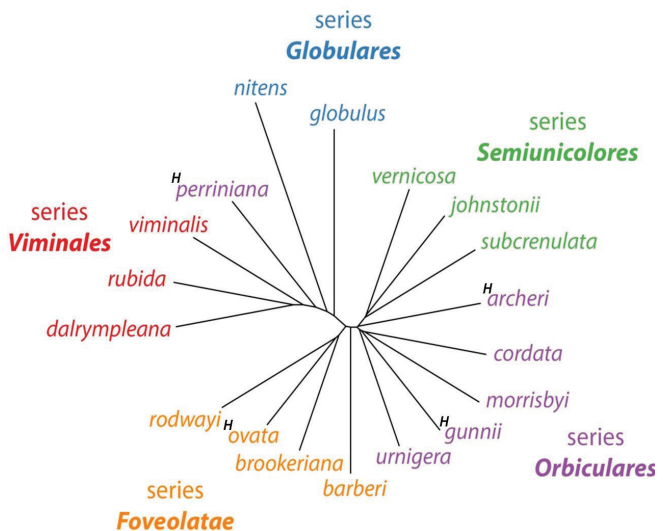


Figure 2: A tree summarising the genetic distances among the 17 Tasmanian members of *Eucalyptus* subgenus *Symphyomyrtus* plus the exotic plantation species *E. nitens*. The unrooted radial tree is derived from 5050 DArT markers and 51 samples (3–4 per species) analysed with Additive Dollo Distance (Woodhams et al. 2013). The pairwise distances among samples are averaged for each pair of taxa. Taxa are coloured by their series-level classification (based on Brooker 2000). The historic misclassification of *E. perriniana* in series *Orbiculares* has been noted in previous molecular studies (McKinnon et al. 2008, Steane et al. 2011). 'H' denotes species that were assessed as being at high risk of exotic gene flow from *E. nitens* in Tasmania (Roberts et al. 2009). The figure is modified from Larcombe et al. (2016b).

500 m buffer distance around the *E. perriniana* population. We monitored the annual rate of exotic hybridisation in this population for the flowering seasons between 2005 and 2012 by growing open pollinated seed collected from up to 100 'sentinel' trees spread throughout the small *E. perriniana* population. The rates of exotic hybridisation have averaged 0.17% (range 0.02% to 0.36% among trees), whereas the average rate of hybridisation with adjacent native eucalypts was estimated to be 21 times greater (Larcombe et al. 2012, and unpublished data). While monitoring is ongoing as more of the new plantation estate reaches flowering and the older plantation is harvested, to date the low level of exotic hybridisation is consistent with that expected in the tail of the pollen dispersal curve (Barbour et al. 2005b). *Eucalyptus perriniana* occurs in an unusual habitat that can be waterlogged for about half of the year, and very dry for the other half. These conditions make seedling recruitment difficult, and seedlings are rare. Until consecutive fires burnt parts of the population in 2007 and 2008, no seedlings (either hybrid or pure *E. perriniana*) had been recorded in the population. However, despite hundreds of trees being burnt, only 26 seedlings were recorded in surveys of the burnt area in 2009; most of

these were establishing around a single tree, and none were hybrids with *E. nitens* (Larcombe et al. 2012). The low levels of exotic hybridisation and seedling establishment indicate that the prescribed buffer distance of 500 m appears appropriate at this stage and exotic hybridisation into this population of *E. perriniana* is manageable by monitoring seedling recruitment after fire events in the reserve.

The case of Eucalyptus globulus in southern Australia

Given the diversity of *Eucalyptus* species on mainland Australia, understanding where barriers to reproduction exist (from a taxonomic/phylogenetic perspective) is of central importance for identifying which species are at risk of exotic gene flow from *E. globulus* plantations. Until recently, it could have been suggested that any of the 484 *Symphyomyrtus* species that were within the pollen dispersal zone of *E. globulus* plantations were potentially at risk of exotic gene flow. However, by combining crossability data from supplementary pollination of 99 species with *E. globulus* pollen (Figure 3) and genome-wide molecular markers, it is clear that phylogenetically related barriers to hybridisation exist within *Symphyomyrtus* (Figure 4 — Larcombe et al. 2015). Specifically, there appears to be a virtually complete barrier to hybridisation between *E. globulus* and *Symphyomyrtus* species in clades III and IV (Figure 4). This finding alone reduces the number of species potentially at risk of exotic gene flow by over 70%

(to 138 of the 484 species). Reproductive barriers between species within the subgenus *Symphyomyrtus* also extend to the more closely related clades I and II (Figure 4), with the probability of successful hybridisation between *E. globulus* and species in clade II being less than 5%. The clade at greatest risk (clade I) includes only species from section *Maidenaria* (68 species) — the section which includes both *E. globulus* and *E. nitens*. The overall trend for a reduction in compatibility with increasing genetic distance means that there is likely to be variation in compatibility within *Maidenaria*, but conceptually all *Maidenaria* species are likely to be at least partially cross-compatible. However, these results are based only on the estimate of seedling survival at nine months (minimum), which is likely to underestimate the strength of the post-dispersal barriers to hybridisation in many cases (see below).

By considering spatial proximity, it is possible to further reduce the number of species that are likely to be at risk of exotic gene flow from *E. globulus* plantations. For example, in a survey of 302 plantations across the *E. globulus* estate from Tasmania to south-west Western Australia, Barbour et al. (2008b) found only 21 *Symphyomyrtus* species growing adjacent to those plantations. In a related study, a desktop analysis of point records for rare and threatened eucalypts found that of the 74 nationally listed rare eucalypt taxa only eight occurred within 1 km of a eucalypt plantation (Barbour et al. 2010), and only four of



Figure 3: Cross compatibility between *E. globulus* and species from across subgenus *Symphyomyrtus* has been estimated using a supplementary crossing approach. This involves applying *E. globulus* pollen to open flowers without isolation so that the exotic pollen can compete with intraspecific pollen. Over 7000 crosses were made on 100 taxa, mainly from subgenus *Symphyomyrtus*. The left photograph shows flowers in a receptive state ready for pollination. The right shows *E. globulus* pollen being applied to a species from section Adnataria at Dean Nicolle's Currency Creek Aboretum.

those were *Symphyomyrtus* species within the *E. globulus* growing region. In Table 2 we have combined spatial information from the above-mentioned studies with clade-level crossability from Larcombe et al. (2015) (Figure 4) to present a risk assessment for the 24 *Symphyomyrtus* species identified to occur near *E. globulus* plantations (combining field and desktop data). Of these species, 12 were found to have minimal risk of gene flow, while four had a high risk (Table 2). It is clear that the species in section *Maidenaria* are most at risk of exotic gene flow from *E. globulus* (Figure 4, and Table 2). However, even for high-risk taxa in section *Maidenaria* there are barriers to hybrid establishment and survival (López et al. 2000b; Larcombe et al. 2014) that are likely to act as barriers to gene flow even when hybridisation is possible (see below).

In the case of the rare species, factors other than exotic gene flow may often be of more immediate concern for the species' conservation. For example, while the largest of the two main populations of the rare Tasmanian endemic *E. morrisbyi* is within one km of a windbreak row of a *E. globulus* plantation, this reserved population has been in rapid demographic decline since around 2009, with fewer than 10% of the adult trees now surviving, and only 25% of these being reproductively active (Wiltshire et al. unpublished data). Thus, the prospect of exotic gene flow

is a minor concern in comparison to the ecological factors causing population collapse in *E. morrisbyi*.

While our initial focus was on pollen-mediated gene flow from *E. globulus* plantations, we have also assessed seed-mediated gene flow (i.e. wildling establishment — Figure 5). Wildling spread from *Eucalyptus* plantations is receiving growing attention internationally (Ritter & Yost 2009; Silva & Marchante 2012; Calviño-Cancela & Rubido-Bará 2013; Catry et al. 2015; dos Santos et al. 2015). In Australia, the spread of *E. globulus* wildlings can be viewed as a biological invasion problem *per se*, but it could also lead to exotic gene flow and introgression if the established wildlings reach reproductive maturity. Larcombe et al. (2013) showed that wildling spread across the Australian estate is relatively low with the vast majority of wildlings occurring within the plantation disturbance zone (Figure 5). Wildlings were more common around older plantations, and associated with high reproductive output, fire and similar climatic conditions to the native range of *E. globulus*. Together, these results seem to indicate that *E. globulus* plantations pose a low invasion risk in Australia. However, data from overseas indicate that invasion by *E. globulus* may increase in older plantation estates, as has been observed in Portugal (A. Águas, unpublished). Such increased invasiveness could be associated with a range of factors including reproductive capacity, lack of co-adapted pests, absence of competition with congeners, and regional management practices. While the cause of high levels of wildling establishment elsewhere remains unclear, there is a need for continued monitoring in the Australian estate.

HYBRID FITNESS — A KEY ISSUE

A knowledge of the full risks and consequences of exotic hybridisation will depend upon understanding the post-dispersal vegetative and reproductive fitness of hybrids, particularly in habitats occupied by species at risk. A key outstanding issue is whether the seedling or sapling hybrids that have already been produced, either artificially (Barbour et al. 2005a; Larcombe et al. 2015; Larcombe et al. 2016b) (Figure 3) or naturally (Barbour et al. 2003; Larcombe et al. 2014) (Figure 1), will survive in competition with the native species and be able to backcross to the native species. Obviously, with long-lived organisms such as eucalypts, and the relatively recent plantation estate, the acquisition of such information is ongoing, particularly in assessment of the fitness of hybrids beyond the first generation. Although the above studies found little evidence for later acting barriers to hybrid growth and survival, they concentrated on early life history stages (up to five years), only considered first-generation (F_1) hybrids, and were assessed mainly under glasshouse, nursery or plantation conditions.

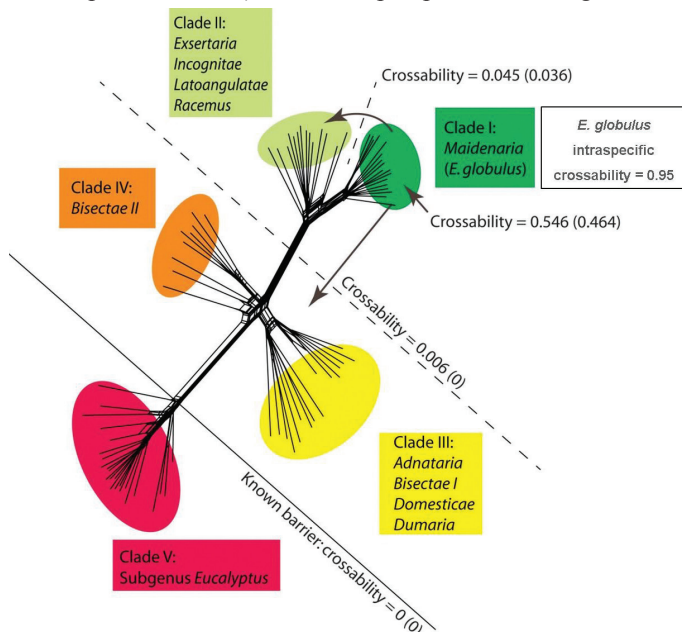


Figure 4: Phylogenetic network based on 78 species and 8350 genome-wide markers showing four main clades in the subgenus *Symphyomyrtus* (I–IV, above the solid line) annotated with the most recent sectional taxonomy (Brooker 2000). The estimated crossability between *Eucalyptus globulus* and various parts of the phylogenetic network is shown (arrows and dashed lines); the first number gives the probability of producing hybrids, and the number in parentheses is the combined probability of hybrid production and survival to nine months of age. The crossability estimates are based on experimental supplementary crossing, where *E. globulus* pollen was applied to the stigma of 99 other species. The figure is modified from Larcombe et al. (2015a).

Table 2: The risk of exotic gene flow, based on crossability (Larcombe et al. 2015) and conservation status (threatened species are in bold; Barbour et al. 2010), for 24 *Symphyomyrtus* species that have been found to occur near *E. globulus* plantations. The risk classification is derived from the criteria suggested by Roberts et al. (2009, where risk equals the product of susceptibility (likelihood) and conservation status (consequence). Taxonomy follows Brooker (2000) unless otherwise indicated.

Section	Series	Species	Distribution (by state)	Clade (expected crossability) ⁶	Risk ⁷
<i>Adnataria</i>	<i>Buxemales</i>	<i>odorata</i> ¹	SA	3 (0)	Minimal
	<i>Heterophloiae</i>	<i>polyanthemos</i> ¹	VIC; NSW;	3 (0)	Minimal
		<i>fasciculosa</i> ¹	SA; VIC	3 (0)	Minimal
	<i>Melliodorae</i>	<i>leucoxylon</i> ¹	SA; VIC; NSW	3 (0)	Minimal
		<i>melliodora</i> ¹	VIC; NSW; QLD	3 (0)	Minimal
<i>Bisectae</i>	<i>Decurvae</i>	<i>decurva</i> ¹	WA	3 (0)	Minimal
	<i>Falcatae</i>	<i>decipiens</i> ¹	WA	3 (0)	Minimal
' <i>Glandulosae</i> ' ²	<i>Cornutae</i>	<i>cornuta</i> ¹	WA	4 (0)	Minimal
	<i>Erectae</i>	<i>occidentalis</i> ¹	WA	4 (0)	Minimal
	<i>Lehmannianae</i>	<i>lehmannii</i> ¹	WA	4 (0)	Minimal
	<i>Levispermae</i>	<i>wandoo</i> ¹	WA	4 (0)	Minimal
		<i>diversicolor</i> ^{1,3}	WA	4 (0)	Minimal
<i>Exsertaria</i>	<i>Rostratae</i>	<i>camaldulensis</i> ¹	SA; VIC; NSW; QLD; NT; WA	2 (3.6%)	Low
	<i>Singulares</i>	<i>rudis</i> ¹	WA	2 (3.6%)	Low
<i>Incognitae</i>		<i>paludicola</i> ⁴	SA	2 (3.6%)	Moderate
<i>Maidenaria</i>	<i>Acaciiformes</i>	<i>fulgens</i> ¹	VIC	1 (46.4%)	Moderate
	<i>Argyrophyllae</i>	<i>alligatrix</i> ⁴	VIC; NSW	1 (46.4%)	High
	<i>Bridgesianae</i>	<i>bridgesiana</i> ¹	VIC; NSW; QLD	1 (46.4%)	Moderate
	<i>Foveolatae</i>	<i>brookeriana</i> ¹	TAS; VIC	1 (46.4%)	Moderate
		<i>ovata</i> ¹	SA; VIC; TAS; NSW; QLD	1 (46.4%)	Moderate
		<i>strzeleckii</i> ⁴	VIC	1 (46.4%)	High
		<i>Globulares</i>	<i>globulus</i> ¹	TAS; VIC	1 (95% ⁵)
	<i>Orbiculares</i>	<i>morrisbyi</i> ⁴	TAS	1 (46.4%)	High
	<i>Viminales</i>	<i>vimalis</i> ¹	SA; VIC; TAS; NSW	1 (46.4%)	Moderate

¹Occurred beside plantations in a survey of 302 *E. globulus* plantations by Barbour et al. (2008b).

²'*Glandulosae*' is an unpublished name (Nicolle 2015), which is synonymous with *Bisectae* II in Steane et al. (2002) and Larcombe et al. (2015a).

³*Eucalyptus diversicolor* was previously classified in section *Latoangulatae* or as its own section (Pryor and Johnson 1971; Brooker 2000; Nicolle 2015). However, recent molecular studies indicate it is not related to other taxa in section *Latoangulatae* (R.C. Jones unpublished) and instead is better placed in section '*Glandulosae*'² (Steane et al. 2007), which is consistent with Larcombe et al. (2015a) in both the phylogenetic placement and crossability with *E. globulus*.

⁴Nationally listed threatened species with point locations within 1 km of an *E. globulus* plantation (Barbour et al. 2010).

⁵The intraspecific crossability of *E. globulus* is 95% using the same 'dabbing' technique for pollination (Patterson et al. 2004).

⁶Clade and crossability are from Larcombe et al. (2015a).

⁷This assessment also does not consider the important pre-mating barrier of flowering time overlap. This is because given the size of the estate and the variation in flowering-time in *E. globulus* plantations and within native populations of other species of section *Maidenaria* (Barbour et al. 2006b; Jones et al. 2011), this issue requires on ground local assessments for high risk cases.

⁸*E. globulus* is at high risk of intraspecific gene flow which can alter genetic composition of native populations; see Introduction.



Figure 5: Wildlings establishing beside an *E. globulus* plantation near Manjimup in south-west Western Australia. At 18 years old, this was one of the oldest plantations surveyed in Larcombe et al. (2013). The arrows highlight that there is a range of wildling age classes present; however, all were in close proximity to the plantation and virtually all were within the harvesting zone (c.10 m from the plantation edge). In most pulpwood plantations, the management of the firebreak surrounding plantations indirectly reduces wildling spread.

Evidence from studies assessing hybrid performance in the wild and in hybrid breeding trials certainly suggest that low hybrid fitness (e.g., due to hybrid inviability or sterility, or to hybrid breakdown reflecting genomic incompatibilities) is likely to be a significant post-mating barrier to exotic gene flow (Costa e Silva et al. 2012; Larcombe et al. 2016a).

There is increasing evidence of selection against exotic hybrids in the wild when compared with open pollinated progeny of the parental native species. In Tasmania, Barbour et al. (2006a) found that *E. ovata* x *nitens* F_1 hybrids (from seed collected from *E. ovata* trees adjacent to *E. nitens* plantations) were 61 % less likely to become established in native forest compared with pure *E. ovata*. After planting hybrid and pure species seedlings in the same environment, they also found reduced growth, increased mortality and poorer plant health in hybrid seedlings compared with their pure siblings at age 4 years (Barbour et al. 2006a), with health remaining lower in the surviving hybrids at age 11 years (unpublished data). Similarly in Victoria, naturally occurring exotic *E. ovata* x *globulus* hybrids that were established following wildfire in a native *E. ovata* forest near a *E. globulus* plantation, showed markedly reduced survival compared with the co-occurring native *E. ovata* seedlings over a five-year period (Larcombe et al. 2014).

Low hybrid fitness has also been reported for many artificial interspecific hybrids of eucalypts, although vigorous hybrid combinations have been found and selected hybrids are used in some overseas plantations particularly in tropical/subtropical regions (discussed in

Potts et al. 2003 and Potts & Dungey 2004). The artificial hybrid combinations we have studied in most detail are *E. ovata* x *globulus* (López et al. 2000b) and *E. nitens* x *globulus* (Potts & Dungey 2004; Volker et al. 2008; Costa e Silva et al. 2012; Larcombe et al. 2016a). As the aim of these studies was a broader understanding of hybrid genetics, the focus was on comparing hybrid performance to both parental species and the mid-parent value using unrelated intraspecific outcrosses of the parental controls. In such studies open pollinated progeny are not suitable as controls (as used in the studies of exotic hybrids discussed above) as they may include self-pollinated and other types of crosses between relatives (Byrne 2008; Mimura et al. 2009; Gauli et al. 2013) which, in the case of eucalypts, can exhibit severe inbreeding depression (López et al. 2000c; Costa e Silva et al. 2010; Costa e Silva et al. 2011). In these hybrid studies, poor F_1 hybrid performance relative to the mid-parent value was observed across all life-cycle stages and accumulated with age. After about age four years, the F_1 hybrids performed markedly worse than both parental taxa in terms of survival and growth of the remaining survivors in both hybrid combinations studied. In the case of *E. nitens* x *E. globulus* this poor hybrid performance occurred on multiple sites and extended to advanced generation hybrids (F_2), including backcrosses (BC_g and BC_n) (Costa e Silva et al. 2012). Reduced hybrid fitness, compared with both pure species, was attributed to outbreeding depression, mainly arising from detrimental effects caused by epistatic incompatibilities amongst loci from different parental genomes and/or disruption of co-adapted complexes that have built up during isolation and speciation (Costa e Silva et al. 2012). Such outbreeding depression represents a significant post-zygotic barrier to gene flow between species, may be important in maintaining species boundaries in nature, and will likely operate to limit exotic gene flow.

The importance of later-age outbreeding depression as a barrier to first and second generation gene flow between species is further emphasised in a recent study of *E. nitens* x *globulus* that integrated assessments of reproductive barriers at various stages of the life cycle, from crossability and germination through to the proportion of plants surviving and reproducing at 20 years of age (Larcombe et al. 2016a — Figure 6). The strong unilateral pre-zygotic barrier to hybridisation evident in this cross and associated with style length (Gore et al. 1990) was lost in the F_1 hybrid, as there was no evidence for a significant reduction in seed set following backcrossing. The loss of this barrier conceptually allows F_1 hybrids to act as a bridge for bi-directional gene flow between these species. However, intrinsic post-zygotic barriers were strong and persistent in all hybrid generations. If such strong and persistent post-

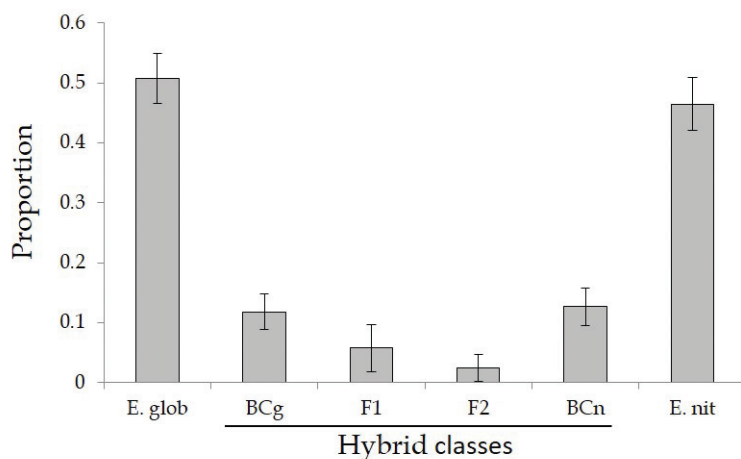


Figure 6: The proportion (\pm se) of plants surviving and having a crop of flowers or capsules at 20 years of age for *Eucalyptus globulus* (*E. glob*), *E. nitens* (*E. Nit*) and their F_1 , F_2 and backcross (BC) hybrids under field trial conditions. The backcross classes (BCg and BCn) combine crosses where each of the pure species (*E. globulus* and *E. nitens*, respectively) are used as both the male and the female parent. The trial was located at Tyenna in southern Tasmania, and details of the design, establishment and analysis can be found in Costa e Silva et al. (2012) and Larcombe et al. (2016b). Standard error bars for the proportion are based on the number of plants of each cross planted (which range from 88 to 202).

zygotic barriers exist among species involved in exotic hybridisation, then barriers arising from poor growth, survival and reproduction of hybrids will be important for preventing gene flow in the longer-term. This pattern observed for interspecific hybridisation contrasts with the positive heterosis observed for inter-provenance crosses within *E. globulus* (Volker et al. 2008; Costa e Silva et al. 2014).

FUTURE ISSUES

As the scale and extent of current, and predicted future climate change has become apparent, there has been a shift in environmental management policy to emphasise the importance of adaptation (Keenan 2015). This shift recognises that, given the rate of global change, many species may not be able to adapt to changing conditions, or migrate quickly enough to keep pace with spatial shifts in suitable habitat (Aitken & Bemmels 2016). This has triggered changes in non-forestry industries such as the revegetation sector, which is moving away from the old tenant of 'local seed is best', to recognise that mixed or even climate-adjusted seed sourcing may be required to successfully revegetate areas over the long term (Prober et al. 2015; Prober et al. 2016). Global change will also have profound repercussions for native forest management, and it has been suggested that 'assisted migration' might be necessary to 'rescue' some species and ecosystems (Burbidge et al. 2011; Hewitt et al. 2011; Weeks et al.

2011; Aitken & Bemmels 2016). Assisted migration involves moving species beyond their current range, or moving populations within a species range (sometimes referred to as 'assisted gene flow' — Aitken & Bemmels 2016). Debate about the need for assisted migration and assisted gene flow in forest trees is ongoing, because it is possible that naturally occurring long-distance gene flow may be enough to facilitate rapid natural adaptation to climate change (Kremer et al. 2012; Costa e Silva et al. 2014). Certainly high levels of such gene flow have been noted among remnant eucalypt populations in highly fragmented landscapes in Western Australia (Sampson & Byrne 2008; Byrne et al. 2008). Additionally, recent empirical studies showing possible cryptic adaptation to soil-types along climate gradients (Steane et al. 2015), and non-linear relationships between trait responses and climate variables (Liepe et al. 2016), highlight that generalisations about the adaptive potential of populations based solely on climatic factors can be misleading, and could even result in translocations being maladaptive.

As is often the case with plantation forestry, plantings from assisted migration as well as those for carbon sequestration or biofuel production, often involve growing trees outside their natural range (Byrne et al. 2011; Byrne & Stone 2011; Shepherd et al. 2011; Lindenmayer et al. 2012). As such, these new industries or forest management approaches have risks of exotic gene flow that are analogous to those found in forestry in terms of genetic exchange with congeners, as well as other sustainable management issues such as minimising unwanted spread (invasiveness). However, the consequences of exotic gene flow may need to be re-assessed in the light of global climate change in order to recognise the goals of assisted gene flow and other adaptation strategies. A change in conservation priorities towards assisted gene flow may or may not become a reality in Australia, although it is already underway in British Columbia (Liepe et al. 2016) and the expansion of this Canadian model to temperate forests globally has been strongly advocated (Aitken & Bemmels 2016). If assisted gene flow is implemented in Australia, then the consequences of gene flow from plantation forestry, whether it be inter- or intra-specific gene flow, would require re-evaluation. In terms of exotic intra-specific gene flow, what is currently perceived as a negative impact could become an environmental/adaptive benefit, providing additional genetic diversity that increases

the capacity of native populations to adapt to rapid global climate change. Similarly, in regard to exotic inter-specific gene flow, if assisted migration (beyond the natural range) of eucalypt species was undertaken on a large scale, the concept of locally exotic eucalypts may become difficult to define, and subsequently exotic gene flow may be viewed with less concern.

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