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Identification of intersectional *Corymbia* hybrids based on seedling morphology improves with parental divergence

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ABSTRACT

Differences in morphology have provided a basis for detecting natural interspecific hybridisation in forest trees for decades but have come to prominence again more recently as a means for directly measuring gene flow from planted forests. Here we examined the utility of seedling morphology for hybrid discrimination in three hybrid groups relevant to the monitoring of gene flow from plantings of *Corymbia* (L.D. Pryor & L.A.S. Johnson ex Brooker) taxa in subtropical Australia. Thirty leaf and stem characters were assessed on 907 8-month old seedlings from four parental and six hybrid taxa grown in a common garden. Outbred F_1 hybrids between spotted gums (*Corymbia citriodora* subspecies *variegata*, *C. citriodora* subspecies *citriodora* and *Corymbia henryi*) tended to more closely resemble their maternal *Corymbia torelliana* parent and the most discriminating characters were the ratio of blade length to maximum perpendicular width, the presence or absence of a lignotuber, and specific leaf weight. Assignment of individuals into genealogical classes based on a multivariate model limited to a set of the more discriminating and independent characters was highest in the hybrid group, where parental taxa were genetically most divergent. Overall power to resolve among outbred F_1 hybrids from both parental taxa was low to moderate, but this may not be a limitation to its likely major application of identifying hybrids in seedlots from native spotted gum stands. Advanced generation hybrids (outbred F_2 and outbred backcrosses) were more difficult to resolve reliably due to the higher variances of hybrid taxa and the tendency of backcrosses to resemble their recurrent parents. Visual assessments of seedling morphology may provide a filter allowing screening of the large numbers needed to monitor gene flow, but will need to be combined with other hybrid detection methods to ensure hybrids are detected.

1. Introduction

Worldwide, forest plantations are being established on an enormous scale to meet demands for wood products. The annual rate of increase in forest plantation establishment was 5 million hectares per year over the 1990–2000 period (FAO, 2010) which leads to a continuous translocation of species (Allendorf et al., 2001). Hybridisation of the translocated species with the native populations may decrease genetic variation, or alter population structure and composition of the native species (Arnold et al., 1999). In general such translocations are unmonitored (Laikre et al., 2010), but in Australia Potts et al. (2003) outlined the need for monitoring and assessment of gene flow from planted exotic hardwoods nearly a decade ago. As a result, monitoring systems are now in place in temperate Australia, where hardwoods, typically eucalypts, have been planted extensively (Gavran and Parsons, 2010).

Long term monitoring of gene flow at plantation boundaries involves periodic assessment of the rates of hybridisation, a process that is increasingly integral to risk management for planting of trees for forestry or restoration purposes (Potts et al., 2003; Byrne et al., 2011; Barbour et al., 2010). Risk management usually requires evaluating taxonomic, biological and geographic criteria within a decision tree framework to quantify the risk associated with each planting (Byrne et al., 2011). The major biological criteria include the likelihood of hybridisation and the rate that hybrids established (Potts et al., 2003; Byrne et al., 2011). Often these parameters must be initially assumed, and management strategies are adaptive, putting in place monitoring processes, and utilising information to inform management responses iteratively once evidence on hybridisation rates comes to hand (Pers. Comm. B Potts). Directly measuring hybridisation rates has confirmed expectations, for example, that hybrid frequency tends to increase in populations with high flowering synchrony, and that pollen tends to move from

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a more abundant pollen source to the populations with less pollen, i.e. fewer flowering trees, (Field et al., 2008, 2011; Lepais et al., 2009).

In temperate Australia, a system based on the visual identification of hybrids using morphological differences has been effective in large-scale screening to detect hybrid eucalypt seedling and provide direct measurements of gene flow (Potts and Reid, 1985, 1988; Barbour et al., 2003). The use of morphological markers has the advantage above other methods in these situations because many thousands of individuals may be screened easily and cheaply to detect even relatively low levels of gene flow provided at least a few reliable discriminating characters can be identified (Barbour et al., 2002; Barbour et al., 2003). If necessary, where high surety is required, morphological markers can also be combined with molecular markers like isozymes or microsatellites, to achieve higher confidence in hybrid detection (Barbour et al., 2003; Field et al., 2009).

Study of hybrid morphology also has ecological significance. In many hybrids, including eucalypts, F_1 hybrid morphology is intermediate relative to its parents, but sometimes the hybrid may resemble one parent, or exceed the range of its parents (e.g. Barbour et al., 2003; Meddings et al., 2003). Although the fitness of hybrids can be depressed, surviving transgressive individuals may have greater fitness in marginal or novel environments than their parents (Arnold and Hodges, 1995; Rosenthal et al., 2003; Rieseberg et al., 2007). This may be important in extending adaptability of a taxon and creating a new ecological space to support hybrid speciation (Rosenthal et al., 2003), or increase its weedy potential, or allow it to act as a bridge for gene flow (Whitney et al., 2006, 2010).

In subtropical Australia, spotted gums (Genus *Corymbia* Section *Maculatae*) and their hybrids are the principal taxa of interest for hardwood plantation forestry (Nichols et al., 2010). Plantations are often established in close proximity to native forests, where spotted gums and other bloodwoods (*Corymbia sp*) are endemic. As these plantations mature, management of gene flow is an increasing concern for forest managers (Barbour et al., 2008).

One concern is the planting of locally exotic spotted gum provenances. For example, northern provenances of *Corymbia citriodora* subsp. *variegata* (CCV) from around Gympie (QLD) are widely planted in northern NSW, where both *Corymbia henryi* (CH) and genetically distinct populations of CCV occur (Shepherd et al., 2008a). Natural interspecific hybridisation between CCV and CH occurs in regions of sympatry and northern and southern provenances of CCV are inter-fertile and have flowering synchrony to some extent (Hill and Johnson, 1995; Ochieng et al., 2008, 2010). *Corymbia henryi* and CCV are distinct in their morphology and although they co-occur at some locations, they tend to occupy different positions in the landscape, suggesting adaptive differentiation (Ochieng et al., 2010). Southern and northern provenances of CCV also show differential adaptability to frost (Larmour et al., 2000), tolerance to pests and diseases (Johnson et al., 2009), and leaf mass area, an ecologically important trait due to its role in tolerance to water stress (Fonseca et al., 2000). This suggests that if gene flow occurs between exotic provenances of CCV when planted in the south of its range, non-adapted genes may be introduced in local CCV or CH populations.

The second concern is with the planting of spotted gum – *Corymbia torelliana* (CT) hybrids. These hybrids have proved to have many benefits for plantation timber production in the subtropics (Lee 2007). The concern with the use of hybrids for plantations is that backcrossing and transfer of weedy attributes from CT to native spotted gums may occur. *Corymbia torelliana* is a native of north Queensland but has been planted widely throughout the subtropics, where it is regarded as an environmental weed in some shires of northern NSW and southern QLD (Hill and Johnson, 1995; NCWAC, 2003; Kingston et al., 2004). *Corymbia torelliana* naturally hybridises with spotted gums in north QLD and can be easily crossed with spotted gums in controlled pollinations (Hill and Johnson, 1995; Dickinson et al., 2010). The ability of CT to invade disturbed environments and the adaptability and vigour of its hybrids (Lee et al., 2005, 2009; Lee, 2007; Nahrung et al., 2010) raise concerns about the translocation of CT and use of hybrids in plantations (Barbour et al., 2008).

In this study, we evaluated the potential of morphology to resolve among genealogical classes by a comparative analysis of seedling attributes in *Corymbia* hybrids with their parental taxa. Seedlings of up to 10 families in each taxon, from usually two provenances, were grown in a common garden and assessed for a range of leaf and stem characters, and the presence or absence of lignotubers. Three hybrid groups (either *Corymbia citriodora* subsp. *citriodora* (CCC, CCV or CH) of interest to plantings of CT or its hybrid in three situations in subtropical Australia were studied. In the context of gene flow from plantations, the transfer of pollen from planted sources onto native spotted gums is the cross direction of most concern. However, crosses with a spotted gum maternal parent were not available at the time of this study, and we were limited to crosses in the reciprocal direction, i.e. with CT maternal parents. A recent study of seed germination showed that controlled crosses with CCV maternal parents are as viable as the reciprocal crosses (Dickinson et al., 2012), strengthening evidence for the possibility for gene flow in both directions. For the CT-CCV hybrid group, advanced generation (outbred F_2 and backcross) hybrids were also examined in addition to the F_1.

2. Materials and methods

2.1. Materials

The materials used in this study were from two section of the Genus *Corymbia*. The first was from Section *Maculatae* (i.e. the spotted gums) represented by three of the four recognised taxa (CCV, CCC and CH; *Corymbia maculata* was not studied) and the second was from section *Torellianae* (CT) (Hill and Johnson, 1995; Parra et al., 2009).

A total of 1834 seeds from 11 parental or hybrid taxa were sown in March 2010 at the DEEDI glasshouse facilities at Gympie, QLD (Table 1). The parental taxa, CCC, CCV, CH, and CT were represented by open-pollinated families from different provenances: CCC from Kirrima, QLD (17°39′S, 146°5′E) and Yeppoon, QLD (23°07′S, 150°44′E); CCV from Richmond Range, NSW (28°40′S, 152°42′E) and Woondum, QLD (26°15′S, 152°49′E); CH from Lockyer, QLD (27°30′S, 152°04′E), Nerang, QLD (27°59′S, 153°20′E) and Myrtle, NSW (29°08′S, 152°05′E); CT from Helenvale, North Queensland (15°43′S, 145°14′E) and Gympie Landrace (no latitude and longitude available but molecular studies attribute this material to be of Kuranda origin (McVey, 2004). Because the parental taxa were open-pollinated, we cannot rule out the possibility of inadvertently including hybrids especially for species that occur in sympatry. Inbreds may likewise have been included in the parental taxa. The hybrid taxa studied were from controlled pollinations and consisted of outbred F_1 interspecific hybrids, CT × CH (F_{1H}), CT × CCV (F_{1V}), CT × CCC (F_{1C}), outbred backcrosses, CT × CCV × CCV (BC_V), CT × CCV × CH (BC_H), CT × CCV × CT (BC_T) and an outbred F_2 (CT × CCV) × (CT × CCV) (F_{2V}). Each parental taxon was represented by 10 families. Where possible, parental control populations were composed of open-pollinated seedlots of trees from the same provenance as the parents used for hybrid crosses but they were not the same individuals (Table 1). In the case of CT, all the maternal parents used in hybrid crosses were from the Gympie Landrace, hence for the CT parental control we choose