Exotic gene flow from plantations to native eucalypts

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Gene flow is a natural process

The movement of genetic material between populations or species. In plants, usually the movement of seed or pollen.
Eucalypt plantations in Australia

Large-scale hardwood plantation expansion starting in 1990s
973,000 ha in 2010

Gavran and Parsons (2008)
Pollen-mediated gene flow is of concern

- eucalypts are the dominant forest type in Australia
- often weak reproductive barriers between species
- pollen dispersal is widespread compared with seed
- minimising gene flow from plantations is an indicator of sustainable forest management

> 700 documented hybrid combinations

Map showing Eucalypt distribution

700+ eucalypt species

Gavran and Parsons 2011
The exotic gene flow process

- Exotic wildlings = weeds
- Plantation
- Native
- F₁
- Limited seed dispersal capacity
- Exotic hybrids
- Genetic contamination
The Australian plantation estate is dominated by *E. nitens* and *E. globulus*

- Together make up ~ 80% of the hardwood estate
- Now planted well outside their native range

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Gavran and Parsons 2011

![E. globulus](image1.png)  ![E. nitens](image2.png)
Eucalyptus nitens in Tasmania – 16 years of exotic gene flow research

- *E. nitens* is exotic to TAS
- Main plantation species (207,000 ha) Gavran and Parsons 2011
- 17 of the 30 native species are possibly at risk
- Morphological markers
E. nitens risk assessment protocol

Flora Technical Note No. 12:
Management of gene flow from plantation eucalypts

<table>
<thead>
<tr>
<th>Hybridisation risk</th>
<th>Planning measures</th>
<th>Monitoring and control measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal risk</td>
<td>No special planning requirements</td>
<td>No formal monitoring requirements</td>
</tr>
<tr>
<td>Low risk</td>
<td>No special planning requirements</td>
<td>Regular monitoring for established hybrid seedlings and hand-weeding programs instigated if hybrids found.</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>No special planning requirements (however note possible measures discussed in the text below)</td>
<td>Regular monitoring for hybrid seedlings plus breeding system monitoring (explained in dot point 7 in the text below). Hand-weeding programs instigated if hybrids found.</td>
</tr>
<tr>
<td>High risk</td>
<td>Do not establish or re-establish eucalypt plantations without consultation with FPA. (Substantial planning and monitoring obligations may be required).</td>
<td></td>
</tr>
</tbody>
</table>

The Flora Technical Note Series provides information for Forest Practices Officers on flora management in production forests. These technical notes are advisory guidelines and should be read in conjunction with the requirements of the Forest Practices Code.
So what about the much larger *E. globulus* estate

- 484 species possibly able to hybridise

- 88% of plantations have adjacent native eucalypts (n = 302)

Barbour et al. 2008, Biological conservation
PhD questions:

1. Which species and groups of species can hybridise with *E. globulus*

2. Case study of an *at-risk* species, *Eucalyptus ovata*:
   1. Do source sink relationships affect the likelihood of gene flow: are small remnants at greater risk?
   2. How common is hybrid establishment?
   3. How fit are hybrids in relation to their pure native siblings?

3. Is seed-mediated gene flow from *E. globulus* plantations a problem?
1: Which species can hybridise with *E. globulus*?

Hybridisation does not occur between genera/subgenera.

About 900 species

- **Angophora**
- **Corymbia**
- **Eudesmia**
- **Symphyomyrtus**
  - 484 species
- **Eucalyptus**

<table>
<thead>
<tr>
<th>Genus &gt; subgenus &gt; section &gt; series</th>
<th>0%</th>
<th>0%</th>
<th>9%</th>
<th>39%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% natural hybrids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1: Which species can hybridise with *E. globulus*?

**Crossing:**
- Currency Creek Arboretum (>900 taxa)
- > 7000 flowers crossed with *E. globulus* pollen
- 100 species
- 13 taxonomic sections
- Subg. *Symphyomyrtus* (96 spp.)
- Subg. *Eucalyptus* (2 spp.)
- Subg. *Eudesmia* (1 sp.)
- *Corymbia* (1 sp.)
1: Which species can hybridise with *E. globulus*?

**Crossing:**
- Currency Creek Arboretum
- > 7000 flowers crossed
- 100 species
- 13 taxonomic sections
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- Subg. *Eucalyptus* (2 spp.)
- Subg. *Eudesmia* (1 sp.)
- *Corymbia* (1 sp.)

**Phylogenetics:**
- Genome-wide DArT markers:
  1. 8350 markers covering all sections but not all species
  2. 5050 markers covering ca. 200 spp. (Sections *Maidenaria*, *Latoangulatae* and *Exertaria*) including the 22 most closely related species in this study

**Dean Nicolle**
*Arboriculture - Botany - Ecology Eucalypt Survey & Research*
1. Two crossing approaches

- “Supplementary” pollination mimics natural pollination
- “Cut-style” pollination avoids (pre-zygotic) incompatibilities in the style and receptivity problems
1. Hybrids identified with morphology and validated with molecular markers

10 microsatellite loci were used to match alleles from each parent in hybrids.
1. Hybrid success reflects phylogenetic relatedness

Hybridisation with *E. globulus* was more common among species from Clades 1 & 2 (22 spp.) than from Clades 3 & 4 (4 spp.).

* Hybrids from CS pollination only (not supplementary pollination)
† Complete hybrid mortality

A total of 616 hybrids identified in 4571 progeny
1. Compatibility declines as genetic distance increases

Genetic distance explains 90% of variation at the section level
1. What is the time frame for reproductive isolation in *Eucalyptus*?

Dated eucalypt phylogeny (Crisp et al. 2011)

- 50% takes 3-10 mya
- 95% takes 10-15 mya
- 100% takes 21-31 mya
1. The risk of exotic gene flow from *E. globulus* plantations

- Previously 484 ‘at risk’ species (within Subg. *Symphyomyrtus*)
- Clades 3 & 4 are isolated, leaving 138 ‘at risk’ species
- The 70 species in Clade 2 have a 45% lower risk than the 68 species in clade 1
2. The case of *Eucalyptus ovata*

- known to hybridise with *E. globulus*
- hybrids have distinctive morphology
- widely distributed in the plantation zone
- common *E. globulus* plantation neighbour
- one of the most *at-risk* species (Barbour *et al.* 2008)
2.1: Landscape context: Does patch size affect hybridisation risk?

What is the effect of patch size in fragmented landscapes?

Pollen dispersal decreases with distance
2.1: Does patch size affect hybridisation risk?

We identified five remnant categories:
1. isolated paddock tree 50 to 200m from plantation edge
2. 1-30 trees surrounded by plantation
3. ~50 trees surrounded by plantation
4. > 100 trees surrounded by plantation
5. > 100 trees continuous native forest adjoining plantation

- open-pollinated seed collected from *E. ovata* in each remnant
- in categories 2, 3 and 4, trees were sampled on the boundary and 50m inside the remnant.
- capsule abundance assessed in the plantation

<table>
<thead>
<tr>
<th>Remnant category</th>
<th>n patches</th>
<th>n trees</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>23</strong></td>
<td><strong>142</strong></td>
</tr>
</tbody>
</table>
2.1: Does patch size affect hybridisation risk?

> 24,000 seedlings screened
1.62% exotic hybrids

Hybrids identified from morphology, confirmed using molecular markers

E. ovata
E. ovata x globulus F1
2.1: Does patch size affect hybridisation risk?

- Patch size and tree position in the landscape effect hybridisation rate
- Minimising fragmentation and maximising embedded remnant size will help maintain genetic integrity
2.2: How frequent is hybrid establishment in the wild?

Targeting high risk sites, because:

• Rare events are hard to survey for
• Obtain a conservative estimate i.e. worst case scenario

Method

• Categorise every seedling (out to 20m) as pure *E. ovata*, pure *E. globulus*, or *E. ovata* x *globulus* hybrid

Conditions:

• *E. globulus* plantation beside *E. ovata* native forest with no native *E. globulus*
• Recruitment from *E. ovata* and plantation *E. globulus* of equivalent age
2.2: How frequent is hybrid establishment in the wild?

- 216 km of plantation edge surveyed in Tasmania, Gippsland and the green triangle
- Only 4 high-risk sites identified
- 12ha surveyed in detail along 4 km of plantation native forest boundary

![Image of E. ovata and E. globulus F1 hybrid seedlings]

<table>
<thead>
<tr>
<th>Number of seedlings</th>
<th>E. ovata</th>
<th>F1 hybrids</th>
<th>E. globulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>547</td>
<td>7</td>
<td>191</td>
</tr>
</tbody>
</table>
2.3: Will hybrids survive in the wild?

In 2006 Robert Barbour paired 80 naturally establishing *E. globulus* x *ovata* hybrids with 80 ♀ *E. ovata* (Barbour et al. 2008).

- In 2010 I found 33 pairs
- 23 pure and 5 hybrids (P<0.001)
- The hybrids are 78% less fit than the pure seedlings
2.3: Will hybrids survive in the wild?

Similar results were obtained by Lopez et al. (2000) in a trail situation using hybrids from controlled crosses, hybrids also flowered asynchronously = secondary barrier

Lopez et al. 2000, Heredity, 85, 242-250
3: What about the wildlings?

Plantations are cyclical: plant – grow – flower – harvest – plant?

Yr1 Yr6 Yr12

538,000 ha now, but what will be replanted?

Wildling establishment => weeds, and a long term exotic pollen source => long term exotic gene flow?
3: How common is wildling establishment and what factors control it?
3: A survey at two geographic scales

1. broad scale survey:
   • Surveyed 269 plantation boundaries (290km)
   • Across all main growing regions
   • To investigate regional and bioclimatic factors

2. fine-scale survey:
   • Density triggered paired plots
   • Local and microsite factors
3: Seedlings mainly established within 10m of the plantation edge

4939 wildlings
## 3: Regional variation

<table>
<thead>
<tr>
<th>Region</th>
<th>No. transects surveyed</th>
<th>Distance surveyed (km)</th>
<th>Total No. wildlings</th>
<th>No. wildlings/km</th>
<th>No. fine-scale pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany</td>
<td>31</td>
<td>56.1</td>
<td>1274</td>
<td>22.7</td>
<td>22</td>
</tr>
<tr>
<td>Manjimup</td>
<td>33</td>
<td>21.5</td>
<td>851</td>
<td>39.6</td>
<td>9</td>
</tr>
<tr>
<td>Grampians</td>
<td>19</td>
<td>17.2</td>
<td>624</td>
<td>36.3</td>
<td>5</td>
</tr>
<tr>
<td>Penola</td>
<td>49</td>
<td>64.2</td>
<td>75</td>
<td>1.2</td>
<td>3</td>
</tr>
<tr>
<td>Portland</td>
<td>40</td>
<td>44.5</td>
<td>1483</td>
<td>33.3</td>
<td>20</td>
</tr>
<tr>
<td>Gippsland</td>
<td>59</td>
<td>60.4</td>
<td>525</td>
<td>8.7</td>
<td>12</td>
</tr>
<tr>
<td>Tasmania</td>
<td>38</td>
<td>26.5</td>
<td>107</td>
<td>4.0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>269</strong></td>
<td><strong>290.4</strong></td>
<td><strong>4939</strong></td>
<td><strong>17.0 (8/ha)</strong></td>
<td><strong>71</strong></td>
</tr>
</tbody>
</table>

We then used a modelling approach to determine factors driving the variation.
3: Wildlings increase with plantation age

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transect length</td>
<td>1</td>
<td>3.82</td>
<td>0.0507</td>
</tr>
<tr>
<td>Region</td>
<td>6</td>
<td>44.87</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plantation age</td>
<td>1</td>
<td>13.69</td>
<td>0.0002</td>
</tr>
<tr>
<td>Precipitation seasonality</td>
<td>1</td>
<td>3.61</td>
<td>0.0576</td>
</tr>
<tr>
<td>Region * Precipitation seasonality</td>
<td>6</td>
<td>32.32</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Total variation exp. = 30%

Typical rotation age is 10 - 15 years
3: Climatic conditions similar to native range seem to promote establishment

- regular rainfall
- high rainfall
- low temperature

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transect length</td>
<td>1</td>
<td>1.9</td>
<td>0.1691</td>
</tr>
<tr>
<td>Region</td>
<td>6</td>
<td>56.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plantation age</td>
<td>1</td>
<td>4.9</td>
<td>0.0264</td>
</tr>
<tr>
<td>Annual mean temperature</td>
<td>1</td>
<td>10.9</td>
<td>0.0009</td>
</tr>
<tr>
<td>Precipitation seasonality</td>
<td>1</td>
<td>10.1</td>
<td>0.0015</td>
</tr>
<tr>
<td>Annual precipitation (log)</td>
<td>1</td>
<td>22.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Region * Plantation age</td>
<td>6</td>
<td>24.3</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

total deviance exp. = 44.8
3: Fire promotes establishment

Graph showing the predicted number of wildlings/km against plantation age (years) for burnt and unburnt conditions.
3: How common is wildling establishment and what factors control it?

2. fine scale survey results:
   - None triggered in Tasmania

<table>
<thead>
<tr>
<th></th>
<th>Gippsland</th>
<th>Grampians</th>
<th>Penola</th>
<th>Portland</th>
<th>Manjimup</th>
<th>Albany</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plots (pairs)</td>
<td>12</td>
<td>5</td>
<td>1</td>
<td>22</td>
<td>9</td>
<td>22</td>
<td>71</td>
</tr>
<tr>
<td>Number of wildlings /plot</td>
<td>9.8</td>
<td>9.8</td>
<td>6</td>
<td>8.4</td>
<td>9.8</td>
<td>6.7</td>
<td>9</td>
</tr>
</tbody>
</table>

Topography; aspect; ground cover classes; disturbance were all non-significant

\( P < 0.0001 \)
Plantation - Fire break management could be helping control the spread of wildlings.

3: How common is wildling establishment and what factors control it?

Fire break management could be helping control the spread of wildlings.
3: But we need to keep monitoring...

In Australia 12% of 2nd rotation
In Portugal 80% 2nd or older

<table>
<thead>
<tr>
<th></th>
<th>Australia</th>
<th>Portugal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km surveyed</td>
<td>290</td>
<td>33</td>
</tr>
<tr>
<td>Wildlings/km</td>
<td>17</td>
<td>245</td>
</tr>
<tr>
<td>Transects</td>
<td>269</td>
<td>96</td>
</tr>
<tr>
<td>FS positive plots</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>FS control plots</td>
<td>71</td>
<td>22*</td>
</tr>
</tbody>
</table>
Main conclusions

1. Genetic barriers to hybridisation exist within *Symphyomyrtus* reducing the number of species at risk by 71%; main threat to *Maidenaria*

2. Patch size and tree position affect hybridisation; maximising remnant size will help maintain genetic integrity

3. Hybrid establishment in the wild is low

4. Hybrid survival in the wild is 78% lower than pure native seedlings

5. Wildling establish is relatively low, and associated with older plantations and high reproductive output
What do the results mean for managing exotic gene flow

1. The barriers to exotic gene flow identified in this project indicate that the genetic risk posed by *E. globulus* plantations is low and will mainly be a concern where:
   1. Species are closely related to *E. globulus*
   2. They occur in small fragmented population/patches
   3. And/or are of conservation significance

2. Wildlings do not currently seem to pose a major risk in managed plantation estates, but the experience in older estates in Portugal shows that continued monitoring is warranted, particularly if current management changes
Future issues: will exotic gene flow be a problem if assisted migration becomes a reality?

What is now considered a risk may be of little concern, or even a target of conservation programs, if assisted migration becomes widespread.