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Gene flow between introduced and native *Eucalyptus* species: exotic hybrids are establishing in the wild

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Abstract. F_1 hybrids between exotic *Eucalyptus nitens* plantations and native *E. ovata* have previously been reported among seedlings grown from open-pollinated seed collected from *E. ovata*, on the island of Tasmania. Such exotic hybrid seedlings have now been found in the wild adjacent to plantations at three locations. The proportion of exotic hybrids in open-pollinated seed collected from nearby mature *E. ovata* was 5.5%. This level compares with only 0.4% for natural hybrids between native species at these sites (*E. ovata*, *E. viminalis* and *E. rodwayi*). Detection of hybrids was initially based on their deviant morphology, which was generally intermediate between parental species. This subjective classification was then successfully verified by morphometric and allozyme analyses. Pure *E. nitens* seedlings (wildlings) were restricted to within 30 m of these plantations, whereas established hybrids were found up to 310 m from the plantations. This pattern of establishment reflects dispersal of exotic seed and pollen respectively. It is likely that the recent expansion of the eucalypt plantation estate in Australia will cause an increase in the frequency of exotic hybrids. However, the long-term impact of such hybridisation is yet to be assessed.

Introduction

Gene flow from introduced species can have an impact on the genetic integrity and survival of native populations (Butler 1994; Rhymer and Simberloff 1996; Anttila *et al.* 1998). Plant species introduced for agricultural, forestry and ornamental purposes, as well as weeds, can all act as potential sources of foreign genes. Indirect examples of gene flow between introduced and native species are documented in studies of seed crop contamination (Bateman 1947), the evolution of agricultural weeds (Small 1984; Van Raamsdonk and Van Der Maesen 1996) and the escape of engineered genes (Ellstrand and Hoffman 1990; Raybould and Gray 1994). However, with the high number of exotic species established around the world, studies of their genetic impact on native populations is becoming increasingly common (Mejnartowicz 1996; Anttila *et al.* 1998). First-generation (F_1) hybrid populations between exotic and native populations represent an initial step towards such introgression (Arnold 1992; Raybould and Gray 1994). The detection of F_1 hybrids has proved the most effective technique for tracking the movement of genes and for assessing the potential for introgression (Pryor 1976; Potts and Reid 1988; Rieger *et al.* 2002).

Gene flow between introduced *Eucalyptus* plantations and native eucalypt species has been recognised as one of the many issues to be managed as part of a sustainable forest production regime in Australia (Commonwealth of Australia 1998; Strauss 2001; Potts *et al.* 2003). More than 502 000 ha of hardwood plantations, almost all from the genus *Eucalyptus*, have been established in Australia (Wood *et al.* 2001). These have been planted with a small number of species and within the range of potentially interbreeding native species (Potts *et al.* 2003). In Tasmania, two eucalypt species are predominantly used for plantation forestry, *E. globulus* and *E. nitens* (Tibbits 1986). Both of these species are in section *Maidenaria*, along with all of Tasmania's native eucalypts that are from subgenus *Sympyomyrtus* (Brooker 2000). However, *E. nitens* is exotic to the island as it is native only to continental Australia (Pederick 1979). Its use in Tasmania may pose a risk of genetic pollution to the native eucalypt species, as the barriers to inter-specific crossing within sections are often weak (Griffin *et al.* 1988; Tibbits 1988, 1989). Hybrids between plantation-grown *E. nitens* and the native species *E. ovata* were previously identified in open-pollinated seed from *E. ovata* (Barbour *et al.* 2002); however, whether these hybrids can establish in the wild was unknown. We here present the first evidence that exotic F_1 hybrid seedlings, arising from fertilisation of native *E. ovata* by plantation-grown *E. nitens* pollen, are establishing in the wild.

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Materials and methods

Field and glasshouse work

Between September 2000 and January 2002, 77 putative *E. ovata* × *nitens* F_1 hybrid seedlings were identified at three locations in northern Tasmania; two at Huntsman in the Meander Valley (41°42′S, 146°35′E), six close to Lilydale (41°14′S, 147°11′E) and 69 at Nunamara (41°22′S, 147°17′E). The putative exotic hybrids at Huntsman were near an *E. nitens* family trial in which some of the trees had been treated with the flowering promoter paclobutrazol (Barbour *et al.* 2002), whereas the remaining exotic hybrids were found near routine plantations that were reproductively mature. The *E. nitens* at the three sites were between 10 and 14 years old when the first exotic hybrids were discovered. The putative exotic hybrids were found in naturally regenerating road-side vegetation strips, cleared areas of forest or rough vehicular tracks, and ranged in height from 10 to 80 cm. All the putative exotic hybrids were found regenerating next to reproductively mature *E. ovata* trees and among pure *E. ovata* seedlings. No other hybrids between the other eucalypt species at each site were identified in the wild. Specimens of the *E. ovata* × *nitens* hybrids, *E. nitens* wildlings established through seed dispersal from the plantations and native *E. ovata* and *E. viminalis*, were lodged in the Tasmanian Herbarium (reference numbers HO 521070, 521071, 521256, 521255, respectively).

The first nine putative hybrids to be discovered were removed from the sites, placed into pots and grown further under glasshouse conditions so that allozyme analysis on fresh glasshouse-grown leaves could be conducted. An exhaustive search of the area around the plantations where the putative hybrids were originally found was then conducted, to locate as many of these plants as possible. The distance between each seedling and the nearest plantation boundary was measured, as well as the distance between any pure *E. nitens* wildlings and the plantations. Open-pollinated seed was collected from the *Symphyomyrtus* species, expected to be reproductively compatible with *E. nitens*, at each of the three sites (Griffin *et al.* 1988). Three native species, *E. ovata*, *E rodwayi* (present at Nunamara only) and *E. viminalis*, were sampled, as well as the exotic *E. nitens*. *E. ovata* was most likely to overlap in flowering time with *E. nitens* (Williams and Potts 1996; Barbour *et al.* 2002) and the most likely seed parent of the exotic hybrids. To confirm this, four trees of each species located closest to the putative hybrids at each site were sampled. At Nunamara, the sampled *E. ovata* and *E. viminalis* trees were within 10 m of the *E. nitens* plantation and within 30 m of the exotic hybrids. The *E. rodwayi* trees sampled were *c.* 200 m from both the *E. nitens* boundary and the exotic hybrids. At Lilydale, the *E. ovata* trees sampled were *c.* 100 m from the *E. nitens* and the canopies of these trees were directly above the exotic hybrids. The *E. viminalis* trees were within 50 m of *E. nitens* and 150–250 m from the exotic hybrids. At Huntsman, *E. ovata* and *E. viminalis* were within 100 m of the *E. nitens* and 20–200 m from the exotic hybrids.

The open-pollinated seed was propagated by scattering it on top of moist potting mix in 17 × 49 × 34 cm polystyrene boxes, under glasshouse conditions and kept well-watered, with different seedlots in different boxes (Barbour *et al.* 2002). Seedlings were grown to at least Node 8, so that they could be morphologically screened for the presence of hybrids. The total number of seedlings of each putative cross-type was recorded. The morphological characters used to identify putative hybrids involving *E. nitens* are outlined in Barbour *et al.* (2002) and shown in Fig. 1. The preliminary identification of hybrids between the native species was based on their morphology being outside the phenotypic range of each species and intermediate between any of the *Symphyomyrtus* species present at each of the sites. Due to the seedling characters of *E. ovata* and *E. rodwayi* being similar, the morphological detection of hybrids between these two species may have been less accurate than for the other cross-types. Once seedlings were classified, most were removed to allow remaining seedlings to grow to an assessable height. However, some seedlings from each cross-type in each family were kept for later morphometric and allozyme analysis. These seedlings also acted as a reference point for the verification of the putative hybrids found in the wild.

Morphometric assessment

Five seedlings from each open-pollinated family were used for the morphometric analysis of the pure species cross-types. Where possible, five seedlings per family were also used for each of the putative hybrid combinations identified; however, insufficient numbers often resulted in extra plants from other families being used or this number not being reached. Also included were pure *E. ovata* and the putative *E. ovata* × *nitens* F_1 hybrid seedlings identified in the wild and the hybrids that were removed from the wild and grown further in the glasshouse. Twenty-one characters were assessed on each seedling (Table 1). Leaf measurements were taken from a photocopy of one of the Node 10 leaves by using a digitising tablet. Stem measurements were done on the internode below Node 10. Stem diameters were taken across the same axis as the leaves coming out of the stem at Node 10 (SD1), at right angles to this line (SD2) and at 45° between the two lines (SD3). For seedlings that had pronounced wings on the stem, such as those of *E. nitens* (Fig. 1*e*), SD1 and SD2 were taken across the stem between the wings and SD3 measured the diameter of the stem including the wings (see Tibbits 1988).

Cross-type means of all characters were calculated. The raw data were then transformed where necessary, to optimise normality and homogeneity of variance criteria (Table 1). This was followed by a canonical discriminant analysis by using the DISCRIM procedure of SAS (Version 8) which aimed to maximise the separation between the four pure species groups. The positions of the putative hybrids in the three-dimensional discriminant space were then calculated to help verify their parentage.

Allozyme assessment

Variation at the PGD-1 allozyme locus was assessed in the propagated open-pollinated seedlots collected from Nunamara, Lilydale and Huntsman, to assist in verification of the putative exotic hybrids between *E. nitens* and the native species, and among the three native species. One seedling from each cross-type per family was used. For *E. rodwayi*, however, 25 seedlings from three families were used as no previous allozyme work had been conducted on this species. Also the number of putative *E. ovata* × *nitens* F_1 hybrid seedlings from each of the *E. ovata* seedlots was increased to an average of six. When individuals from a particular cross-type or family were lacking, then the numbers were left short or made up from other families. In addition, the putative *E. ovata* × *nitens* hybrids found in the wild were screened at the PGD-1 locus, along with wild samples of pure *E. nitens*, *E. viminalis* and *E. ovata*. Pure-species seedlings from Huntsman were not assayed as this site had been previously assessed (Barbour *et al.* 2002).

The starch gel electrophoresis technique used a morpholene citrate buffer system following Moran and Bell (1983). For each gel, the samples were aligned into blocks containing six samples, with every fourth being a known sample homozygous for PGD-1^1. The staining technique followed Wendel and Weeden (1989). Allozymes at PGD-1 were scored from one to five, as in Barbour *et al.* (2002), with one being the most anodal.

Results

Morphological characters of putative hybrids

The putative *E. ovata* × *nitens* F_1 hybrid seedlings found in the wild were easily identified by their deviant morphology

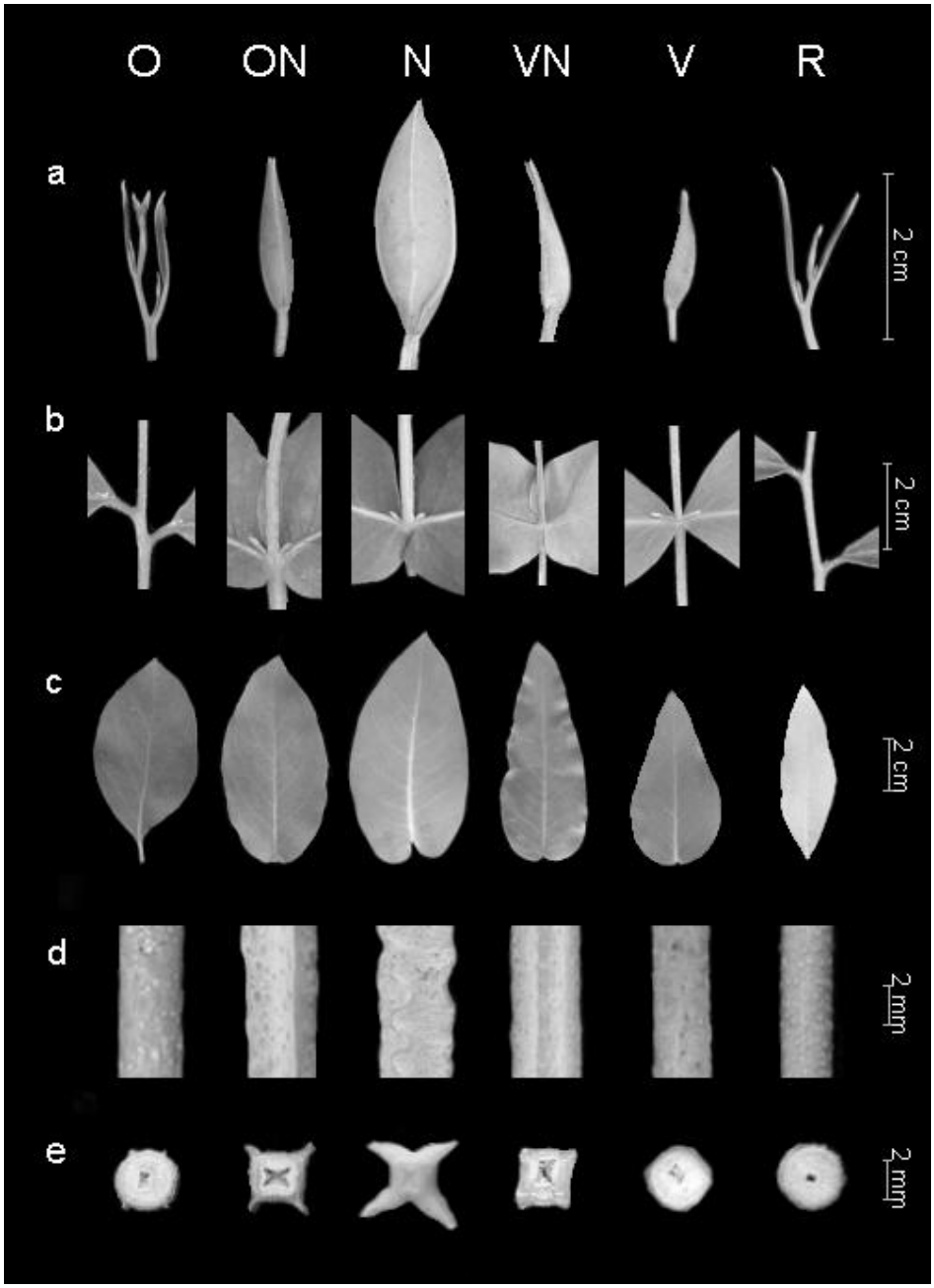

Fig. 1. Seedling morphology (at Node 10) of *Eucalyptus ovata* (O), *E. ovata* × *nitens* F_1 hybrids from *E. ovata* seedlots (ON), *E. nitens* (N), *E. viminalis* × *nitens* from *E. viminalis* seedlots (VN), *E. viminalis* (V) and *E. rodwayi* (R). Variations in (*a*) bud, (*b*) node (leaves truncated), (*c*) leaf, (*d*) longitudinal stem and (*e*) cross-sectional stem morphology display clear species-specific differences between *E. nitens* and the native species. This allowed for morphological identification of F_1 hybrids between *E. nitens* and the native species, followed by their morphometric and molecular verification. It should be noted that the ON hybrids may or may not display intra-nodes at Node 10; however, if they do, the intra-node is much smaller than that for *E. ovata*.

which appeared intermediate between pure *E. nitens* and *E. ovata* seedlings growing in the same area. They were similar to the *E. ovata* × *nitens* F_1 hybrids identified previously among glasshouse-grown open-pollinated seedlings (Barbour *et al.* 2002).

The open-pollinated progeny grown in the glasshouse displayed significant ($P < 0.001$) separation of the pure species at both the univariate (Table 1) and multivariate (Fig. 2) levels. All the pure-species seedlings displayed tight clustering in the discriminant space and the greatest

Table 1. Characters used in the morphometric analysis of the open-pollinated progeny and the plants found in the field
Included are the transformations (Transf.: Log, logarithmic; Sqrt, square root) used for the analysis, along with the *F*-ratios and *P*-values of the significance test for the difference among the four pure species

Code	Description	Transf.	*F*-ratio	*P*-value
Bud character				
LFUS	Length of fusing of the apical bud leaves (mm) (see *E. nitens* in Fig. 1 for an example)	Log	299.9	0.0001
Leaf character				
CORD	Length of cordate lobes; length of extension of the cordate lobes of the lamina past the base of the petiole–lamina join (mm)	Sqrt	46.7	0.0001
LA	Leaf angle; the axilary angle made by the midrib and the stem (°)	Sqrt	7.2	0.0001
LAML	Length of lamina (mm)	Log	595.2	0.0001
LAMW	Lamina width at the widest point (mm)	Log	372.9	0.0001
LGL	Leaf glaucousness (1–8); converted from Cauvin *et al.* (1987) (1–2 = green, 3–5 = subglaucous, 6–8 = glaucous)	Log	228.9	0.0001
LP	Leaf plane; cross-sectional angle of the leaf from horizontal (°)	Log	92.5	0.0001
LTA	The acute angle of the leaf tip (°)	Sqrt	26.8	0.0001
LWP	Length along midrib to the widest point (mm)	None	119.3	0.0001
PETL	Length of petiole (mm)	Log	98.3	0.0001
Plant character				
HT10	Height to Node 10 (mm)	None	5.3	0.0016
LAT05	Number of laterals from Nodes 0–5	Sqrt	16.5	0.0001
LAT610	Number of laterals from Nodes 6–10	None	19.4	0.0001
Stem character				
INTER10	Inter-node length below Node 10 including lower intranode (mm)	Sqrt	534.7	0.0001
INTRA10	Intra-node length at Node 10 (mm)	None	89.2	0.0001
SGL	Stem glaucousness (1–2; 1 = absent, 2 = present)	None	12.7	0.0001
SQU	Stem squareness; the cross-sectional shape of the stem (i.e. Fig. 1*e*) (1–4; 1 = round, 2 = square but no wings, 3 = wings but still flat between each wing, 4 = wings with little or no flat section between wings)	None	181.5	0.0001
SRE	Stem rectangularity (stem diam. 1/stem diam. 2—see text) (mm)	Log	6.5	0.0003
SRO	Stem roundness (stem diam. 1/stem diam. 3—see text) (mm)	Sqrt	429.1	0.0001
SV	Stem verrucae (1–2; 1 = absent, 2 = present)	None	17.3	0.0001
WW	Waviness of wings (1–3; 1 = waves absent, 3 = maximum)	Log	201.3	0.0001

morphological difference occurred between *E. ovata* and *E. nitens* (Fig. 2*a*; Table 2). The putative *E. ovata* × *nitens* hybrids from the wild lay outside of the pure species groups and their distribution in the discriminate space was similar to the same hybrids identified in the open-pollinated families of *E. ovata* and *E. nitens* (Fig. 2). There were two distinct morphological groups of *E. ovata* × *nitens* hybrids. One was intermediate between the parents (Group 1) while the other fell more in between *E. viminalis* and *E. nitens* (Group 2). The seedlings in Group 2 deviated from an intermediate position because they had not developed an intra-node by Node 10. Intra-node development is one of the main indicators of vegetative phase change in these species, an ontogenetic process in which the juvenile foliage is replaced by adult foliage. The transition occurs at about Node 4–6 in *E. ovata*, but much later in *E. nitens* and *E. viminalis*. All plants were measured at Node 10 because this provided strong discrimination of the parental species. However, this node was within the transition stage of the hybrids and the Group 2 hybrids had yet to develop intra-nodes.

Four other putative hybrid combinations were identified within the open-pollinated seedlots: *E. rodwayi* (♀) × *ovata*; *E. ovata* (♀) × *viminalis*; *E. nitens* (♀) × *viminalis*; and *E. viminalis* (♀) × *E. nitens* (Fig. 2*b*, *c*). These hybrids were intermediate between the pure-species clusters or biased towards one of the putative parents. The bias seen in the majority of the *E. ovata* × *viminalis* hybrids towards *E. viminalis* was again a result of their ontogeny as explained for the hybrids between *E. ovata* and *E. nitens*. The *E. nitens* × *viminalis* hybrids, for example, were intermediate with the exception of one, from an *E. nitens* female, that lay within the *E. viminalis* cluster (Fig. 2*b*).

Verification of hybrids

Eucalyptus nitens displayed the highest frequency of the PGD-1^1 allozyme (94.4%), with *E. ovata* (2.2%) and *E. viminalis* (9.5%) having relatively low frequencies (Table 3). PGD-1^1 can therefore verify the F_1 hybrids between *E. nitens* and these native species at these sites. *E. rodwayi* displayed a higher proportion of the PGD-1^1 allozyme (20%) than the other native species, making the marker less effective at verifying hybridisation between this species and *E. nitens* at Nunamara.

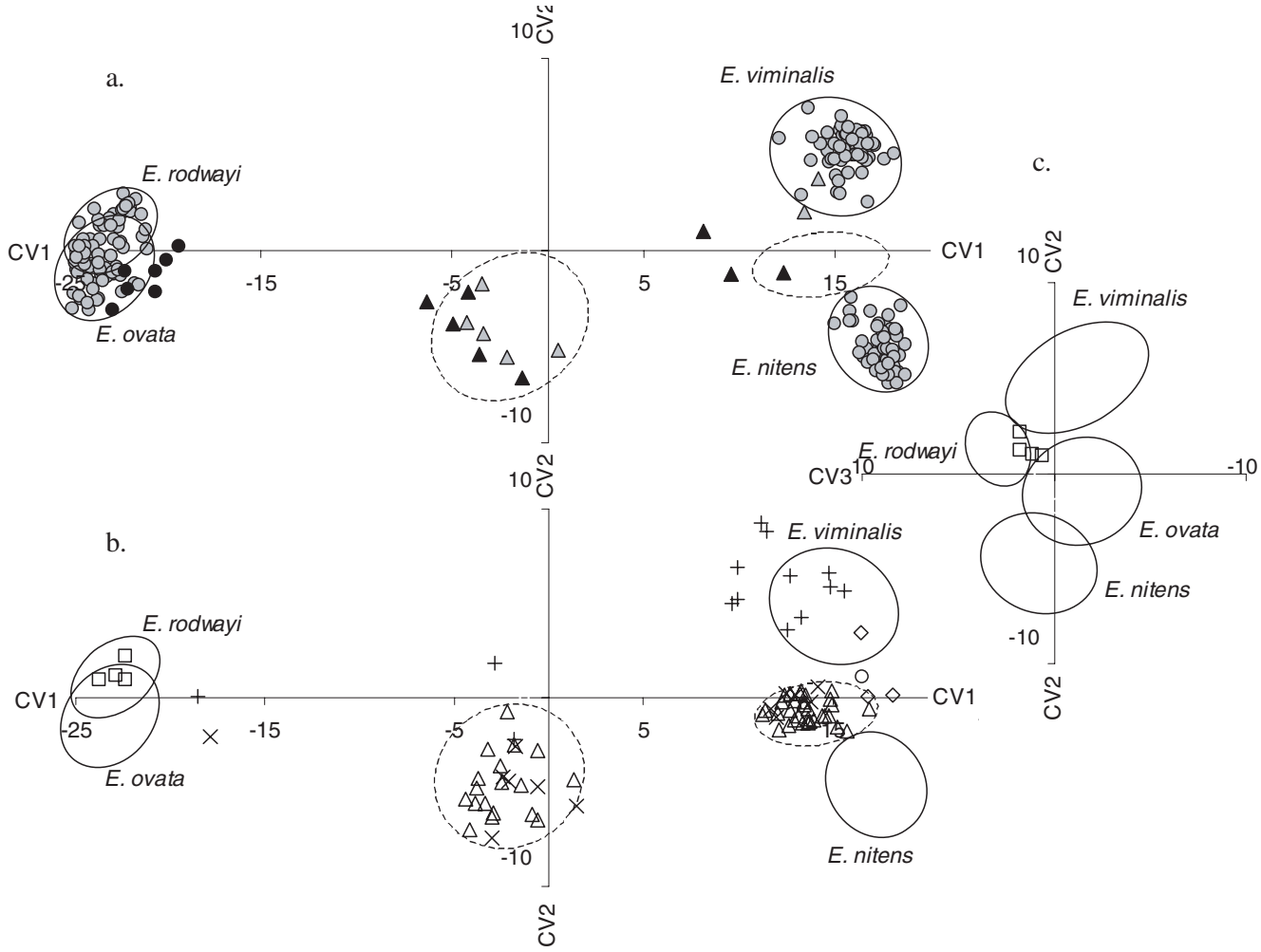

Fig. 2. Morphological variation (canonical discriminant analysis) of putative F_1 hybrids among *Eucalyptus nitens*, *E. viminalis*, *E. ovata* and *E. rodwayi*. Plot (*a*) compares the putative *E. ovata* × *nitens* hybrids (▲, field-sampled and △, grown further in the glasshouse) and pure *E. ovata* (●) indentified in the field, with the pure species (◍) and distributions of the *E. ovata* × *nitens* hybrids (broken ellipsoids) that were grown from open-pollinated seed under glasshouse conditions. Plots (*b*) and (*c*) display all the putative F_1 hybrid combinations found among the open-pollinated seedlots, i.e. from *E. nitens* (×, *E. nitens* × *ovata*; ◇, *E. nitens* × *viminalis*), *E. ovata* (△, *E. ovata* × *nitens*; +, *E. ovata* × *viminalis*), *E. viminalis* (○, *E. viminalis* × *nitens*) and *E. rodwayi* (□, *E. rodwayi* × *ovata*), relative to the limits of the pure species' distributions (ellipsoids with solid lines).

The PGD-1^1 allozyme occurs at a frequency of 48% in the putative *E. ovata* × *nitens* F_1 hybrids from the open-pollinated families of *E. ovata*. Of the putative hybrids, 83% had PGD-1^1, either in the heterozygote (67%) or homozygote (17%) state (Table 3). This result verifies the hybrid status of these plants since PGD-1^1 is frequent in *E. nitens* but rare in *E. ovata* (Table 3). Little difference was seen in allozyme composition between the two morphological groups of putative hybrids or the putative hybrids obtained from *E. nitens* females, which argues they are all F_1 hybrids between *E. ovata* and *E. nitens*.

Almost all of the putative *E. ovata* × *nitens* hybrids found in the wild were heterozygous between the *E. nitens* PGD-1^1 allozyme and the slower native *E. ovata* allozymes, PGD-1^3 or PGD-1^5 (94%; Table 3). This, combined with the majority of the plants being morphologically outside pure-species ellipsoids and intermediate between the pure parental species (Fig. 2), confirms their original classification. The two wild hybrids seen within and next to the *E. viminalis* cluster in the morphometric plot (Fig. 2) were heterozygous for PGD-1^1 and PGD-1^3. This result is consistent with their classification as *E. ovata* × *nitens* hybrids, since both allozymes are rare in *E. viminalis* (Table 3).

Putative F_1 hybrids between *E. nitens* and *E. viminalis* were detected in open-pollinated seedlots of both *E. nitens* and *E. viminalis*. All of the *E. nitens* (♀) × *viminalis* hybrids, including the seedling within the *E. viminalis* morphometric cluster (Fig. 2), were PGD-1^3 and PGD-1^5 heterozygotes. PGD-1^5 is only found at high frequency in *E. viminalis*, is rare in *E. ovata* and has not been recorded in *E. nitens*. All of

Table 2. Untransformed means for *Eucalyptus ovata*, *E. viminalis*, *E. rodwayi*, *E. nitens* and hybrids involving *E. nitens* as the pollen donor for each of the characters used in the morphometric analysis

For description of characters, see Table 1. OP, open-pollinated

Character assessed	OP *E. nitens*	OP *E. ovata* × *nitens*	Field *E. ovata* × *nitens*	OP *E. ovata*	OP *E. rodwayi*	OP *E. viminalis* × *nitens*	OP *E. viminalis*
Bud character							
LFUS (mm)	26.7	13.7	5.5	0.0	0.0	20.4	15.6
Leaf character							
CORD (mm)	6.0	2.0	0.7	0.0	0.0	1.7	1.3
LA (°)	71.1	84.3	56.9	106.4	101.6	125.0	108.1
LAML (mm)	79.8	76.6	56.2	7 0.5	44.8	61.1	47.6
LAMW (mm)	40.7	38.5	27.0	34.7	11.4	23.4	25.7
LGL (1–8)	5.2	1.2	1.5	1.2	1.0	3.0	2.5
LP (°)	9.4	12.4	14.0	18.3	10.2	8.0	15.5
LTA (°)	87.2	79.4	93.9	83.2	54.9	69.6	69.2
LWP (mm)	26.4	27.3	22.5	29.1	18.6	13.0	12.8
PETL (mm)	0.2	0.4	0.9	7.2	1.2	0.2	0.1
Plant character							
HT10 (mm)	35.2	38.6	10.9	38.4	26.4	34.6	32.7
LAT05	0.0	0.3	0.8	0.1	0.7	0.0	0.7
LAT610	0.4	0.8	0.5	2.6	2.3	0.0	1.8
Stem character							
INTER10	32.1	45.7	26.7	46.5	35.4	26.0	39.7
INTRA10	0.0	1.1	1.3	16.3	10.4	0.0	0.0
SGL (1–2)	1.9	1.1	1.0	1.0	1.0	2.0	1.2
SQU (1–4)	3.7	3.0	2.9	1.3	1.0	2.0	1.1
SRE (mm)	1.0	1.0	1.1	1.0	1.0	1.1	1.0
SRO (mm)	0.5	0.7	0.7	0.9	1.0	0.8	1.0
SV (1–2)	1.8	1.6	1.8	1.3	1.8	1.0	1.6
WW (1–3)	2.1	1.2	2.0	1.0	1.0	1.0	1.0
Number of seedlings	60	50	8	60	25	1	60

these hybrids came from a single *E. nitens* tree that must have been homozygous for PGD-1[3]. The reciprocal *E. viminalis* × *nitens* hybrid detected in a *E. viminalis* seedlot was heterozygous for PGD-1[1] PGD-1[5], as expected. These results, combined with the generally intermediate morphology of the seedlings, verify their hybrid status.

Most putative *E. ovata* (♀) × *viminalis* hybrids were heterozygous for PGD-1[5], a rare allozyme in *E. ovata*, which is consistent with that expected from such hybrids. These certainly cannot be confused with the *E. ovata* × *nitens* hybrids from the same family, since none had the common PGD-1[1] allozyme of *E. nitens*. While the putative *E. rodwayi* (♀) × *ovata* hybrids tended to be intermediate between the pure species in morphology (Fig. 2), the lack of clear morphological and allozyme differentiation between seedlings of the pure species does not allow verification of their hybridity.

Frequency of hybrids in the open-pollinated seedlots

In total, 11486 seedlings were propagated from the open-pollinated seed and morphologically classified as being either pure species or F_1 hybrid (Table 4). *E. ovata* × *nitens* hybrids were found in the open-pollinated seed of all the *E. ovata* trees that were sampled in close proximity to the exotic wild hybrids. These hybrids were more common (averaging 5.5%, ranging from 1 to 23% across sampled trees) than any hybrid combinations between the native species (mean for all native species is 0.4%) and *E. nitens* × *ovata* hybrids from *E. nitens* on the edge of the plantations (1.8%). The effect of protandry on the frequency of hybridisation was evident in reciprocal hybrid combinations as hybrids were invariably more frequent in the open-pollinated seedlots collected from the earlier flowering species [e.g. *E. ovata* (♀) × *nitens* >*E. nitens* (♀) × *ovata*; *E. ovata* (♀) × *viminalis* >*E. viminalis* (♀) × *ovata*; Table 4].

Distribution of exotic hybrids and E. nitens *wildlings in the wild*

The frequency distribution of the *E. ovata* × *nitens* hybrids identified in the wild indicated that the majority were found within 20 m of the plantation boundary, but their distribution extended up to 310 m (Fig. 3). In comparison, all *E. nitens* wildlings were found within 30 m of the plantation boundaries and most occurred within 10 m.

Table 3. PGD-1 genotype frequencies (%) in *Eucalyptus nitens*, *E. ovata*, *E. viminalis*, *E. rodwayi* and their putative F_1 hybrids from open-pollinated seedlots and plants identified in the wild

The open-pollinated data from Huntsman and Tasmania-wide are from Barbour *et al.* (2002). The majority of the putative *E. ovata* × *nitens* F_1 hybrids were intermediate between the two parental species, verifying their F_1 hybrid classification. Genotype 11 refers to PGD-1^1 PGD-1^1, genotype 13 refers to PGD-1^1 PGD-1^3 and so on. Underlined numbers indicate the most frequent genotype for each cross-type/location. The ♀ symbol indicates which species was the female in a particular cross-type

Cross-type/location	Number of plants	Genotype (increasingly cathodal to the right)								
		11	13	14	15	33	35	44	45	55
Pure species and E. nitens hybrids										
E. nitens										
Huntsman	107	87	13							
Lilydale, Nunamara & Huntsman	8	88	13							
Field plants	10	90	10							
E. ovata (♀) × *nitens*										
Huntsman	121		75		7	12	5			
Lilydale, Nunamara & Huntsman	24	17	63		4	17				
Group 1	9	11	67	22						
Group 2	12	25	58	17						
Field plants	16		75		19	6				
E. viminalis (♀) × *nitens*										
Lilydale, Nunamara & Huntsman	1				100					
E. ovata										
Tasmania-wide	121	1	10		1	64	21			4
Huntsman	122					64	27			9
Lilydale, Nunamara & Huntsman	8				13	75	13			
Field plants	15					80	20			
E. viminalis										
Tasmania-wide	198	2			8	5	4			82
Huntsman	65	2			8		5		3	83
Lilydale, Nunamara & Huntsman	8				25			13		63
Field plants	13	8								92
E. rodwayi										
Nunamara	25	4	20		12	8	16		8	32
Other hybrid combinations										
E. nitens (♀) × *ovata*	4		100							
E. nitens (♀) × *viminalis*	5						100			
E. ovata (♀) × *viminalis*	8					13	75			13
E. rodwayi (♀) × *ovata*	4					50	25			25

Discussion

Morphological and allozyme analysis has shown that F_1 hybridisation between plantation and native *Eucalyptus* is occurring (Barbour *et al.* 2002; present study) and the present study shows that these hybrids are establishing in the wild. Natural hybridisation between *E. ovata* and *E. viminalis* and between *E. rodwayi* and *E. ovata* has been previously recorded in the literature (Williams and Potts 1996). However, the present study reports the discovery of a new hybrid entity within the Tasmanian biota.

Eucalypt species are often highly differentiated in seedling morphology and the general intermediacy of their hybrids makes detection relatively easy (Pryor 1976; Potts and Reid 1988, 1990; Tibbits 1988; Delaporte *et al.* 2001; Stokoe *et al.* 2001; Barbour *et al.* 2002). Certainly, all the F_1 hybrid cross-types that were identified appeared to be generally intermediate on visual inspection, although the level of intermediacy varied between trait and hybrid combinations. In the morphometric analysis, both reciprocal crosses of *E. nitens* × *viminalis*, *E. ovata* × *rodwayi* and a large proportion of both reciprocal crosses of *E. nitens* × *ovata*, displayed generally intermediate positions between their parental species. However, exceptions were found within both reciprocal crosses of *E. nitens* × *ovata*, including a few of the hybrids from the wild and in the *E. ovata* × *viminalis* hybrids, both of which showed a strong bias towards *E. viminalis* and *E. nitens*. The reason for this mainly lay in the ontogenetic stage of development at which the seedlings were measured. Many of the hybrids were measured close to a transitional stage in their ontogeny, which was not the case for the parental species.

The 5.5% of exotic hybrids identified in the open-pollinated seedlots of *E. ovata* was comparable to the

Table 4. Frequency (%) of each cross-type found in open-pollinated seedlots of *Eucalyptus nitens*, *E. ovata*, *E. viminalis* and *E. rodwayi* at Huntsman, Lilydale and Nunamara

E. rodwayi was only sampled at Nunamara. Classification of the seedlings was conducted by using clear morphological characters, then verified by morphometric and allozyme analyses. The number of families of each species found to produce hybrids with other native species (—, not applicable for *E. nitens*) and between the exotic *E. nitens* and the native species is shown (four families were grown per species at each site). Interspecific F_1 hybrids arising from *E. nitens* pollen fertilising *E. ovata* were the most frequently recorded hybrid cross-type at each site (**bold**). Intraspecific crosses are underlined. The female species are in expected order of flowering time (top to bottom), so that the influence of protandry can be seen on the proportions of hybrids in these reciprocally crossing species (Williams and Potts 1996; Barbour *et al.* 2002)

Seed (♀) parent/site	Pollen (♂) parent				No. of families with hybrids		Number of seedlings
	E. ovata	*E. nitens*	*E. viminalis*	*E. rodwayi*	Native	Exotic	
E. ovata							
Huntsman	95.6	**4.1**	0.3		2	4	1017
Lilydale	82.3	**16.3**	1.4		3	4	1337
Nunamara	97.3	**2.7**	0.0	0.0	1	4	4701
E. nitens							
Huntsman	0.7	99.3	0.0		—	1	682
Lilydale	0.0	98.4	1.6		—	1	310
Nunamara	3.2	96.8	0.0	0.0	—	3	917
E. viminalis							
Huntsman	0.0	0.0	100.0		0	0	471
Lilydale	0.0	0.1	99.9		0	1	1168
Nunamara	0.0	0.0	100.0	0.0	0	0	883
E. rodwayi							
Nunamara	0.9	0.0	0.0	99.1	1	0	446
Total							
E. ovata	94.2	**5.5**	0.3	0.0	6	12	7055
E. nitens	1.8	98.0	0.3	0.0	0	5	1909
E. viminalis	0.0	0.0	100.0	0.0	0	1	2522

4.2% reported by Barbour *et al.* (2002) where *E. ovata* trees had been sampled within 300 m of an *E. nitens* family trial in which the flowering of some trees had been artificially enhanced. In both cases, all sampled *E. ovata* trees produced *E. ovata* × *nitens* hybrids. Also, the levels of exotic hybridisation were considerably greater than the levels of natural hybridisation seen among the native species in the present study (0.4%) and those reported in the literature (averaging 1.3% across 11 species; Potts and Wiltshire 1997). However, both studies may have biased upward the frequency of hybridisation with *E. nitens*. In the present case, sampling was focused on trees that were in close proximity to exotic wild hybrids and, in the previous case, a plantation from which rare hybrids had been detected in open-pollinated seed of *E. nitens*. The degree of flowering-time overlap appears to be a major determinant of whether a species or population will be involved in hybridisation (Pryor 1976; Barbour *et al.* 2002).

Several lines of evidence argue that most of the exotic hybrids found in the wild are the product of *E. nitens* pollen dispersing into the range of native *E. ovata*, rather than from dispersal by seed of the reciprocal cross from *E. nitens* females that have been pollinated by *E. ovata*. First, the exotic hybrids were in close proximity to mature *E. ovata* trees that were shown to have the same hybrid cross-type in their open-pollinated seed (5.5%) and from which hybrid seed could have easily dispersed. Second, the level of hybridisation in open-pollinated seed of *E. nitens* was lower than in *E. ovata* (1.8% in the present study; 0.15% in the study by Barbour *et al.* 2002). Third, all the *E. ovata* × *nitens* hybrids identified in the wild were found regenerating next to pure *E. ovata* seedlings, rather than *E. nitens* seedlings. Finally, the hybrids extend well beyond the distance over which *E. nitens* seed dispersal occurs, as judged by the distribution of wildlings. The later result is consistent with the limited dispersal of eucalypt seed, most of which is dispersed within two tree heights (Cremer 1966; Potts and Reid 1988).

Eucalypt pollen is animal-dispersed and is thought to be highly leptokurtic in its dispersal from a source (Potts and Wiltshire 1997). The majority of the exotic hybrids were seen within 20 m of the plantations, but a long tail in their frequency extended through to 310 m. In the previous study of hybridisation between plantation *E. nitens* and native *E. ovata*, relatively high levels of hybridisation were seen in open-pollinated seedlots from trees within 200 m from a plantation (16% for one tree), while by 300 m hybridisation had dropped to 0.4% (Barbour *et al.* 2002). Similar results, albeit limited, have also been found in studies of natural hybridisation (Potts 1990; Potts and Wiltshire 1997).

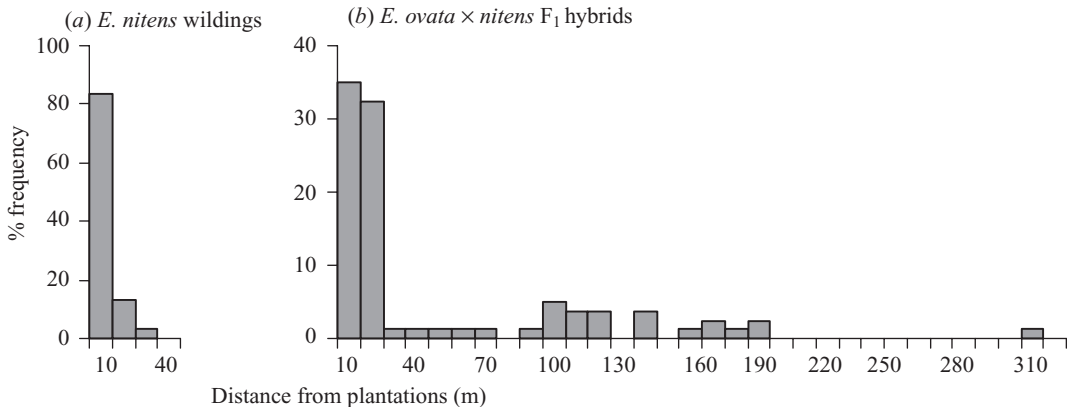

Fig. 3. Frequency distribution of (*a*) *Eucalyptus nitens* wildings (n = 61) and (*b*) established *E. ovata* × *nitens* F_1 hybrids (n = 77) against distance from the boundary of *E. nitens* plantations.

Pollinators of *E. nitens* in Tasmania are small insects, with birds and larger insects such as bees and bumble bees not being active pollinators of this species (Hingston *et al.* 2002). Consequently, the pollen dispersal curve for *E. nitens* is likely to be more restricted than that for species that have larger insects and birds dispersing their pollen.

There has been a recent expansion of the eucalypt plantation estate in Tasmania, which suggests exotic hybrids may become more common. Approximately 3300 ha of *Eucalyptus* plantations were established by 1984, whereas by 2000 the total was nearly 110000 ha, with *E. nitens* constituting 70–80% of the estate (Wood *et al.* 2001). In Tasmania, *E. ovata* and *E. viminalis* frequently occur adjacent to *E. nitens* plantations (R. Barbour, unpubl. data). We have found exotic hybrids at three such sites and further survey is required to determine whether more of these sites exist. The *E. ovata* × *nitens* hybrid seedlings were established next to plantations that were 10–14 years old. While flowering can occur in *E. nitens* plantations as early as at the age of 5 years (G. Dutkowski and D. Williams, unpubl. data; Moncur *et al.* 1994), flowering in dense plantations is usually reduced from that with open-grown trees (Williams 2000). The extent to which the expanded plantation estate will increase the level of exotic hybridisation will therefore depend on numerous factors, including harvest age, stocking density and overlap in flowering time with adjacent compatible species (Potts *et al.* 2003).

The biological impact of gene flow from *Eucalyptus* plantations may be both ecological and genetic. The competitive effects of exotic pollen may reduce intra-specific crossing rates and the regenerative strength of a species (Brown and Mitchell 2001; Song *et al.* 2002). Even without considering the genetic effects of backcrossing, hybrids may directly out-compete parental species (Arnold *et al.* 2001) and even cause community-level changes to biodiversity (Whitham *et al.* 1999). Such considerations are most relevant to the conservation of threatened species (Levin *et al.* 1996).

It has been suggested that hybrids are most successful in novel or disturbed habitats (Anderson 1948; Arnold 1997) and therefore may not be competitive in pure-species habitats (Pryor 1953). The habitat in which the exotic hybrids were found was, however, the same disturbed habitat in which pure *E. ovata* was naturally regenerating. Most eucalypts rely on disturbance for natural regeneration (Ashton 2000). As the exotic hybrids were found regenerating among pure *E. ovata* seedlings and rarely on their own, they clearly were able to colonise the habitat of native *E. ovata*, at least up to this early stage of their life cycle.

One of the most important areas of research now lies in studying the fitness of exotic hybrids and their ability to reach reproductive maturity and backcross with native populations (Arnold 1997). This is relevant in the present case as many plant communities that *E. ovata* dominates have a high conservation priority in Tasmania (Comprehensive, Adequate and Representative reserve system Scientific Advisory Group, unpubl. data) mainly because of agricultural clearing, although *E. ovata* itself is not a rare or endangered taxon. Research has shown that both first- and advanced-generation hybrid breakdowns are common in *Eucalyptus* (Potts and Dungey 2003; Potts *et al.* 2003) and can be effective barriers to inter-specific gene flow (Potts and Wiltshire 1997). For example, F_1 hybrids between *E. ovata* and *E. globulus*, a species closely related to *E. nitens*, showed poor survival by flowering age, compared with the pure species (Lopez *et al.* 2000*b*). Furthermore, while these two species overlap in their flowering time, the flowering of their F_1 hybrid does not overlap with either species (Lopez *et al.* 2000*a*). The *E. ovata* × *nitens* hybrid seedlings, still at least 5–10 years from reproductive maturity, may well behave in a similar manner and strongly reduce the potential for backcrossing to *E. ovata*.

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References

Anderson E (1948) Hybridization of the habitat. *Evolution* **2**, 1–9.

Anttila CK, Daehler CC, Rank NE, Strong DR (1998) Greater male fitness of a rare invader (*Spartina Alterniflora*, Poaceae) threatens a common native (*Spartina foliosa*) with hybridization. *American Journal of Botany* **85**, 1597–1601.

Arnold ML (1992) Natural hybridisation as an evolutionary process. *Annual Review of Ecology and Systematics* **23**, 237–261.

Arnold ML (1997) 'Natural hybridization and evolution.' (Oxford University Press: Oxford, UK)

Arnold ML, Kentner EK, Johnston JA, Cornman S, Bouck AC (2001) Natural hybridisation and fitness. *Taxon* **50**, 93–104.

Ashton DH (2000) Ecology of eucalypt regeneration. In 'Disease and pathogens of eucalypts'. (Eds PJ Keane, GA Kile, FD Podger, BN Brown) pp. 47–61. (CSIRO Publishing: Collingwood, Vic.)

Barbour RC, Potts BM, Vaillancourt RE, Tibbits WN, Wiltshire RJE (2002) Gene flow between introduced and native *Eucalyptus* species. *New Forests* **23**, 177–191.

Bateman AJ (1947) Contamination of seed crops. 1. Insect pollination. *Journal of Genetics* **48**, 257–275.

Brooker MIH (2000) A new classification of the genus *Eucalyptus* L'Her. (Myrtaceae). *Australian Systematic Botany* **13**, 79–148.

Brown BJ, Mitchell RJ (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. *Oecologia* **129**, 43–49.

Butler D (1994) Bid to protect wolves from genetic pollution. *Nature* **370**, 497.

Cauvin B, Potts BM, Potts WC (1987) *Eucalyptus*: hybridation artificielle—barrieres et hérédité des caracteres. In 'Annales de recherches sylvicoles'. pp. 255–303. (Association Forêt-Cellulose: Paris)

Commonwealth of Australia (1998) 'A framework of regional (subnational) level criteria and indicators of sustainable forest management in Australia.' (Department of Agriculture, Fisheries and Forestry: Canberra, Australia)

Cremer KW (1966) Dissemination of seed from *Eucalyptus regnans*. *Australian Forestry* **30**, 33–37.

Delaporte KL, Conran JG, Sedgley M (2001) Interspecific hybridization within *Eucalyptus* (Myrtaceae): subgenus *Symphyomyrtus*, sections *Bisectae* and *Adnataria*. *International Journal of Plant Sciences* **162**, 1317–1326.

Ellstrand NC, Hoffman CA (1990) Hybridisation as an avenue of escape for engineered genes. *Bioscience* **40**, 438–442.

Griffin AR, Burgess IP, Wolf L (1988) Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'Herit.—a review. *Australian Journal of Botany* **36**, 41–66.

Hingston AB (2002) Pollination ecology of *Eucalyptus globulus* Labill. ssp. *globulus* and *E. nitens* (Deane & Marden) Marden (Myrtaceae). PhD Thesis, University of Tasmania.

Levin DA, Francisco-ortega J, Jansen RK (1996) Hybridization and the extinction of rare plant species. *Conservation Biology* **10**, 10–16.

Lopez GA, Potts BM, Gore PL (2000*a*) The inheritance of flowering time in interspecific F_1 hybrids of *Eucalyptus*. In 'Hybrid breeding and genetics of forest trees. Proceedings of QFRI/CRC-SPF symposium, 9–14 April 2000, Noosa, Queensland, Australia'. (Eds HS Dungey, MJ Dieters, DG Nikles) pp. 453–456. (Queensland Department of Primary Industries: Brisbane)

Lopez GA, Potts BM, Tilyard PA (2000*b*) F_1 hybrid inviability in *Eucalyptus*: The case of *E. ovata* × *E. globulus*. *Heredity* **85**, 242–250.

Mejnartowicz L (1996) Cisovka—The relic population of *Abies alba* and its relationship to man-made silver-fir stands in Bialowieza primeval forest. *Acta Societatis Botanicorum Poloniae* **65**, 319–328.

Moncur MW, Hand FC, Ramsden NG (1994) Environmental and cultural effects on flowering and seed production of plantation grown *Eucalyptus nitens*. Report for the Tasmanian Forest Research Council, Inc. Division of Forestry, CSIRO, Canberra.

Moran GF, Bell JC (1983) *Eucalyptus*. In 'Isozymes in plant genetics and breeding'. (Eds SD Tanksley, TJ Orton) pp. 423–441. (Elsevier: Amsterdam)

Pederick LA (1979) Natural variation in shining gum (*Eucalyptus nitens*). *Australian Forest Research* **9**, 41–63.

Potts BM (1990) The response of eucalypt populations to a changing environment. *Tasforests* **2**, 179–193.

Potts BM, Dungey HD (2003) Hybridisation of *Eucalyptus*: key issues for breeders and geneticists. *New Forests*, in press.

Potts BM, Reid JB (1988) Hybridisation as a dispersal mechanism. *Evolution* **42**, 1245–1255.

Potts BM, Reid JB (1990) The evolutionary significance of hybridisation in *Eucalyptus*. *Evolution* **44**, 2151–2152.

Potts BM, Wiltshire RJE (1997) Eucalypt genetics and genecology. In 'Eucalypt ecology: individuals to ecosystems'. (Eds J Williams, J Woinarski) pp. 56–91. (Cambridge University Press: Cambridge)

Potts BM, Barbour RC, Hingston AB, Vaillancourt RE (2003) Turner review: genetic pollution of native eucalypt gene pools—identifying the risks. *Australian Journal of Botany* **51**, 1–25.

Pryor LD (1953) Genetic control in *Eucalyptus* distribution. *Proceedings of the Linnean Society of New South Wales* **78**, 8–18.

Pryor LD (1976) 'Biology of eucalypts.' (Edward Arnold: London)

Raybould AF, Gray AJ (1994) Will hybrids of genetically modified crops invade natural communities? *Trends in Ecology and Evolution* **9**, 85–89.

Rhymer JM, Simberloff D (1996) Extinction by hybridisation and introgression. *Annual Review of Ecology and Systematics* **27**, 83–109.

Rieger MA, Lamond M, Preston C, Powles SB, Roush RT (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* **296**, 2386–2388.

Small E (1984) Hybridisation in the domesticated-weed–wild complex. In 'Plant biosystematics'. (Ed. WF Grant) pp. 195–210. (Academic Press: San Diego, CA)

Song ZP, Lug BR, Zhu YG, Chen JK (2002) Pollen competition between cultivated and wild rice species (*Oryza sativa* and *O. rufipogon*). *New Phytologist* **153**, 289–296.

Stokoe RL, Shepherd M, Lee DJ, Nikles DG, Henry RJ (2001) Natural inter-subgeneric hybridization between *Eucalyptus acmenoides* Schauer and *Eucalyptus cloeziana* F.Muell (Myrtaceae) in southeast Queensland. *Annals of Botany* **88**, 563–570.

Strauss SY (2001) Benefits and risks of biotic exchange between *Eucalyptus* plantations and native Australian forests. *Austral Ecology* **26**, 447–457.

Tibbits WN (1986) Eucalypt plantations in Tasmania. *Australian Forestry* **49**, 219–225.

Tibbits WN (1988) Germination and morphology of progeny from controlled pollinations of *Eucalyptus nitens* (Deane & Maiden) Maiden. *Australian Journal of Botany* **36**, 677–691.

Tibbits WN (1989) Controlled pollination studies with shining gum (*Eucalyptus nitens* (Deane and Maiden) Maiden). *Forestry* **62**, 111–126.

Van Raamsdonk LWD, Van Der Maesen LGG (1996) Crop-weed complexes: the complex relationship between crop plants and their wild relatives. *Acta Botanica Neerlandica* **45**, 135–155.

Wendel JF, Weeden NF (1989) Visualisation and interpretation of plant isozymes. In 'Isozymes in plant biology'. (Eds DE Soltis, PA Soltis) pp. 5–45. (Chapman and Hall: London)

Whitham TG, Martinsen GD, Floate KD, Dungey HS, Potts BM, Keim P (1999) Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. *Ecology* **80**, 416–428.

Williams DR (2000) Flowering and seed production in *Eucalyptus nitens*. PhD Thesis, University of Tasmania.

Williams K, Potts BM (1996) The natural distribution of *Eucalyptus* species in Tasmania. *Tasforests* **8**, 39–164.

Wood MS, Stephens NC, Allison BK, Howell CI (2001) 'Plantations of Australia—a report from the National Plantation Inventory and the National Farm Forest Inventory (abridged version).' Bureau of Rural Sciences, Canberra.

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