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Inheritance of freezing resistance in interspecific F_1 hybrids of *Eucalyptus*

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Summary. The inheritance of freezing resistance in interspecific F_1 hybrid families of *Eucalyptus* encompassing 27 different species combinations and a range of levels of hardiness was examined. Freezing resistance was assessed by determining the temperatures required to cause either 30% (T30), 40% (T40), or 50% (T50) leakage of electrolytes from excised leaf discs subjected to artificial freezing. Highly significant variation in freezing resistance occurred between species; the maximum difference between parents in any specific combination was over 9 °C (*E. gunnii* × *E. globulus*). Freezing resistance was inherited in a predominantly additive manner in interspecific hybrids, although there was a tendency towards partial dominance toward the more sensitive species in some combinations (e.g., *E. nitens* × *E. globulus, E. nitens* × *E. camaldulensis, E. gunnii* × *E. globulus*). The full expression of this genetic variation appeared to increase with hardiness and in some cases appeared to vary with ontogeny. Estimates of individual narrow-sense heritability of freezing resistance for pure *E. nitens* families were $h^2 = 0.66 \pm 0.44$ and 0.46 ± 0.44. Across all species combinations examined, the heritability of F_1 family means estimated from midparent regression was $h^2 = 0.76 \pm 0.06$ and $h^2 = 0.89 \pm 0.06$ for T40 and T50 values, respectively. The advantage of using selected parents for interspecific hybridization is demonstrated and the implications of these results for breeding for freezing resistance in *Eucalyptus* are discussed.

Key words: Inheritance – Freezing/frost resistance – Interspecific hybrids – *Eucalyptus*

Introduction

Improvement of freezing resistance in *Eucalyptus* is important in many parts of the northern hemisphere where the winter minimum temperatures are near the limit for its growth (Pryor 1957a; Potts and Potts 1986; Cauvin 1988; Reddy and Rockwood 1989). It is also important in some temperate regions of Australia where there is interest in extending the environmental range of fast-growing but freezing-sensitive species such as *E. globulus* and *E. regnans* (Griffin et al. 1982). Pryor (1957a) considered that four lines of research should be explored in breeding eucalypts for freezing resistance, viz., assessing (1) species, (2) provenances and families within species, (3) natural hybrids, and (4) manipulated hybrids. While wide variation in freezing resistance has been demonstrated at the interspecific (e.g., Davidson and Reid 1985) and intraspecific (e.g., Griffin et al. 1982; Tibbits and Reid 1987a; Cauvin 1988) levels in the genus, the use of hybridization as a means of breeding more resistant strains of eucalypts has received little attention in Australia. However, this strategy has been investigated in some northern hemisphere countries. As early as 1934, attempts were made in Russia to produce freezing-resistant, yet productive, forms of *Eucalyptus* through artificial hybridization (Pilipenka 1969). Although some data were obtained on the relative freezing resistance of the numerous hybrids produced, most of this valuable genetic material was lost due to severe winters. More recently, workers in France have concentrated on interspecific hybridization between fast-growing species (e.g., *E. globulus, E. nitens, E. dalrympleana,* and *E. macarthurii*) and the highly resistant species *E. gunnii* (Cauvin et al. 1987; Potts et al. 1987).

Despite the long-standing interest in the evolutionary (Pryor 1976) and applied (Pryor 1957a) role of eucalypt hybrids, relatively little quantitative data exists on the inheritance of freezing resistance in hybrids of *Eucalyptus*. The present article uses the electroconductivity method (Raymond et al. 1986; Hallam and Tibbits 1988) to detail the inheritance of freezing resistance in hybrid families encompassing 27 different species combinations and a range of levels of hardiness.

Materials and methods

Hybrid seedlings and parental controls from several pollination programs (e.g., Tibbits 1988; Potts and Savva 1989) were used in this study. Open-pollinated, intraspecific outcrossed, or self-pollinated seed collected from the parents of the hybrids were used as controls. The major series of artificial freezing experiments undertaken are listed in Table 1. Freezing resistance was assessed from the leakage of cellular electrolytes from leaf discs that had been subjected to a range of subzero temperatures in an air-filled chamber or an ethylene-glycol bath (Raymond et al. 1986; Hallam and Tibbits 1988). One or two replicate discs (8 or 12 mm) were cut from leaves for each of the temperatures tested and, depending on the experiment, the number of replications per plant ranged from one to four per temperature (see Table 1). Following Hallam and Tibbits (1988), the degree of cell damage at each temperature was estimated using the relative conductivity index, ct/ck, where ct is the electrolyte conductivity recorded 24 h after exposure to a given temperature and ck is the conductivity of the same sample following complete tissue death after heating the sample to ca. 70 °C.

Freezing resistance was compared on the basis of the temperature required to cause 30, 40, or 50% loss of cellular electrolytes. Previous studies have indicated that leaf death commences at relative conductivity indices of 30% and is usually complete by 50% (Tibbits and Reid 1987b). For experiments 1 and 2 the temperature causing 50% loss of cellular electrolytes (T50) was estimated from linear interpolation as described by Tibbits and Reid (1987b). In all other experiments, linear regressions of the probit-transformed ct/ck indices on frost temperature were fitted for each individual (averaged over replicates) using the REG procedure of SAS (SAS Institute 1987). The temperature causing a given percentage loss of electrolytes was then predicted from the regression equation obtained for each plant. Some families were not killed by even the lowest frost temperature and, to avoid extrapolating beyond the range of electrolyte leakage observed, T30 or only T40 values are compared in some cases. The significance of the difference in T30–T50 values was tested using the general linear models procedure of SAS (SAS Institute 1987). The least-squares means and their standard errors were estimated for each family in the same procedure, and specific a priori comparisons were tested. The deviation of each F_1 hybrid family from the corresponding midparent value was tested using a two-tailed *t*-test and, where parental progenies differed significantly, the degree of dominance (*d*) of freezing resistance in the F_1 hybrid was calculated as

$$d = (F_1 - mp)/(P2 - mp),$$

where mp is the midparent value and P2 is the value of the most resistant parent such that –1 or +1 corresponds to complete dominance toward the least or most resistant parent, respectively, and 0 represents no dominance. To plot the response curves of relative conductivity against frost temperature, family least-squares means were estimated at each temperature and compared using the GLM procedure of SAS. These analyses were based on angular transformed ct/ck indices and means were back-transformed to plot response curves.

Experiments 1 and 2

Assessments of freezing resistance were made after seedlings had been hardened for 4 and 9 weeks in a growth cabinet at 18 °C day/2 °C night, 8-h photoperiod (ca. 300 μmol m^{-2} s^{-1}). Families were arranged in single-seedling randomized blocks within the growth cabinets, with the position of the blocks and seedlings within blocks rerandomized at regular intervals. Usually, six healthy seedlings (taller than 20 cm) were compared per family and a single disc from each seedling was used at each temperature. The individual narrow-sense heritability of T50 values and the genetic and phenotypic correlations between T50 values after 4 and 9 weeks hardening were calculated for the six *E. nitens* families, using the least-squares program written by Harvey (1988). Five of the *E. nitens* families were self-pollinated full sib and one was open-pollinated, but this was isolated from other *E. nitens* and was probably largely self-pollinated. In both analyses the coefficient of relatedness used was 0.5.

Experiments 3 and 4

Seedlings were planted in November 1986 in a 1.5 ha field trial on a level site located near West Ridgley in northwest Tasmania. Hybrids and parental controls were planted in one-half of the trial separate from the *E. nitens* controls to avoid competitive effects. Each half of the trial contained twelve 6 × 6 randomized blocks, in which families were arranged in single-tree plots at 4 × 4 m spacing.

Experiments 5 and 6

Seedlings were grown in paper or plastic pots in the container nursery of APPM Forests at Ridgley, Tasmania. From February onwards they were kept on raised beds and exposed to natural conditions, except that shade-cloth was lowered over seedlings each night. Seedlings were kept in family groups, with group positions randomized on two occasions.

Experiments 7, 8, and 9

Seedlings were planted in September 1988 in two adjoining field trials located near West Ridgley, northwest Tasmania. Trial 1 (Experiment 7) comprised eight replicates with families arranged in five-tree linear plots. Trial 2 (Experiments 8 and 9) comprised five replicates with families arranged in three-tree linear plots. In each trial, trees were at 3 × 3 m spacing with plots across rows, and within each replicate, families were grouped into blocks of major hybrids to avoid competition between fast- and slow-growing hybrids. Within each block families were randomized, and both replicates and blocks were surrounded by edge rows where possible. From each family studied, individuals were sampled as evenly as possible across replicates, and the replicate effect was removed in analyses of both the T30–T50 values and the relative conductivity index. Usually 10–12 trees per family were sampled except where indicated in Table 1. Ten unselected individuals of the species *E. nitens* were also sampled from within the trials and included in the frosting experiment for comparison with the *E. gunnii* × *E. globulus* hybrids.

Results

Effect of hardening on F_1 hybrids and parental progenies (Experiment 1)

Four weeks after hardening in a controlled environment, significant ($P < 0.05$) differences between the control progenies of *E. nitens* and control progenies of the pollen species were only found in the case of *E. cordata* and *E. johnstonii* (Table 2). The mean of their F_1 hybrids tended

Table 1. Details of artificial freezing experiments for assessing hybrids. The temperatures used to assess freezing resistance of the excised leaf discs are indicated and the figures in parentheses following hybrid combinations are the number of full-sib hybrid families and total number of hybrid progeny, respectively.

Age	Hardening conditions	Temperatures (°C)	Hybrid assessed
Experiment 1a (potted seedlings, one replication/plant)			
6 months	4 weeks	−4.0, −5.0, −6.0	*E. nitens* × *E. cordata* (1,6) *E. nitens* × *E. globulus* (1,6) *E. nitens* × *E. johnstonii* (1,6) *E. nitens* × *E. morrisbyi* (1,6) *E. nitens* × *E. viminalis* (1,6)
Experiment 1b			
7 months	9 weeks	−4.0, −5.0, −6.0, −7.0, −8.0	as above
Experiment 2a (potted seedlings, one replication/plant)			
6 months	4 weeks	−4.0, −5.0, −6.0, −7.0	*E. nitens* × *E. gunnii* (6,36)
Experiment 2b			
7 months	9 weeks	−5.2, −6.2, −7.3, −8.3, −9.6	*E. nitens* × *E. gunnii* (6,36)
Experiment 3 (field trial, one replication/plant)			
	natural		
12 months	Feb. 1987	−2.7, −4.1, −5.5	*E. nitens* × *E. globulus* (1,8)
14 months	Apr. 1987	−4.3, −5.9, −7.3	*E. nitens* × *E. gunnii* (1,8)
16 months	Jun. 1987	−4.2, −5.4, −6.6, −8.1, −9.4	
18 months	Aug. 1987	−4.9, −6.4, −7.9, −9.4, −10.8	
20 months	Oct. 1987	−2.9, −4.3, −5.8, −7.0	
Experiment 4 (field trial, one replication/plant)			
	natural		
17 months	Jul. 1987	−4.9, −6.0, −7.0, −7.9	*E. nitens* × *E. globulus* (8,28)
Experiment 5a (potted seedlings, two leaves/plant, one repl./leaf)			
6 months	natural Jul. 4, 1989	−5.0, −7.5, −10.0	*E. nitens* × *E. botryoides* (1,5) *E. nitens* × *E. camaldulensis* (1,5) *E. nitens* × *E. dunnii* (1,3) *E. nitens* × *E. saligna* (1,1) *E. nitens* × *E. rudis* (1,5)
Experiment 5b			
7 months	natural Aug. 1, 1989	−4, −6, −8, −10, −12, −14	*E. nitens* × *E. neglecta* (3,19) *E. nitens* × *E. parvifolia* (4,18) *E. nitens* × *E. perriniana* (3,13) *E. nitens* × *E. rubida* (2,16)
Experiment 6 (field trial, two leaves/plant, two repl./leaf)			
	natural		
18 months	Sep. 4, 1989	−5, −6.5, −8.0, −9.5, −11, −12.5	*E. nitens* × *E. pulverulenta* (3,10) *E. nitens* × *E. ovata* (1,5) *E. nitens* × *E. rodwayi* (1,5)
Experiment 7 (field trial, two leaves/plant, two repl./leaf)			
18 months	natural		
	Aug. 11, 1989	−4.5, −6.0, −7.5, −9.0, −10.5, −12.0	*E. nitens* × *E. camaldulensis* (2,18)
Experiment 8 (field trial, two leaves/plant, one repl./leaf)			
9 months	natural		
	July 5–6, 1989	−6, −7.1, −8.2, −9.3, −10.4, −11.5, −14, −16	*E. gunnii* × *E. globulus* (2,22) *E. globulus* × *E. gunnii* (2,4)
Experiment 9 (field trial, two leaves/plant, one repl./leaf)			
12 months	natural		
	Sep. 7, 1989	−2, −4, −6, −8, −10, −12, −14, −16, −18	*E. morrisbyi* × *E. urnigera* (1,10) *E. morrisbyi* × *E. gunnii* (1,10) *E. gunnii* × *E. urnigera* (1,4) *E. globulus* × *E. urnigera* (2,7) *E. ovata* × *E. urnigera* (1,10) *E. ovata* × *E. gunnii* (1,1) *E. ovata* × *E. perriniana* (1,1)

Table 2. Mean T30, T40, or T50 temperatures for parental control progenies and their F_1 hybrid. The significance of the difference between parental progenies and the difference of the F_1 hybrid mean from the midparent value (mp) is indicated. Where the parental progenies differ significantly in their freezing resistance, the degree of dominance of the freezing resistance of the F_1 hybrid has been calculated (0 = no dominance, −1 or +1 = complete dominance toward the least and most freezing-resistant parent, respectively).

Expt.	Cross (female × male)		T value	Female parent	F_1	Pollen parent	Difference between		Degreee of dominance
							parents	F_1 vs mp	
1a	*E. nitens* × *E. cordata*		T50	−4.8	−4.9	−3.7	**	NS	1.18
	E. nitens × *E. johnstonii*		T50	−4.8	−3.8	−3.8	*	NS	−1.00
	E. nitens × *E. viminialis*		T50	−4.8	−4.6	−4.7	NS	NS	–
	E. nitens × *E. morrisbyi*		T50	−4.8	−4.4	−4.9	NS	NS	–
	E. nitens × *E. globulus*		T50	−5.1	−5.4	−5.4	NS	NS	–
1b	*E. nitens* × *E. cordata*		T50	−6.2	−6.2	−5.0	*	NS	1.17
	E. nitens × *E. johnstonii*		T50	−6.2	−5.8	−5.3	NS	NS	–
	E. nitens × *E. viminialis*		T50	−6.2	−5.8	−6.1	NS	NS	–
	E. nitens × *E. morrisbyi*		T50	−6.7	−5.7	−5.6	**	NS	−0.82
	E. nitens × *E. globulus*		T50	−7.9	−6.8	−6.8	*	NS	−1.00
2a	*E. nitens* × *E. gunnii*	(119 × 312)	T50	−5.6	−5.7	−7.1	***	NS	−0.87
		(121 × 312)	T50	−5.5	−6.6	−7.1	***	NS	−0.38
		(127 × 312)	T50	−5.3	−7.4	−7.1	***	*	1.33
		(123 × 312)	T50	−5.1	−6.6	−7.1	***	NS	0.50
		(128 × 312)	T50	−6.2	−6.2	−7.1	*	NS	−1.00
		(130 × 312)	T50	−5.3	−6.8	−7.1	***	NS	0.67
2b	*E. nitens* × *E. gunnii*	(119 × 312)	T50	−6.9	−7.6	−9.9	***	NS	−0.53
		(121 × 312)	T50	−6.5	−8.7	−9.9	***	NS	0.29
		(127 × 312)	T50	−6.1	−9.3	−9.9	***	**	0.68
		(123 × 312)	T50	−6.4	−8.4	−9.9	***	NS	0.14
		(128 × 312)	T50	−7.1	−8.3	−9.9	***	NS	−0.14
		(130 × 312)	T50	−6.4	−8.6	−9.9	***	NS	0.26
3a	*E. nitens* × *E. globulus*	February	T50	−4.2	−3.9	−3.3	**	NS	0.33
	(123 × 281)	April	T50	−4.9	−4.1	−4.5	NS	NS	–
		June	T50	−5.3	−4.5	−4.3	*	*	−0.60
		August	T50	−7.2	−5.9	−5.6	*	**	−0.62
		October	T50	−4.0	−4.3	−3.8	NS	NS	–
3b	*E. nitens* × *E. gunnii*	February	T50	−4.2	−4.1	−5.3	**	***	−1.18
	(123 × 312)	April	T50	−4.9	−4.9	−6.6	**	***	−1.00
		June	T50	−5.3	−6.3	−6.8	**	NS	0.33
		August	T50	−7.2	−7.6	−9.6	**	***	−0.67
		October	T50	−4.0	−4.5	−4.5	NS	NS	–
4	*E. nitens* × *E. globulus*	(122 × 281)	T50	−10.1	−6.3	−5.8	**	NS	−0.77
		(123 × 281)	T50	−7.4	−6.3	−5.8	NS	NS	–
		(128 × 281)	T50	−8.9	−7.4	−5.8	*	NS	0.03
		(127 × 281)	T50	−6.8	−5.4	−5.8	NS	NS	–
		(121 × 282)	T50	−6.5	−5.1	−4.3	NS	NS	–
		(123 × 282)	T50	−7.4	−5.9	−4.3	*	NS	−0.03
		(130 × 282)	T50	−6.9	−4.4	−4.3	*	NS	−0.92
		(133 × 282)	T50	−7.9	−5.9	−4.3	**	NS	0.11
5a	*E. nitens* × *E. botryoides*		T40	−11.1	−8.8	−7.8	NS	NS	–
	E. nitens × *E. camaldulensis*	(375 × CAM1)	T40	−11.1	−10.0	−8.6	NS	NS	–
	E. nitens × *E. dunnii*		T40	−11.1	−7.9	−7.6	*	NS	−0.83
	E. nitens × *E. rudis*		T40	−11.4	−7.2	–	–	–	–
	E. nitens × *E. saligna*		T40	−11.1	−8.0	−7.0	*	–	−0.51
5b	*E. nitens* × *E. rubida*	(536 × RUB)	T40	−6.4	−7.9	−8.0	NS	NS	–
		(375 × RUB)	T40	−7.5	−9.1	−8.0	NS	NS	–
	E. nitens × *E. neglecta*	(527 × NEG1)	T40	−7.6	−8.6	−9.9	NS	NS	–
		(375 × NEG1)	T40	−7.5	−9.0	−9.0	NS	NS	–
	E. nitens × *E. parvifolia*	(375 × PAR1)	T50	−8.8	−10.6	−13.4	*	NS	−0.22
		(527 × PAR1)	T50	−8.9	−11.1	−13.4	*	NS	−0.02
		(536 × PAR1)	T50	−7.7	−10.5	−13.4	*	NS	−0.02
6	*E. nitens* × *E. ovata*		T50	−6.3	−6.8	−6.7	NS	NS	–
	E. nitens × *E. rodwayi*		T50	−7.4	−7.2	−7.7	NS	NS	–
	E. nitens × *E. pulverulenta*	(121 × PUL2)	T50	–	−7.6	−7.3	–	–	–
	E. nitens × *E. pulverulenta*	(123 × PUL1)	T50	−7.8	−6.9	−7.3	NS	NS	–
	E. nitens × *E. pulverulenta*	(121 × PUL1)	T50	−6.3	−7.5	−7.3	NS	NS	–

Table 2 (continued)

Expt.	Cross (female × male)		T value	Female parent	F_1	Pollen parent	Difference between		Degreee of dominance
							parents	F_1 vs mp	
7	*E. nitens* × *E. camaldulensis*	(375 × 351)	T40	−11.5	−7.6	−7.2	**	*	−0.81
		(374 × 351)	T40	−14.1	−8.6	−7.2	***	***	−0.59
8	*E. gunnii* × *E. globulus*	(GUN16 × GLOB5)	T40	−17.5	−10.0	−5.2	***	NS	−0.22
		(GUN15 × GLOB23)	T40	−19.9	−8.8	−5.4	***	*	−0.53
		(GLOB23 × GUN7)	T30	−4.0	−8.3	−18.9	***	–	−0.42
		(GLOB24 × GUN7)	T30	−4.4	−10.0	−18.9	***	NS	−0.23
9	*E. morrisbyi* × *E. urnigera*	(MOR2 × URN4)	T50	−7.2	−6.1	−4.3	**	NS	0.25
	E. morrisbyi × *E. gunnnii*	(MOR2 × GUN7)	T50	−7.2	−8.8	−14.1	***	*	−0.53
	E. ovata × *E. urnigera*	(OV11 × URN4)	T50	−4.5	−4.4	−4.3	NS	NS	–
	E. globulus × *E. urnigera*	(GLOB4 × URN4)	T50	−5.6	−4.1	−4.3	NS	NS	–
	E. gunnii × *E. urnigera*	(GUN15 × URN4)	T50	−9.0	−6.3	−4.3	***	NS	−0.13

NS $P<0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

toward the most (*E. nitens*) and least (*E. johnstonii*) freezing-resistant parent, respectively, but did not differ significantly from the midparent value. Five weeks later, the overall level of hardiness had increased by 1.8 °C and the pattern of significance of the difference between the parental progenies had changed. *E. globulus, E. morrisbyi,* and *E. cordata* were all significantly ($P<0.05$) less resistant to freezing than *E. nitens*. The dominance of *E. nitens* × *E. cordata* toward *E. nitens* was still observed, but the freezing resistance of both *E. nitens* × *E. globulus* and *E. nitens* × *E. morrisbyi* F_1 hybrids was virtually identical to the less resistant pollen parent (Table 2). At neither level of hardiness did the *E. viminalis* or the *E. nitens* × *E. viminalis* families differ significantly from *E. nitens*.

E. nitens × *E. gunnii* F_1 *hybrids (Experiment 2)*

Significant differences ($P<0.001$) in mean T50 values were observed between the single *E. gunnii* and all *E. nitens* families at both levels of hardiness (Table 2), and the difference tended to increase with increased hardiness of the plants. Significant differences were also observed among both the six *E. nitens* families (4 weeks $P<0.01$; 9 weeks $P<0.05$) and among the F_1 hybrid families from the same set of *E. nitens* females crossed with a single *E. gunnii* pollen (4 weeks $P<0.02$; 9 weeks $P<0.01$). The individual narrow-sense heritability of the T50 values was relatively high for estimates obtained from the self-pollinated *E. nitens* (4 weeks $h^2=0.66\pm0.44$; 9 weeks $h^2=0.46\pm0.44$), although their standard errors were also high due to the small number of families and progenies tested. Significant levels of additive genetic variation thus appear to be expressed in *E. nitens* (4 weeks 0.43 °C and 9 weeks 1.3 °C). These results compare with a heritability of T50 values of $h^2=0.49$ and levels of additive genetic variation among random samples of open-pollinated progenies from within natural populations of *E. nitens* (between-population effect removed) at 0.14 °C. These parameters were calculated from the variance component estimates of Tibbits and Reid (1987 a), assuming a coefficient of relatedness of 0.4 for open-pollinated progenies of *Eucalyptus* (Griffin and Cotterill 1988). The genetic correlations between the T50 values at each level of hardiness were high (both $\geq 0.99\pm0.23$). However, there was no significant correlation between the relative freezing resistance of the *E. nitens* self-pollinated families and their F_1 hybrids with *E. gunnii*, which is difficult to explain.

Not only did the *E. gunnii* family maintain a higher level of freezing resistance than the *E. nitens* in the relatively unhardened state in both the controlled environment and the field studies (Expt. 3), but its actual rate of hardening was greater than any of the *E. nitens* or hybrid families. The change in the T50 value with hardening from 4 to 9 weeks was -2.8 °C for *E. gunnii* and -1.1 °C (±0.08) for the *E. nitens* families, with the F_1 hybrids intermediate (-1.9 °C ±0.06). In the relatively unhardened state there was only 1.6 °C difference between the *E. nitens* and *E. gunnii*, however, with the greater rate of hardening the difference increased to 3.3 °C after 9 weeks of hardening. In all cases the mean freezing resistance of the F_1 hybrids was intermediate between either parent, and in all but one case it did not differ significantly from the midparent value (Table 2). In the one exception (127 × 312), a significant deviation above the midparent value toward the *E. gunnii* occurred at both levels of hardening. However, the average degree of dominance of freezing resistance of the *E. nitens* × *E. gunnii* F_1 hybrid families did not differ significantly from zero after either 4 weeks (mean $=0.04\pm0.381$; $n=6$) or 9 weeks of hardening (mean $=0.12\pm0.169$; $n=6$).

Seasonal variation in freezing resistance (Experiment 3)

The pattern of hardening was further monitored for *E. nitens* × *E. globulus* (123 × 281) and *E. nitens* × *E. gunnii* (123 × 312) F_1 hybrid families and their parental controls in field trials over virtually a full year. As observed in Experiment 2, the difference in freezing resistance between the parental species increased with increasing levels of hardening. The difference in freezing resistance reached a maximum in August (*E. nitens* versus *E. globulus*, –1.6 °C; *E. nitens* versus *E. gunnii*, –2.4 °C), after which plants rapidly dehardened. By October, summer levels of freezing resistance had been reached and no significant differences occurred among any of the families (Table 2). The full expression of genetic differences in freezing resistance does not seem to occur until maximum levels of hardiness are achieved.

In virtually all months in which the parental progenies differed significantly ($P < 0.05$) in freezing resistance, the F_1 hybrids with both *E. globulus* and *E. gunnii* were intermediate between parental means, although the exact degree of dominance in the F_1 appeared to be quite variable. In several months the means of both hybrids differed significantly from the mid-parent value, and in all instances this was in the direction of the least resistant parent (Table 2). In contrast, in the earlier controlled environment experiment (Table 2; Expt. 2), the same *E. nitens* × *E. gunnii* F_1 family deviated ($P < 0.05$) toward the more resistant species at both levels of hardening.

E. nitens × *E. globulus* F_1 hybrids (Experiment 4)

In five of the eight *E. globulus* × *E. nitens* combinations, the parents differed significantly in T50 values, and the freezing resistance of the *E. nitens* families ranged from 1 to 4.3 °C lower than the comparable *E. globulus* family (Table 2). In all cases where the parental control progenies differed significantly, the F_1 hybrid mean was intermediate between the parental means (Table 2). While on average there was a slight deviation toward the less resistant parent *E. globulus* (mean degree of dominance $= -0.32$; $SE = 0.218$; $n = 5$), this was not significant and no F_1 family mean differed significantly from the midparent value.

Other hybrids with *E. nitens* (Experiments 5, 6, and 7)

The T50 values for a range of F_1 hybrids with *E. nitens* and corresponding parental control progenies grown as potted plants in the nursery are shown in Table 2. A significant difference in freezing resistance only occurred between the *E. nitens* families and the *E. parvifolia* (T50, $P < 0.05$) and *E. camaldulensis* (T40, $P < 0.01$) families. The *E. parvifolia* family was over 4 °C more freezing resistant than the *E. nitens* families, with the three F_1 hybrid families intermediate and not significantly different from the midparent values (Table 2). The *E. camaldulensis* family was 4.3–6.9 °C less resistant to freezing than the *E. nitens* families, and each F_1 hybrid family showed significant ($P < 0.05$) dominance toward the less resistant parent. Large differences in freezing resistance were also found between *E. perriniana* and *E. nitens*, but no estimates of T30–50 values are presented, as the *E. perriniana* was not damaged over the range of temperatures examined (Fig. 1). However, the F_1 hybrid was intermediate in its response and reciprocal crosses did not appear to differ significantly (Fig. 1 b).

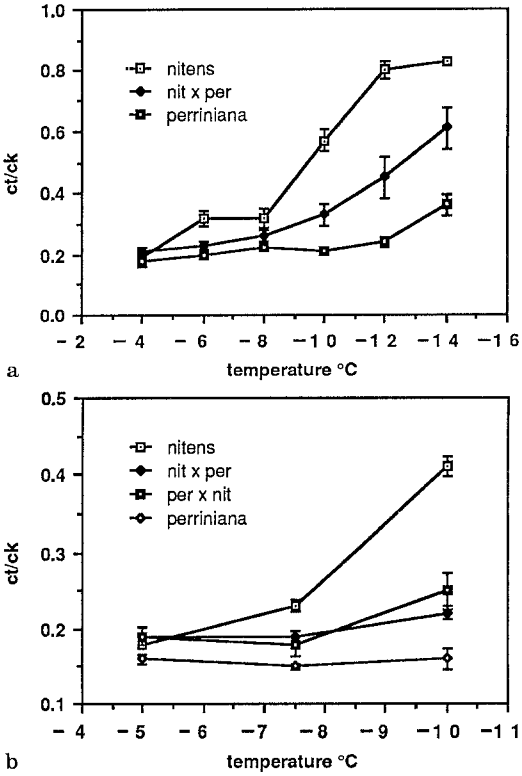

Fig. 1 a and b. Relative conductivity (ct/ck) plotted against temperature for families of *E. nitens*, *E. perriniana*, and **a** their F_1 hybrid with *E. nitens* as female and **b** reciprocal F_1 hybrids. (standard error bars are indicated)

E. gunnii × *E. globulus* F_1 hybrids (Experiment 8)

Highly significantly ($P < 0.001$) differences in freezing resistance were demonstrated between the *E. globulus* and *E. gunnii* families (Table 2, Fig. 2). The *E. gunnii* families were extremely freezing resistant and the difference between parental species of over 9 °C was the largest observed in all experiments. The T30 values for the *E. globulus* families ranged from −3.9 to −4.4 °C compared to −12.7 to −18.9 °C for the *E. gunnii* families, and one of the *E. gunnii* families (GUN7) exhibited virtually no damage even at −16 °C. In all cases, the F_1 hybrids were intermediate but deviated significantly ($P < 0.02$) from the midparent value toward *E. globulus* (Table 3). How-

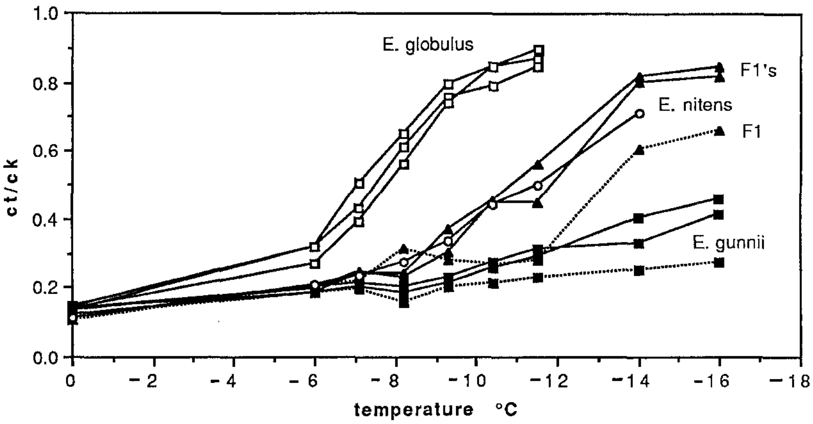

Fig. 2. Mean relative conductivity (ct/ck) plotted against frost temperature for *E. globulus*, *E. gunnii*, and F_1 hybrid families and a pooled *E. nitens* control. F_1 hybrids with the freezing resistant *E. gunnii* parent (GUN7) are indicated (....)

Table 3. The average degree of dominance of freezing resistance in F_1 hybrid families of different species combinations. Only crosses in which the parental controls differed significantly in freezing resistance have been assessed. Where crosses had been screened on several occasions, results for the most frost-hardened time and lethal temperatures for the highest measure of electrolyte leakage have been used. Negative values indicate partial dominance toward the less resistant parent

Cross-type (female × male)	Degree of dominance				
	Mean	SE	n	Signif. of *t*	Experiment
E. nitens[a] × *E. cordata*	1.17	–	1	–	1b
E. nitens[a] × *E. johnstonii*	−1.00	–	1	–	1a
E. nitens[a] × *E. morrisbyi*	−0.82	–	1	–	1b
E. nitens[a] × *E. globulus*	−0.46	0.181	7	$P < 0.05$	1b, 3, 4
E. nitens × *E. gunnii*[a]	−0.02	0.213	6	NS	2b, 3
E. nitens[a] × *E. dunnii*	−0.83	–	1	–	5a
E. nitens[a] × *E. saligna*	−0.51	–	1	–	5a (one F_1 only)
E. nitens × *E. parvifolia*[a]	−0.09	0.067	3	NS	5b
E. nitens[a] × *E. camaldulensis*	−0.70	0.110	2	NS	7
E. gunnii[a] × *E. globulus*	−0.36	0.070	4	$P < 0.02$	8
E. morrisbyi × *E. gunnii*[a]	−0.53	–	1	–	9
E. morrisbyi × *E. urnigera*	0.25	–	1	–	9
E. gunnii[a] × *E. urnigera*	−0.13	–	1	–	9
Cross-type mean	−0.25	0.154	13	NS	

[a] Most freezing-resistant parent

ever, in all cases, the F_1 hybrid families were more ($P < 0.05$) freezing resistant than the comparable *E. globulus* control, but only the F_1 families with *E. gunnii* as the male were more ($P < 0.01$) freezing resistant than the *E. nitens* samples included in the trial ($n = 10$; T30 = −7.6 ± 0.89; T40 = −10.2 ± 1.32; T50 = −12.3 ± 1.73) (Table 2, Fig. 2). These F_1 hybrids were among the few derived from crosses using *E. globulus* as the female (Potts and Savva 1989), but rather than reflect a maternal effect, their higher resistance to freezing is due to the pollen parent (GUN7 – Table 2, Fig. 2). The open-pollinated family derived from this pollen parent was more than 4 °C more resistant than the other *E. gunnii* families (GUN16 and GUN15), which is directly reflected in the higher resistance of the F_1 hybrids (Fig. 2).

F_1 hybrids with some Tasmanian endemic species (Experiment 9)

The *E. gunnii* families were significantly more resistant to freezing than most of the other pure species families examined (Table 2). The poor freezing resistance of the *E. urnigera* family (URN4) is surprising as this species grows in subalpine habitats. However, this specific pollen was collected from a green variant at the lower altitudinal limit (600 m) of the species range. In the three cases where parental families differed significantly ($P < 0.01$) in resistance to freezing, the F_1 hybrid families were intermediate (Table 2, Fig. 2), and only the T50 value of the *E. morrisbyi* × *E. gunnii* F_1 deviated significantly ($P < 0.05$) from the midparent value.

The *E. gunnii* families from GUN7 and GUN15 were the same as sampled in July for Experiment 8. Experiment 9 was undertaken in September, and marked dehardening in the order of 10° and 6 °C (based on T30), respectively, had occurred in both families in the 2-month interval. Their rate of dehardening over this period of 0.75–1.25 °C/week was even greater than the dehardening that occurred between August and October in 1987 in other families (Table 2, Expt. 3). In addition, despite lower levels of hardiness, these two *E. gunnii* families still differed in freezing resistance by 4–5 °C, consistent with the results obtained in July.

Midparent regressions

The linear regression of the F_1 hybrid mean on the midparent value was highly significant ($P < 0.001$) for both T40 ($F_1 = -1.22 + 0.76 \cdot \mathrm{mp}$; $r = 0.92$) and T50 (Fig. 3) values, indicating that the mean freezing resistance of

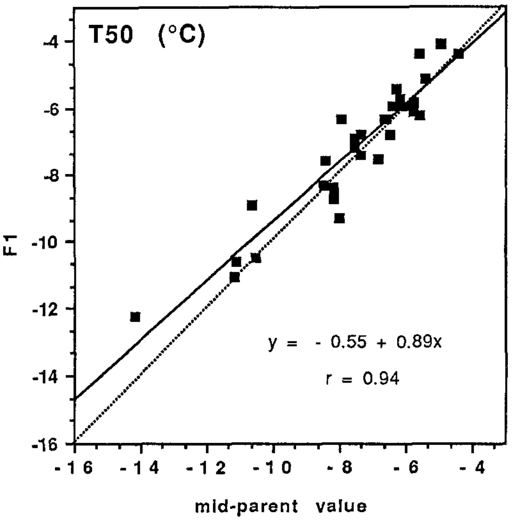

Fig. 3. Estimates of temperatures causing 50% electrolyte leakage (T50) in interspecific F_1 hybrids of *Eucalyptus* plotted against the midparent values. The regression lines and lines indicating a 1:1 relationship between the F_1 hybrid mean and the midparent value are shown. Crosses were combined from all experiments and where repetitions of a specific cross was undertaken, only the results from the most hardened situation were included

these interspecific F_1 hybrid families is highly heritable. The heritability of these F_1 hybrid family means estimated from midparent regressions (Falconer 1986) is $h^2 = 0.76 \pm 0.06$ (T40) and $h^2 = 0.89 \pm 0.06$ (T50), suggesting that a large additive genetic component underlies the control of freezing resistance in these interspecific hybrids. The coefficients of determination of the F_1 on midparent regressions are high ($R_2 = 85$ and 88% for T40 and T50, respectively) indicating that the mean freezing resistance of the F_1 hybrid family can be accurately predicted from the midparent value. However, the regression lines deviate from a 1:1 relationship, and the mean freezing resistance of the F_1 hybrids tends to deviate slightly toward the least freezing-resistant parent, particularly at lower midparent values (Fig. 3). The latter arises from partial dominance toward the less freezing-resistant parent species in many of the crosses where the species differ widely in freezing resistance, such as *E. gunnii* × *E. globulus* and *E. nitens* × *E. camaldulensis* (Table 3). Nevertheless, the variation in the deviation of the F_1 family means from midparent values was relatively small when compared to the variance in parental deviations (approx. one-tenth). Over all families for which the parental controls differed significantly in freezing resistance, the average degree of dominance in the F_1 hybrid was -0.24 (± 0.130; $n = 26$; $P < 0.05$) in the direction of the less resistant species.

Discussion

These results suggest that the significant variation in freezing resistance among eucalypt species is under the control of a predominantly additive gene system, which supports previous findings with *Eucalyptus* (Pilipenka 1969; van Wyk 1976; Cauvin et al. 1987). Intermediate inheritance of freezing resistance in interspecific eucalypt hybrids has also been reported in *E. pulverulenta* × *E. grandis* (Paton 1981), *E. gunnii* × *E. grandis* (Cauvin 1988), *E. gunnii* × *E. cordata* (Cauvin et al. 1987), and other eucalypt species (Pilipenka 1969). The present study indicates that in many cases, full expression of this genetic variation does not occur until maximum levels of hardiness are achieved, which is consistent with results from provenance studies (Tibbits and Reid 1987a). In fact, a large component of the variation in freezing resistance among species and their hybrids appears to be related to variation in the rate of hardening, which the present study suggests is also inherited in a predominantly additive manner in F_1 hybrids.

Our conclusions, based on the artificial frosting of leaf discs, agree with results based on observations of the relative survival of parents and F_1 hybrids in natural frosts (Pilipenka 1969; Cauvin et al. 1987). Pilipenka (1969) recorded damage following natural frosts (-7.5 to -9 °C) over three seasons, and concluded that F_1 hybrids between a frost-sensitive and a relatively resistant species have a higher level of resistance than the less resistant parent. Similarly, Cauvin et al. (1987) reported significantly reduced survival of all F_1 hybrid types with the frost resistant species *E. gunnii*, as compared to intraspecific crosses of *E. gunnii*, following the extreme frost of 1985 in France (Potts and Potts 1986). However, observations on survival in field trials may not reflect freezing resistance per se, but rather a combination of both freezing resistance and the ability to outgrow the more damaging microenvironments of inversion layers (i.e., "frost resilience" – according to Reddy and Rockwood 1989).

In the present study there was no case of interspecific hybrids being significantly more freezing resistant than the more resistant parent, i.e., better-parent heterosis (Table 2). While the inheritance of freezing resistance was predominantly additive and significant variation from the midparent value was relatively uncommon, some notable variation in the degree of dominance did occur both within and between hybrid types. For example, while the average degree of dominance in the six *E. nitens* × *E. gunnii* crosses did not differ significantly from zero, the degree of dominance varied from -0.53 to 0.68 after 9 weeks hardening, and one F_1 hybrid family consistently deviated ($P < 0.05$) from the midparent value toward the more resistant parent. There is insufficient replication within each hybrid type in the present study to assess the full significance of variations in dominance in these F_1 hybrids, or to determine whether this is due to specific combining effects expressed at the individual or species level. However, in a comprehensive study of the inheritance of freezing resistance within a eucalypt species, van Wyk (1976) reported little specific combining effect in a diallel of *E. grandis*, but it is conceivable that these effects may exist in interspecific hybrid combinations.

Pilipenka (1969) indicated that when the more sensitive species is used as the female, the F_1 hybrids had slightly lower freezing resistance than the reciprocal cross, and he cited *E. dealbata* × *E. viminalis* and *E. camaldulensis* × *E. viminalis* as examples. A reciprocal effect could explain the higher dominance in the *E. nitens* crosses with *E. gunnii* and *E. parvifolia* in the present study, but is not consistent with the results obtained for the *E. gunnii* × *E. globulus* crosses. In addition, no difference was observed in the only reciprocal F_1s examined (i.e., *E. nitens* × *E. perriniana* hybrid, Fig. 1). However, there is some indication that the degree of dominance may change due to ontogenetic, environmental, or physiological effects, which also requires further investigation. For example, one *E. nitens* × *E. gunnii* F_1 (123 × 281) showed significant partial dominance on three occasions when monitored in a field trial, whereas when younger and growing in a controlled environment, the same plants showed no dominance. Ontogenetic variation in freezing resistance has been noted in eucalypts (Paton 1981). This may have implications for early screening of freezing resistance, and it is important that juvenile-mature correlations for this trait are clearly established. Pilipenka (1969) notes several eucalypt F_1 hybrid combinations in which the degree of dominance in morphological traits appears to change with ontogeny. He classified F_1 hybrids into three main classes: those in which (1) there is complete or almost complete dominance of the characteristics of one parent, (2) characters are inherited in an intermediate manner, and (3) the pattern of combination is complex, with the degree of dominance varying in different periods of ontogeny. Nevertheless, the predominantly intermediate inheritance of traits in F_1 hybrids of *Eucalyptus* has been reported for many morphological (Pryor 1957 b, 1976; Cauvin et al. 1987; Tibbits 1988) and physiological traits (freezing resistance, present study), which is consistent with trends noted in numerous plant genera for many of the ecological and taxonomic characteristics that differentiate species predominantly controlled by additive gene systems (Grant 1971).

Conclusions

Implications for breeding

Manipulation of the freezing resistance in breeding programs through interspecific hybridization is clearly an option, and this study indicates that interspecific hybrids can be produced which have significantly greater freezing resistance to that of the less resistant parent. Hybridization of species with complementary traits may result in synergistic effects in specific environments where neither parental species is well adapted (Sedgley and Griffin 1989). The F_1 hybrid between *E. globulus* and *E. gunnii* is of particular interest in this respect, as it would allow the combination of genes of one of the faster growing, high pulp yielding species with the genes of one of the most freezing-resistant species in the genus (Pryor 1957 a; Cauvin et al. 1987). Despite the partial dominance toward *E. globulus* observed in the present study, the F_1 hybrids were still as resistant to freezing as *E. nitens* (Fig. 1), the species currently used to replace *E. globulus* on frost-prone sites. Considerable variation in resistance to freezing has been demonstrated within *E. gunnii* (Cauvin 1988; present study), and the large difference in freezing resistance of the *E. gunnii* parents in the present study was a provenance effect that was directly reflected in the resistance of their F_1 hybrids (Fig. 2). Greater gains in the freezing resistance of F_1 hybrids could clearly be made by intensive selection within parental species prior to hybridization. However, the more freezing-resistant species tend to be slow growing and, with predominantly additive inheritance of growth rate in many interspecific hybrids (Cauvin et al. 1987), it is likely that gains in freezing resistance through F_1 hybridization may only be made at the expense of other traits.

Any commitment to the use of interspecific hybrids in tree breeding programs will necessitate a concurrent development of clonal propagation, since the operational production of F_1 seed appears prohibitive. It is possible that outstanding fast-growing, freezing-resistant genotypes may be found in later hybrid generations which can be selected for clonal propagation (Cauvin et al. 1987). It is important that advanced generation hybrid material be explored to determine the genetic and physiological limits to recombination of freezing resistance with other economic traits. Pilipenka (1969) observed that open-pollinated second generation hybrids on average had freezing resistance comparable to the first generation hybrids. However, he also noted variation in freezing resistance within second generation hybrids that was closely associated with morphology, suggesting relatively strong character coherence in the F_2 generation (Hartley 1965). Such coherence is expected for traits under polygenic control (Hartley 1965), yet Pilipenka did record cases of recombination where plants had the morphology of the resistant species but the resistance to freezing of the sensitive species. Paton (1981) found no evidence of a genetic or physiological association between freezing resistance and glaucousness in F_2 or backcross hybrid progenies between the green, freezing-sensitive species, *E. grandis* and the glaucous, resistant species *E. pulverulenta*. In fact, of the 21% of seedlings recovered with the green phenotype of *E. grandis* in Paton's F_2, one-third had the high freezing resistance of the *E. pulverulenta* (Paton 1981 – Table 3). While the control of traits associated with vigor is likely to be under much more complex genetic control than glaucousness, these results are encouraging and suggest that the large-scale screening of advanced generation hybrid progenies for rare recombinants may be a fruitful approach if a cloning option exists. Indeed, rooting ability may also be under additive genetic control in interspecific hybrids, and improved cloning potential may be a biproduct of crosses using the freezing-resistant species *E. gunnii*, which is readily propagated from hardwood cuttings (Potts and Potts 1986).

Interspecific hybridization has not been widely adopted as an approach to breeding in extreme winter climates such as in France, where even species such as *E. gunnii* are at their limits. No reduction in the freezing resistance can be tolerated (as occurred in most of the F_1 hybrids examined), and current approaches are focusing on intense clonal selection within resistant species such as *E. gunnii* (Cauvin 1988). Significant genetic variation in freezing resistance has been found within *E. nitens* (Tibbits and Reid 1987a; present study) and other commercial, but relatively freezing-sensitive, species (Griffin et al. 1982), which indicates that considerable genetic gains may also be made by classical programs of recurrent or mass selection within pure species (Griffin 1988; Reddy and Rockwood 1989). Of particular concern in the use of a hybridization strategy is the possible decrease in vigor with increasing taxonomic distance between parents (Potts et al. 1987). An optimal degree of genetic divergence for the expression of heterosis (at least relative to midparent values) has been hypothesized (Potts et al. 1987) and this effect is currently being investigated.

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