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Hybridizing ability and heterosis between *Eucalyptus urophylla* and *E. tereticornis* for growth and wood density over two environments

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Abstract

Interspecific hybrids of *Eucalyptus urophylla* × *E. tereticornis* in a factorial-mating design were used to analyze general hybridizing ability (*GHA*), specific hybridizing ability (*SHA*) and heterosis for height (*H*;

0.5, 1.5, 2.5, 4 and 7.5 years in age), diameter at breast height (*D*; ages 1.5, 2.5, 4 and 7.5) and wood density (*WD*; age 7.5) across two environments. The *GHA* variances were significant for all traits, and those of the *SHA* were also significant for most of the traits but with less magnitude, indicating the greater importance of additive gene effects in explaining the phenotypic variation among hybrids. The narrow-sense heritability (h^2) and dominance (d^2) estimates with hybrid growth and wood density ranged between 0.02 ± 0.05 (d^2 in $D_{7.5}$) and 0.23 ± 0.10 (d^2 in $H_{1.5}$) over the different ages, suggesting weak additive and dominant effects on these traits. Additive genetic correlations of growth with wood density were weak at age 7.5. Female general combining ability (*GCA*) based on maternal open-pollinated families was not necessarily a good indicator of *GHA* as their cor-

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relations were 0.48 ($P=0.17$), 0.65 ($P=0.04$) and -0.56 ($P=0.10$) for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$, respectively. A great proportion of hybrids showed positive female-parent heterosis (FPH) at age 7.5, with the highest relative FPH of 47.2% in $D_{7.5}$. This study demonstrates the heterosis between inter-sectional species and could have implications for *E. urophylla* × *E. tereticornis* hybrid breeding.

Key words: Hybridizing ability, heterosis, growth, wood density, *Eucalyptus*.

Introduction

The tree genus *Eucalyptus* L'HÉRIT (family Myrtaceae) comprises more than 780 species and subspecies of 10 subgenera (SLEE et al., 2006). Interspecific hybrids can often be readily produced, spontaneously or by manipulation, between closely related species within a subgenus, and hybrid superiority has been frequently observed in *Eucalyptus* (POTTS and DUNGEY, 2004). With the technical progress in vegetative propagation, many *Eucalyptus* hybrids can be easily macro- (rooted cuttings) and/or micro-propagated (tissue culture), thereby enabling the utility of superior hybrid individuals for large-scale clonal cultivation (ELDRIDGE et al., 1993). In the past few decades, significant gains in productivity, uniformity and wood properties have been realized in eucalypts mostly due to the intensive plantation of hybrid clones. For example, the new hybrid varieties being planted largely in China are achieving an average wood yield of 20 m³/ha/year, almost three times that of the traditional species (BUEREN, 2004).

In quantitative genetics theory, the parental effects on hybrid performance could be analyzed by combining ability, including general and specific combining ability (GCA and SCA , respectively), in which GCA is the average performance of a parent (line) in hybrid combinations and SCA represents the deviation in performance of a parental combination from the average expectation over the parents (lines) involved (SPRAGUE and TATUM, 1942; MELCHINGER and GUMBER, 1998). GCA is primarily a measure of additive genetic effects, which are amenable to selection, whereas SCA includes non-additive effects (ROJAS and SPRAGUE, 1952). Thus, combining ability analysis is conducive to define the pattern of genetic effects influencing quantitative traits and identify potentially superior parents and hybrid combinations in breeding practices. Similarly, NIKLES and NEWTON (1991) proposed the concepts of general and specific hybridizing ability (GHA and SHA , respectively) to measure the parental additive and non-additive genetic effects, respectively, for interspecific hybrid crosses. So far, GHA and SHA have been evaluated in a number of forest tree taxa, e.g. European larch (*Larix decidua* MILL.) and Japanese larch (*L. kaempferi* (LAMB.) CARR.) (PLÁQUES, 2004) as well as flooded gum (*E. grandis* HILL ex MAIDEN) and Timor mountain gum (*E. urophylla* S. T. BLAKE) (RETIEF and STANGER, 2009). Moreover, the correlation between GCA and GHA estimates, coupled with the magnitude of SHA , will provide fundamental information which can be used to optimize breeding strategies (VOLKER et al., 2008; RETIEF and STANGER, 2009).

The superior performance of hybrids over their parents or parental lines is defined as heterosis or hybrid

vigor (SHULL, 1952). The phenomenon of heterosis has been widely exploited in plant breeding practices since its discovery at the beginning of the 20th century (SHULL, 1908). In *Eucalyptus*, though hybrid depression was occasionally observed (e.g. *E. nitens* (DEANE and MAIDEN) MAIDEN × *E. globulus* LABILL.; VOLKER et al., 2008; COSTA E SILVA et al., 2012), heterosis has been demonstrated in economically important traits such as growth and processing characteristics as well as stress tolerance and resistance (e.g. MEDDINGS et al., 2001; GAN et al., 2004; HE et al., 2012a). Moreover, a number of approaches have been exploited to capture the heterosis for industrial plantations, such as mass production of F₁ hybrid seed of heterotic parents (MARTIN, 1989) and mass clonal propagation of outstanding individuals (WATT et al., 1995). Thus far, great success in heterosis exploitation in *Eucalyptus* has been achieved in tropical and sub-tropical plantation forestry with the widespread use of *E. urophylla* × *E. grandis* clones in South America, Asia and South Africa (GRIFFIN et al., 1988; POTTS and DUNGEY, 2004). However, the magnitude of heterosis and its underlying genetic mechanisms are poorly understood.

Eucalyptus trees are widely grown around the world for pulp production. Growth and wood density are very important traits in terms of pulp productivity per unit area. For such traits, several reports have estimated the GHA and SHA effects on inter-specific F₁ hybrids of *Eucalyptus*, such as *E. urophylla* × *E. grandis* for growth (RETIEF and STANGER, 2009) and *E. nitens* × *E. globulus* for both growth and wood density (VOLKER et al., 2008). However, the previous reports are based merely on single-site field trials, which may result in the environmental effects and thus biased the GHA and SHA estimates. Furthermore, the parental species analyzed previously are mostly of intra-sectional combinations, e.g. within sections *Latoangulatae* BROOKER (RETIEF and STANGER, 2009) and *Maidenaria* L. D. PRYOR and L. A. S. JOHNSON ex BROOKER (VOLKER et al., 2008) of subgenus *Symphyomyrtus* (SCHAUER) BROOKER according to the classification of BROOKER (2000). In this context, inter-sectional hybrids need to be examined in *Eucalyptus* for broadening the information on parental hybridizing ability as well as that of the magnitude of the observed heterosis.

Here we present the GHA , SHA and heterosis analyses in inter-sectional hybrids between *E. urophylla* and *E. tereticornis* SMITH (sections *Latoangulatae* and *Exsertaria* L. D. PRYOR and L. A. S. JOHNSON ex BROOKER under subgenus *Symphyomyrtus*, respectively; BROOKER, 2000) for growth and wood density over two environments. *E. urophylla* is native to Indonesia and East Timor while *E. tereticornis* is native to Australia and Papua New Guinea (ELDRIDGE et al., 1993). In addition to the favorable features of fast growth and versatile wood properties, the two species are favorably complementary in some other important traits. For instance, *E. urophylla* is characterized in early flowering (WU et al., 1996) and resistance to gall wasp (*Leptocybe invasa* FISHER and LA SALLE; THUA et al., 2009), and some provenances of *E. tereticornis* are advantageous in resistance to or tolerance of salinity (MARCAR, 1989), bacteri-

al wilt (*Ralstonia solanacearum* SMITH; DIANESE and DRISTIG, 1993) and frost (LIN and LI, 2003). Thus, *E. urophylla* × *E. tereticornis* hybrids may combine these characteristics and have great potentials in heterosis exploitation.

Materials and Methods

Plant material

A total of 62 crosses derived from an incomplete 10 × 10 factorial mating design of *E. urophylla* × *E. tereticornis* were used as per HE et al. (2012a), and 59 crosses were finally maintained while three died during the field trials. The parental trees were selected from previous provenance/family trials or seedling plantations as described earlier (HE et al., 2012b). Ten maternal open-pollinated (OP) families were also included, seeds of which were harvested from the maternal trees of the original trials or plantations.

Field trial and trait measurement

Seedlings of the crosses and OP families were field planted in July 2003 in two environments, namely, Yong'an County in northern Fujian Province (117°23' E, 25°53' N and 260–300 m above sea level) and Changtai County in southern Fujian (117°52' E, 24°46' N and 225–260 m above sea level). The Yong'an trial site is relatively cold (mean annual temperature 18.7°C and absolute minimum -7.0°C) with deep soil, approximately 15 degree slope and annual rainfall 1564 mm, whereas the Changtai site is warm (mean annual temperature 19.6°C and absolute minimum -1.7°C) with shallow red soil, about 20 degree slope and annual rainfall 1466 mm. The field trial for all the crosses and OP families was set in a randomized complete block design with four replicates of four-tree row plots. Site preparation and tending operations were as described previously (HE et al., 2012a).

Measurements were made for height (H , m) at 0.5, 1.5, 2.5, 4 and 7.5 years of age, diameter at 1.3 m height (D , cm) at ages 1.5, 2.5, 4 and 7.5 and wood density at age 7.5 ($WD_{7.5}$). Wood density was indirectly measured by a bark-to-bark drilling of each standing tree at 1.3 m height using the Resistograph F-400S (Instrumenta Mechanik Labor GmbH, Wiesloch, Germany). The Resistograph system has been demonstrated to be reliable and efficient for assessing relative wood density of live trees in tree improvement programs (ISIK and LI, 2003). The Resistograph measurements were restricted to trees with $D_{7.5}$ greater than 8 cm, and visible limbs and knots were avoided to eliminate outlier values. The average amplitude (%) of each measurement was calculated over the first 5 cm from the bark due to the increasing trend of resistance amplitude with the drilling depth (BOUFFIER et al., 2008). The average amplitude was square-root transformed to a normal distribution for subsequent analyses.

Statistical analyses

For a given trait, the significance level of difference between hybrids and the maternal OP families was tested with t -test using Proc MEANS procedure in software

SAS/STAT® 6.0 (SAS Institute Inc., Cary, NC, USA). A combined analysis of variance (ANOVA) was conducted based on plot means using Proc GLM in SAS/STAT® 6.0 following the below mixed linear model:

$$Y_{ijk} = \mu + G_i + E_j + R_k(E_j) + (GE)_{ij} + e_{ijk}$$

where Y_{ijk} is the trait value of the i th genotype (hybrid or maternal open-pollinated family) in the k th replication under the j th environment, μ is an overall mean, G_i is the effect of the i th genotype, E_j is the effect of j th environment, $R_k(E_j)$ is the effect of the k th replication within the j th environment (fixed), $(GE)_{ij}$ is the genotype × environment interaction, and e_{ijk} is the residual error. For the hybrid population, the genotype sum of squares was partitioned further into the variation due to female, male and female × male interaction. Proc VARCOMP (method REML) of SAS/STAT® 6.0 was used to estimate the variance components. The variances due to female and male parents are equivalent to the GHA effects, and the female × male interaction is equivalent to the SHA effects, which are similar to the GCA and SCA effects, respectively, for intra-species hybrids (HALLAUER and MIRANDA, 1981).

Similar to the case of pure-species, narrow-sense heritability (h^2) and dominance estimate (d^2) were evaluated as:

$$h^2 = 2(\sigma_{GHA(F)}^2 + \sigma_{GHA(M)}^2) / \sigma_P^2$$

$$d^2 = 4\sigma_{SHA}^2 / \sigma_P^2$$

$$\sigma_P^2 = \sigma_{GHA(F)}^2 + \sigma_{GHA(M)}^2 + \sigma_{SHA}^2 + \sigma_E^2 + \sigma_{GHA(F) \times E}^2 + \sigma_{GHA(M) \times E}^2 + \sigma_{SHA \times E}^2 + \sigma_e^2$$

where $\sigma_{GHA(F)}^2$, $\sigma_{GHA(M)}^2$, σ_{SHA}^2 , σ_E^2 , $\sigma_{GHA(F) \times E}^2$, $\sigma_{GHA(M) \times E}^2$, $\sigma_{SHA \times E}^2$, σ_e^2 and σ_P^2 are the variance components of female GHA , male GHA , SHA , environment, female GHA × environment interaction, male GHA × environment interaction, SHA × environment interaction, error and total phenotype, respectively. Standard errors of h^2 and d^2 were estimated using the delta method (LYNCH and WALSH, 1998).

Phenotypic (r_p) and additive genetic (r_g) correlation coefficients between traits across the two environments were estimated for the hybrid population using Proc MIXED (method REML) following the SAS code in APPENDIX C of HOLLAND (2006), modified by the deletion of SET effect from the related procedures. The additive genetic correlations were based on male covariance and covariance components derived from the mixed linear model mentioned above.

The estimates of maternal GCA (GCA_F), female GHA (GHA_F), male GHA (GHA_M) and parental SHA were generated with best linear unbiased predictors (BLUP) of individual family, female, male and hybrid, respectively, using Proc MIXED procedure (method REML) of SAS/STAT® 6.0 based on plot means. For each hybrid, while mid-parent heterosis (MPH) could not be estimated due to the absence of paternal OP families, absolute female-parent heterosis ($AFPH$) and relative female-parent heterosis ($RFPH$) were calculated as follows:

$$AFPH = Y_{ij} - Y_i$$

$$RFPH = AFPH / Y_i$$

Table 1. – Mean values of the traits measured for *E. urophylla* × *E. tereticornis* hybrids and maternal open-pollinated (OP) families grown in two environments.

Trait	Environment	Hybrid			OP family		
		Mean	CV (%)	LSD (5%)	Mean	CV (%)	LSD (5%)
$H_{0.5}$ (m)	Yong'an	0.75	41.2	0.40	0.76	50.8	0.29
	Changtai	0.93	46.7	0.26	0.62	43.5	0.20
	Both	0.84**	44.9	0.26	0.69	45.9	0.18
$H_{1.5}$ (m)	Yong'an	5.56	25.1	1.89	5.08	39.3	1.56
	Changtai	5.50	36.4	1.13	5.33	24.9	1.00
	Both	5.53	29.7	1.19	5.21	30.5	0.93
$D_{1.5}$ (cm)	Yong'an	4.86	29.5	1.95	4.02	48.2	1.58
	Changtai	4.43*	44.5	1.20	4.49	31.8	1.08
	Both	4.64*	35.8	1.22	4.27	37.8	0.96
$H_{2.5}$ (m)	Yong'an	7.79	27.7	2.91	7.57	40.2	2.54
	Changtai	8.61	33.6	1.97	6.83	32.3	1.78
	Both	8.22**	30.7	1.92	7.20	34.6	1.56
$D_{2.5}$ (cm)	Yong'an	9.16	21.4	2.93	6.74	44.6	2.59
	Changtai	7.26*	40.1	1.73	8.23	26.1	1.70
	Both	8.17*	31.9	1.99	7.46	35.4	1.63
H_4 (m)	Yong'an	13.40	19.1	3.30	9.93	33.8	3.33
	Changtai	11.11*	29.0	2.52	12.27	21.8	2.41
	Both	12.15***	25.3	2.56	10.98	29.4	2.28
D_4 (cm)	Yong'an	10.73**	26.6	3.80	8.58	43.5	3.57
	Changtai	9.19	39.9	2.77	9.52	31.0	2.84
	Both	9.89*	33.4	2.75	8.99	37.1	2.33
$H_{7.5}$ (m)	Yong'an	16.71	25.3	4.88	13.78	34.2	4.32
	Changtai	14.68*	32.4	4.23	15.08	26.4	4.33
	Both	15.62*	28.4	3.80	14.36	29.9	3.00
$D_{7.5}$ (cm)	Yong'an	13.16	29.6	5.30	11.37	41.8	4.38
	Changtai	12.06*	42.4	3.89	11.48	33.5	4.10
	Both	12.57*	35.1	3.78	11.42	37.0	2.96
$WD_{7.5}$ (10 ⁻¹)	Yong'an	3.88	16.1	0.81	4.17	15.6	0.61
	Changtai	4.29	17.6	0.64	3.65	15.0	0.63
	Both	4.10	16.9	0.60	3.93	16.3	0.47

*, ** and *** Significant in *t*-test at 0.05, 0.01 and 0.001 levels, respectively.

where Y_{ij} is the mean of the hybrid between the *i*th female and the *j*th male parents, and Y_i is the mean of *i*th maternal OP family. As the female *E. urophylla* outperforms generally the male *E. tereticornis* in growth, *AFPH* and *RFPH* could be treated as the better-parent heterosis.

Results

Mean performance

For growth traits *H* and *D*, the Yong'an environment showed generally higher mean values for hybrids but less means for maternal OP families as compared with

Changtai (*Table 1*). Also, the hybrid population outperformed consistently the OP progeny in growth after age 1.5 in Yong'an whereas the OP out-grew the hybrids in *H* or *D* over all ages in Changtai. However, coefficients of variation (*CV*) for growth of both populations were large and ranged from 19.1% (H_4 for hybrids in Yong'an) to 50.8% ($H_{0.5}$ for OP progeny in Yong'an), indicating a wide variation in growth within each population. For wood density $WD_{7.5}$, similar contrasting pattern was observed for mean performance of the two populations between the two environments, that is, being higher with OP families in Yong'an but with hybrids in Changtai.

Table 2. – Mean squares from the combined analyses of variances and the h^2 and d^2 estimates for 10 traits of the hybrids grown in two environments.

Source	df	$H_{0.5}$	$H_{1.5}$	$D_{1.5}$	$H_{2.5}$	$D_{2.5}$	H_4	D_4	$H_{7.5}$	$D_{7.5}$	$WD_{7.5}$
Environment (E)	1	6.39***	2.25	42.28***	211.11***	792.87***	996.71***	427.08***	844.48***	212.30**	27.71***
Replicate/E	6	1.57***	44.63***	42.24***	34.96***	35.09***	60.87***	77.93***	104.49***	119.46***	8.14***
Hybrid	58	0.35***	9.26***	8.46***	13.31***	14.27***	17.99***	22.17***	49.38***	49.99***	1.03***
GHA_F	9	0.52***	11.98***	14.31***	19.16***	23.44***	20.41**	31.17***	51.32**	68.34***	0.74*
GHA_M	9	0.42***	12.59***	11.09***	12.71*	14.61**	20.24**	23.68*	31.80*	34.91*	1.06**
SHA	40	0.24***	5.48***	4.91***	9.33**	8.97**	11.90*	12.62	23.94	20.87	0.57*
Hybrid × E	56	0.16	3.95**	3.48*	11.62***	10.64***	9.53	9.77	25.13*	23.05	0.40
GHA_F × E	9	0.18	4.64*	5.61*	15.75**	12.30*	7.23	13.25	30.66	30.41	0.22
GHA_M × E	9	0.16	4.14	2.62	7.75	10.38*	11.10	6.50	31.04	25.79	0.41
SHA × E	38	0.15	3.24	3.24	9.32*	7.41	8.08	6.10	27.34*	20.76	0.35
Pooled error	- ^a	0.13	2.40	2.44	5.75	5.38	7.61	9.81	17.53	18.16	0.40
h^2 estimate		0.07±0.05	0.15±0.09	0.11±0.07	0.09±0.05	0.11±0.06	0.10±0.06	0.12±0.06	0.13±0.06	0.17±0.07	0.11±0.07
d^2 estimate		0.17±0.07	0.23±0.10	0.22±0.09	0.07±0.06	0.11±0.07	0.09±0.07	0.07±0.06	0.09±0.07	0.02±0.05	0.13±0.07

*, ** and *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

^a Value of df for pooled error varied with trait from 900 in $WD_{7.5}$ to 1228 in $H_{0.5}$.

When averaged over both environments, hybrids had consistently better performance than the OP families in all the traits studied, and their differences were significant in t -test except for $H_{1.5}$ and $WD_{7.5}$ (Table 1). CV val-

ues were relatively high for growth traits H and D (25.3–45.9%) over all ages as compared with that of $WD_{7.5}$ (16.9% for hybrids and 16.3% for OP families). The hybrids showed smaller CVs exclusively in growth

Table 3. – Phenotypic correlations (r_p , above the diagonal) and additive genetic correlations (r_g , below the diagonal) between traits of growth and wood density at age 7.5.

Trait	$H_{7.5}$	$D_{7.5}$	$WD_{7.5}$
$H_{7.5}$		0.86±0.01	0.48±0.05
$D_{7.5}$	1.09±0.15 ^a		0.49±0.05
$WD_{7.5}$	0.68±0.48	0.64±0.36	

^a The value was significant in r_g using a threshold of ±2 standard errors.

Table 4. – Maternal GCA (GCA_F), female GHA (GHA_F) and male GHA (GHA_M) estimates for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$.

	GCA_F			GHA_F			GHA_M			
	$H_{7.5}$	$D_{7.5}$	$WD_{7.5}$	$H_{7.5}$	$D_{7.5}$	$WD_{7.5}$	$H_{7.5}$	$D_{7.5}$	$WD_{7.5}$	
Female	(m)	(cm)	(10 ⁻¹)	(m)	(cm)	(%)	Male	(m)	(cm)	(10 ⁻¹)
UX-01	0.39	0.16	0.07	-1.50**	-1.34**	-0.17*	T25-08	0.83*	1.58**	0.11
UX-30	-0.17	-0.41	-0.08	0.10	0.54	0.11	T25-09	-0.05	0.15	-0.07
U32-23	0.38	0.69	0.27*	0.97	1.06*	-0.10	T25-10	-0.19	-0.50	-0.03
U31-38	2.05*	2.17*	0.10	0.20	0.47	-0.04	T25-11	-0.04	-0.13	0.02
U89-16	-1.95*	-1.92*	-0.31*	-0.04	-0.43	0.09	T25-12	0.20	-0.01	0.01
UX-20	-2.09*	-2.19*	0.09	-1.29*	-1.43**	-0.02	T43-01	-0.45	-0.51	-0.06
UX-07	0.91	1.18	0.01	1.00*	0.74	-0.02	T44-20	-0.02	-0.19	-0.12
UX-04	-0.94	-0.98	0.04	-0.26	-0.26	0.14	T60-09	0.22	0.47	0.07
UX-02	0.66	0.64	-0.12	0.42	0.29	-0.03	TX-04	-0.03	-0.04	0.10
U31-41	0.76	0.66	-0.07	0.40	0.42	0.04	T43-31	-0.47	-0.83	-0.03

*, ** and *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

whilst the OP families demonstrated a slightly higher CV in $WD_{7.5}$.

Variance, h^2 and d^2 estimates

The combined ANOVA analyses over two environments indicated significant differences in all traits among genotypes and between environments for the hybrids (Table 2), suggesting the diversity of the genotypes and their variability in environmental response. The GHA variances due to either female or male parents were also significant for all traits with a relatively high magnitude as compared to those of SHA , although SHA variances were

significant for majority of the traits. It appears that additive gene effects are more important in explaining phenotypic variations of growth and wood density in the *E. urophylla* × *E. tereticornis* population while the significance of dominant gene effects declines in growth with time. Moreover, the GHA mean squares due to the females were considerably larger than those of the males, though it was uncertain to what extent this difference was genetically meaningful. Furthermore, the interaction between genotype and environment varied with trait, e.g. significant GHA_F × environment interaction for growth over ages 1.5 and 2 but non-significant GHA_M

Table 5. – Parental SHA estimates for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$.

SHA					SHA					
		$H_{7.5}$	$D_{7.5}$	$WD_{7.5}$			$H_{7.5}$	$D_{7.5}$	$WD_{7.5}$	
Female	Male	(m)	(cm)	(10^{-1})	Female	Male	(m)	(cm)	(10^{-1})	
UX-01	T25-08	-0.36	0.03	0.02	U89-16	T25-08	1.15	1.49	0.12	
	T25-09	-0.42	-0.21	-0.06		T25-09	0.86	0.86	0.22	
	T25-10	-1.75*	-1.85*	-0.22		T25-10	-0.62	-0.91	0.02	
	T25-11	-0.37	-0.95	-0.14		T25-11	-0.60	-1.24	0.01	
	T25-12	-0.41	-0.69	-0.04		T25-12	0.90	1.42	-0.05	
	T43-01	-1.50	-0.78	-0.16		T44-20	0.02	-0.49	0.09	
	T44-20	-1.03	-0.60	-0.17		T43-31	-0.69	-1.39	0.11	
	T60-09	-1.65	-1.54	-0.14		UX-20	T25-08	-0.31	-0.01	0.12
	TX-04	-0.95	-1.25	-0.04			T25-09	-0.78	-0.93	-0.13
	T43-31	-0.09	-0.51	-0.08			T25-10	-0.68	-1.15	0.04
UX-30	T25-09	-0.58	0.11	-0.08	T25-11		-0.49	-0.64	0.06	
	T25-11	0.44	0.96	0.19	T25-12		-0.53	-1.06	0.10	
	T43-01	-0.67	-1.19	-0.09	T43-01	-1.90*	-2.06*	-0.13		
	T44-20	0.76	0.49	0.05	T44-20	0.12	0.09	0.04		
	T60-09	-0.16	0.94	0.17	T43-31	-1.62	-1.66	-0.18		
TX-04	0.59	0.92	0.30*	UX-07	T25-08	2.79**	2.76**	0.12		
U32-23	T25-08	1.18	1.89*		0.13	T25-09	-0.08	0.18	-0.20	
	T25-09	0.84	1.03		-0.03	T25-10	1.43	1.01	0.03	
	T25-12	1.34	1.25		-0.03	T25-11	0.30	0.67	0.07	
	T44-20	-0.04	-0.13		-0.19	T25-12	-0.01	-0.55	-0.06	
	T60-09	1.85*	1.98*		0.15	T43-01	0.24	-0.12	0.06	
	TX-04	0.08	0.55		-0.24*	T44-20	0.91	0.67	-0.14	
	T43-31	0.06	-0.17		-0.18	T60-09	1.12	0.70	0.07	
U31-38	T43-01	0.73	1.22		-0.09	T43-31	-0.20	0.10	0.02	
	T44-20	-0.82	-0.85		-0.32*	UX-04	T25-09	-0.22	-0.14	-0.01
	T60-09	0.70	1.03	0.06	T43-01		-0.65	-0.44	0.15	
	TX-04	0.11	0.23	0.27*	TX-04		0.42	0.41	0.21	
T43-31	-0.30	-0.70	0.18							
U31-41	T43-01	0.53	0.46	0.04	UX-02	T44-20	0.50	0.30	-0.13	
	T43-31	-0.07	-0.18	0.06		T60-09	0.59	0.65	0.06	

*, ** and *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

or $SHA \times$ environment for most traits. Additionally, for the maternal OP families, significant differences were found among families in all the traits as well as between environments in $WD_{7.5}$ and growth over ages 0.5–4 except for $H_{1.5}$ and D_4 (data not shown).

The narrow-sense heritability (h^2) estimates with hybrid growth and wood density ranged between 0.07 ± 0.05 ($H_{0.5}$) and 0.17 ± 0.07 ($H_{7.5}$) over the different ages (Table 2), suggesting that both growth and wood density were under a low level of additive genetic control. The d^2 estimates for both trait types were also small, with the lowest 0.02 ± 0.05 in $D_{7.5}$ and the highest 0.23 ± 0.10 in $H_{1.5}$, suggesting a weak effect of dominance on these traits. It seems that growth has an increasing h^2 with advancing age while a reverse trend holds true for d^2 . In addition, h^2 values estimated from the OP families were markedly larger than those of hybrids, ranging from 0.22 in H_4 to 0.70 in $H_{1.5}$ (data not shown).

Phenotypic and additive genetic correlations

Additive genetic correlations of $H_{7.5}$ and $D_{7.5}$ with $WD_{7.5}$ for the hybrid population across the two environments were 0.68 ± 0.48 and 0.64 ± 0.36 , respectively, which were non-significant using a threshold of ± 2 standard errors, though phenotypic correlations were moderately strong and positive (Table 3). The non-significant genetic correlations may suggest that selection in one trait is unlikely to have any effect on the hybrid performance in the alternative trait. Additionally, there were

very strong phenotypic and genetic correlations either between H and D at a specific age or between ages for a given growth trait H or D (data not shown).

Maternal GCA, parental GHA and SHA estimates

Maternal GCA , parental GHA and SHA effects were estimated for growth and wood density only at age 7.5 (Tables 4 and 5). The females U32-23 and UX-07 and male T25-08 were the best general combiners for $H_{7.5}$ and/or $D_{7.5}$ with highly significant positive GHA effects. For $WD_{7.5}$, the females UX-04 and UX-30 and males T25-08 and TX-04 exhibited GHA effects no less than 0.10 (10^{-1}) though no GHA effect was positively significant. When overall hybridizing ability for both growth and wood density was considered, female UX-30 and male T25-08 appeared to be good combiners in improving both types of traits. In addition, maternal GCA effect was not necessarily a good indicator of GHA , e.g. the correlations between GCA and GHA being 0.48 ($P=0.17$), 0.65 ($P=0.04$) and -0.56 ($P=0.10$) for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$, respectively.

For $H_{7.5}$ and $D_{7.5}$, the combination UX-07 \times T25-08 had the highest SHA values (2.79 m and 2.76 cm, respectively; Table 5) and also provided the best growth performance (20.98 m and 17.79 cm, respectively; Table 6). For wood density $WD_{7.5}$, UX-30 \times TX-04 and U31-38 \times TX-04 had the highest SHA values [0.30 and 0.27 (10^{-1}), respectively; Table 5] as well as the highest overall values in this trait [4.48 and 4.54 (10^{-1}), respectively].

Table 6. – The best five hybrids in female-parent heterosis (FPH) for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$.

Trait	Rank in FPH	Hybrid	Mean \pm SD	AFPH	RFPH (%)
$H_{7.5}$ (m)	1	U89-16 \times T25-08	17.54 \pm 3.88	6.24	42.2
	2	U89-16 \times T25-09	16.85 \pm 4.12	5.56	37.6
	3	UX-07 \times T25-08	20.98 \pm 1.95	5.46	36.9
	4	U89-16 \times T44-20	16.12 \pm 3.88	4.82	32.6
	5	UX-20 \times T44-20	16.36 \pm 3.70	4.78	32.3
$D_{7.5}$ (cm)	1	U89-16 \times T25-08	14.62 \pm 4.75	5.49	47.2
	2	UX-07 \times T25-08	17.79 \pm 3.54	4.92	42.2
	3	UX-30 \times TX-04	14.48 \pm 5.04	4.27	36.7
	4	U89-16 \times T25-12	13.30 \pm 4.82	4.18	35.9
	5	UX-30 \times T60-09	14.38 \pm 4.84	4.18	35.9
$WD_{7.5}$ (10^{-1})	1	U89-16 \times T25-12	4.33 \pm 0.32	0.82	22.0
	2	U89-16 \times T43-31	4.31 \pm 0.49	0.81	21.7
	3	U89-16 \times T25-10	4.27 \pm 0.71	0.77	20.6
	4	U89-16 \times T25-08	4.27 \pm 0.56	0.77	20.5
	5	UX-30 \times TX-04	4.48 \pm 1.03	0.76	20.2

AFPH, absolute female-parent heterosis; RFPH, relative female-parent heterosis.

Heterosis

The female-parent heterosis (*FPH*) of each hybrid was estimated for growth and wood density at age 7.5. The magnitude of heterosis varied with trait and hybrid. The best five hybrids in *FPH* for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$ are shown in Table 6. Compared with maternal OP families, 45, 48 and 37 hybrids showed positive *FPH* estimates for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$ (data not shown), with the highest relative *FPH* (*RFPH*) being 42.2% (U89-16 × T25-08), 47.2% (U89-16 × T25-08) and 22.0% (U89-16 × T25-12), respectively. On average, the *FPH* values observed were all positive, being 1.64 m (*RFPH* 11.1%), 1.50 cm (12.8%) and 0.12 (10⁻¹; 3.0%) for $H_{7.5}$, $D_{7.5}$ and $WD_{7.5}$, respectively.

Discussion

The generally better performance of *E. urophylla* × *E. tereticornis* hybrids relative to *E. urophylla* pure-species OP progeny, along with the relatively large number of female-parent heterotic hybrids, could provide evidence for heterosis even between inter-sectional species. Further, *E. urophylla* × *E. tereticornis* hybrids are easy to propagate vegetatively, especially through tissue culture (LIU and WANG, 2005). These will therefore warrant the efforts in hybrid breeding with respect to heterosis exploitation and genetic gain realization.

The contrasting comparison of hybrid performance between the two environments may be a reflection of the difference in adaption between the two population types. *E. urophylla* × *E. tereticornis* hybrids are usually more cold tolerant and adaptive to the cold winter besides favorable rainfall and soil conditions in Yong'an, whereas *E. urophylla* OP families exhibited rapid growth in low-latitude regions (ELDRIDGE et al., 1993) and could be more competitive at the warm but less fertile site of Changtai. Also, such an environmental difference, coupled with significant hybrid × environment interaction at most ages, may indicate essentially that the expression of hybrid superiority is often highly dependent on the environmental conditions which may limit performance of one or other of the pure-species parents (POTTS and DUNGEY, 2004). Similarly, MALAN and VERRYIN (1996) noticed environmental sensitivity of *E. grandis* derived hybrids in wood properties, and LEE et al. (2001) observed marked environmental variation in survival and growth of eucalypt hybrids. Climatic and edaphic conditions of the local environment as well as adaptability of hybrids are most likely to account for such differences. This may also evoke the necessity of multiple environments for comprehensive assessment of hybrid performance and for reliable estimation of genetic parameters.

The h^2 and d^2 estimates for hybrid growth are within the low range as compared to earlier reports with interspecific hybrids in *Eucalyptus*. BOUVET and VIGNERON (1995) observed h^2 of 0.00 to 0.45 for H and D up to 48 months in *E. urophylla* × *E. grandis* and *E. urophylla* × *E. pellita* F. MUELL. GAN et al. (2004) obtained h^2 values ranging from 0.34 to 0.41 for 5-year-old H and D in *E. urophylla* × *E. tereticornis*, *E. urophylla* × *E. camaldulensis* DEHNH. and *E. urophylla* × *E. pellita*. VOLKER

et al. (2008) reported h^2 of 0.42–0.61 and d^2 0.00–0.52 for D measured at one site over ages 2 to 10 in *E. nitens* × *E. globulus*. These authors used only single-site data, in which the additive and dominant genetic effects would include the site factors and therefore be inflated, as evidenced by multiple-location trials of *E. grandis* × *E. tereticornis* and *E. grandis* × *E. camaldulensis* hybrids (MADHIBHA et al., 2013). Nevertheless, the relatively stable h^2 values over ages are in accordance with the results obtained from hybrids of *E. nitens* × *E. globulus* (VOLKER et al., 2008), *E. urophylla* × *E. grandis* (BOUVET and VIGNERON, 1995; RETIEF and STANGER, 2009), *E. grandis* × *E. tereticornis* and *E. grandis* × *E. camaldulensis* (MADHIBHA et al., 2013). Moreover, the h^2 and d^2 estimates for hybrid wood density are comparable to previous reports, e.g. h^2 0.20 and d^2 0.02 for Pilodyn-based wood density on one site at age 6 in *E. nitens* × *E. globulus* (VOLKER et al., 2008), and the non-significant interaction of genotype by environment identified in the present study might partially explain such a comparability even with different number of trial sites.

Given that the estimates of additive genetic variance in pure-species and hybrid populations are not theoretically comparable (GORDON, 1999), h^2 values derived here from hybrid populations could have limited usages in prediction of hybrid performance and genetic gains following a classical quantitative genetic model. Alternatively, the generally low correlations between *GCA* and *GHA* would suggest that pure-species breeding values are not useful for identifying parents that produce hybrids with faster growth and higher wood density. By comparison, VOLKER et al. (2008) observed in *E. nitens* × *E. globulus* similar correlations between maternal *GCA* and *GHA* in diameter (0.35–0.69 across ages) but a considerably higher correlation in wood density (0.68 at age 6). Nonetheless, the significant correlation between *GCA* and *GHA* for $D_{7.5}$ (0.65; $P=0.04$) may indicate that pure-species selection prior to hybridization, at least for maternal *E. urophylla*, would be efficient in producing outstanding hybrids for diameter trait rather than height and wood density.

The positive *FPH* values verified with *E. urophylla* × *E. tereticornis* would suggest hybrid superiority or heterosis for inter-sectional combination of species with so large geographic and genetic distance. However, outbreeding depression or hybrid abnormality has been observed frequently in eucalypts, particularly for species from the section *Maindenaria* (POTTS and DUNGEY, 2004; VOLKER et al., 2008; COSTA E SILVA et al., 2012). Thus, it seems that heterosis may arise between certain species regardless of their origin from the same or different sections. However, it should be noted that such superiority may be combination specific as there are still some inferior hybrids, which could involve the best parents.

The usefulness of the hybrid superiority has to be carefully considered as it is unlikely to deploy the superior hybrids through a seed orchard strategy when clonal forestry has been largely preferred for those species and hybrids amenable to mass vegetative propagation. Clonal deployment of the elite individuals from the superior hybrids may be an option, but their vegetative

propagation ability and their propagule (ramet) performance have to be tested. YU et al. (2011) have identified different quantitative trait loci (QTLs) controlling growth and rooting ability in *E. urophylla* × *E. tereticornis* hybrids, suggesting the difference in genes responsible for the two types of traits. Alternatively, such elite individuals could be applied to further hybridization to produce next-generation hybrids, but the operational potential has to be explored as severe depression may occur in F₂ hybrids (COSTA E SILVA et al., 2012).

In conclusion, the significant effects of *GHA* and *SHA* and the sound number of heterotic hybrids imply that selection of parents for hybridizing ability and heterosis has a potential to increase growth and wood density in *E. urophylla* × *E. tereticornis* hybrid breeding.

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