

# Strong, independent, quantitative genetic control of the timing of vegetative phase change and first flowering in *Eucalyptus globulus* ssp. *globulus* (Tasmanian Blue Gum)

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Analyses of large open-pollinated and controlled-cross field trials of *Eucalyptus globulus* ssp. *globulus* show quantitative genetic independence of the times of first flowering and the abrupt change in leaf form. The onset of each of these critical developmental events is under moderate to strong additive genetic control in this taxon, with individual narrow-sense heritabilities of 0.4–0.6 and 0.2–0.9, respectively, and little nonadditive genetic control. This strong additive genetic control and the independence of these processes provide the genetic environment for rapid heterochronic microevolution.

**Keywords:** genetic correlation, heritability, heteroblasty, heterochrony, microevolution, ontogeny.

## Introduction

A knowledge of the quantitative genetic control of the timing of developmental processes is basic to understanding a major mode of evolution, heterochrony. Heterochrony involves changes in the timing or rate of such processes (McKinney & McNamara, 1991), especially changes in the order of developmental events (Klingenberg, 1998). It may be a means of rapid morphological evolution, because it only requires alteration of the timing of events, rather than the loss or creation of structures. For example, in plants with marked ontogenetic changes in morphology, small genetic changes can result in marked alterations to the phenotype of mature plants (Wiltshire *et al.*, 1994). The potential for heterochronic microevolution is dependent upon the strength of genetic control, and the degree of quantitative genetic independence, of the timing of different developmental processes.

Many plants show an abrupt vegetative phase change (Guerrant, 1988). These heteroblastic plants produce juvenile foliage for some time but, after a brief transition, produce significantly different new foliage. This means that two critical, discrete, developmental events (vegetative phase change and first flowering) can

be clearly observed, making them especially suited to studies of the quantitative genetics of the timing of developmental events. Klingenberg (1998) implied that different developmental processes were linked, based on studies of animals. However, recent work on heteroblastic plants suggests that the timing of first flowering and that of vegetative phase change are genetically (Wiltshire *et al.*, 1994, 1998; Abedon *et al.*, 1996) and physiologically (Hasan & Reid, 1995) independent. However, such studies are rare (Wiltshire *et al.*, 1998), partly because of the long life cycles of most species with stable, distinct vegetative phases (Poethig, 1990).

This study uses data from four large field trials to examine the quantitative genetic control of the timing of the striking change in foliage in *Eucalyptus globulus* ssp. *globulus* (Fig. 1) and of first flowering. This forest tree (henceforth referred to as ssp. *globulus*) is widespread in south-eastern Australia (Jordan *et al.*, 1993) and has bisexual flowers and a mixed mating system (Hardner & Potts, 1995; Hardner *et al.*, 1998). It is a member of a large genus, of  $\approx 700$  species (Williams & Brooker, 1997), in which heterochronic evolution appears to have been important (Potts & Wiltshire, 1997). High levels of genetic differentiation occur among populations of ssp. *globulus* for the timing of both transition to adult foliage (Dutkowski & Potts, 1999) and first flowering (Chambers *et al.*, 1997). At one extreme, slow-growing plants from an exposed coastal clifftop (Wilson's Promontory)

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**Fig. 1** The transition from juvenile (top left) to adult leaf shape (bottom right) along consecutive nodes of a branch of *Eucalyptus globulus* ssp. *globulus*. The first leaf illustrated grew at  $\approx 1.5$  m height. Juvenile leaves are sessile, opposite and glaucous, and grow transversely on quadrangular stems. Adult leaves are petiolate, alternate and shiny green, and are pendulous on cylindrical stems.

change to adult foliage and may produce flower buds in the first year of growth. At the other extreme, fast-growing trees from populations in the north-east of the island of Tasmania may still be vegetatively and reproductively juvenile after 4 years. However, all healthy individuals in natural populations and in field trials eventually undergo vegetative phase change. All healthy individuals probably also flower, although this is harder to determine because flowering is suppressed in field trials after canopy closure (often at about 4 or 5 years).

Two experiments are examined: one comprising a factorial crossing scheme and related open-pollinated families planted in a single field trial; the other comprising open-pollinated progeny from native stand seed collections from throughout the geographical range of the subspecies and its intergrades with other subspecies planted in three trials. These experiments are used to determine: (i) the relative levels of additive and non-additive genetic control of first flowering and vegetative phase change; (ii) the value of open-pollinated progeny in estimating additive genetic variation (see Hodge *et al.*, 1996); (iii) the hierarchical partitioning of quantitative genetic variation in natural populations; and (iv) the degree of genetic covariation between vegetative phase change and first flowering. This work is unique in segregating the additive and nonadditive variation for vegetative phase change and flowering traits of natural populations of a species.

## Materials and methods

### *Genetic material and trials*

*Experiment 1: the West Ridgley factorial trial* The West Ridgley factorial trial included controlled-cross progeny from a virtually complete factorial crossing scheme using 26 male parents and eight female parents. The male parents were from natural populations at Taranna, south-eastern Tasmania (16 trees), and on King Island, northern Tasmania (10 trees). The female parents were trees in a seed orchard grown from open-pollinated seed from natural populations at Taranna (four trees), King Island (three trees) and south Flinders Island (one tree). The trial also contained progeny from open-pollinated seed collected from the same trees that provided pollen for the controlled crosses. The trial was established in a randomized, resolvable, incomplete block design with four complete replicates and 15 incomplete blocks per replicate. Each incomplete block contained between 10 and 14 families used in this study. Each family was represented in each replicate by five trees side by side in line plots (Hodge *et al.*, 1996). Selfs and abnormal trees, such as runts and multistems, were excluded from the analyses, leaving 168 controlled-cross families (2127 trees) and 24 open-pollinated families (284 trees) for each trait. Trial and crossing details are given in Hodge *et al.* (1996). The trial also contained some unrelated material not considered in this work.

*Experiment 2: the range-wide open-pollinated family trials* Three field trials (West Ridgley open-pollinated, Massy Greene and Latrobe) were grown from open-pollinated seed collected from almost 600 trees in natural populations throughout the range of ssp. *globulus*. Details of this collection and trials are given in Potts & Jordan (1994) and Jordan *et al.* (1994). The seed collections were classified into a geographical hierarchy of 49 localities of about 10 km radius and 13 races (Potts & Jordan, 1994; Dutkowski & Potts, 1999). Most families were represented in each trial. The trials followed a resolvable incomplete block design with five complete replicates, 23–25 incomplete blocks per replicate and 20 families per block. Families within blocks were planted in two-tree contiguous plots. Abnormal plants and trees from three atypical populations, Lighthouse (Wilson's Promontory), Mt Dromedary and Port Davey (see Dutkowski & Potts, 1999), were excluded from the analyses.

### Traits measured

Vegetative and reproductive phase changes in *Eucalyptus globulus* ssp. *globulus* are most likely to be initiated by a form of developmental age related to the number of nodes set rather than absolute age or size. This has been shown for *Eucalyptus tenuiramis* (Wiltshire & Reid, 1992) and appears to be the case for *E. globulus* ssp. *globulus* (G. J. Jordan & B. M. Potts, unpubl. data). However, it was not practicable to count the number of nodes for the very large number of plants in these experiments, so a number of surrogates (Table 1) was used. Two separate measures were used as surrogates for the timing of vegetative phase change. First, the height of phase change was measured as the height, in metres, of the first petiolate leaf growing on the main stem. The transition from juvenile to adult foliage occurs over a number of nodes (see Fig. 1) and can vary slightly in character from plant to plant. The first petiolate leaf almost always develops near the middle of the transition, so provides a repeatable measure of the phase change.

The second surrogate was vegetative juvenility at 2 years, a binary trait (0 = presence of adult foliage; 1 = absence) derived from the height to phase change at 2 years. The binary trait is assumed to represent plants reaching a threshold in an underlying continuous trait and to estimate the genetic and nongenetic variation in this underlying trait. In the case of vegetative juvenility, the underlying trait is the time to vegetative phase change, although not necessarily on a linear scale. Large values of vegetative juvenility and height to phase change each reflect extensions of the juvenile phase, but they are affected differently by growth rates: the environmental correlations of measures of early growth

**Table 1** Traits analysed in *Eucalyptus globulus* ssp. *globulus*, means and transformations used to remove correlations between means and standard deviations

Trait	Source data	Trial	Mean
Height to phase change	$\sqrt{(\text{Height to phase change})}$	WRFA	2.055 $\sqrt{\text{m}}$ (CP)
		MG	1.977 $\sqrt{\text{m}}$ (OP)
Vegetative juvenility	Absence of phase change at 2 years	MG	0.10
		WRFA	0.44 (CP)
Reproductive juvenility	Absence of flowering by 4 years	MG	0.63 (OP)
		LA	0.65
		WRFA	0.89
		WROP	0.85

For binary traits, the means are equivalent to incidences. Separate means are given for controlled-cross (CP) and open-pollinated (OP) data in the West Ridgley factorial trial. WRFA, West Ridgley factorial; MG, Massy Greene; WO, Woolnorth; WROP, West Ridgley open-pollinated.

with the height to phase change are positive, and those with vegetative juvenility are negative (G. J. Jordan & B. M. Potts, unpubl. data). These environmental correlations reflect associations that are independent of genotype. This is consistent with the hypothesis that the underlying determinant of vegetative phase change is the number of nodes set, because greater numbers of nodes set and longer internodes in fast-growing plants will decrease the age but increase the height of phase change.

Only four out of five replicates planted at Massy Greene were scored for cost reasons. The height of vegetative phase change was measured at 2, 3 and 4 years after planting in both the West Ridgley factorial and the Massy Greene trials, and at 5 years at Massy Greene. A few plants (<1%) were still juvenile at Massy Greene at 5 years. Because treating these plants as missing values could bias the results, the height of phase change was estimated by multiplying the height at 4 years by 1.5 to allow for growth. Vegetative juvenility was analysed at Massy Greene but not at West Ridgley because of low incidence at the times of measurement (<7%).

The only surrogate for reproductive phase change was reproductive juvenility (the absence of flowering at a given age), a binary trait that reflects the time of first flowering in a similar way to that in which vegetative juvenility reflects the time to phase change. This trait had negative environmental correlations with measures of early growth (G. J. Jordan & B. M. Potts, unpubl. data), so the manner in which growth rate affects development is similar to that in vegetative juvenility. Trees in the range-wide, open-pollinated family trials were scored as reproductively adult (0) at 4 years if capsules, flowers or flower buds were present at 4 years,

or (only at Massy Greene) if either flowers or flower buds were present at 2 or 3 years. Reproductive juvenility in the West Ridgley factorial trial was based on the presence of flowers at 3 years.

### Statistical analyses

The program ASREML, which uses the average information algorithm and sparse matrix technology (Gilmour *et al.*, 1995, 1997), was used to calculate restricted maximum likelihood variances and covariances for the random effects in the mixed models used. These variances and covariances are uniquely attributable to levels in the hierarchical sections of the designs used here. Those relating to the family, locality or race are purely genetic. Incomplete block variances and covariances reflect mesoenvironmental effects on phenotype independent of genotype. Residual effects reflect both genetic and environmental variation.

*West Ridgley factorial trial* Three separate types of analysis were performed on the West Ridgley factorial data. Cross type (provenance for open-pollinated data, the combination of maternal and paternal provenance for controlled-cross data) and replicate were treated as fixed. The incomplete blocks within replicates effects were treated as random. Plot within replicate effects were small and ignored in these analyses. The first type of analysis used only the controlled-cross data and fitted an individual tree model with a matrix of relationships derived from the pedigree of each tree to estimate additive and nonadditive (SCA) variances (Gilmour *et al.*, 1997). The second type of analysis followed a bivariate individual tree model with the same effects as the univariate model for each trait and unconstrained covariances between traits. The third type used a parent model of all data to estimate covariances between controlled-cross and open-pollinated parents and among-family variances from the open-pollinated data. The genetic effects, female (both controlled-cross and open-pollinated), male and family (controlled-cross only) were treated as random. Covariances were calculated between open-pollinated females and controlled-cross males. An individual tree model was not used here because of biases induced by the unknown male parents for the open-pollinated progeny. Additive and non-additive genetic variances derived from the parent model were consistent with those calculated with the individual tree model. Univariate analyses of binary traits used binomial models with a probit link function.

The significance of variance and covariance components was tested by likelihood ratio tests assuming an approximate  $\chi^2_{0.5}$  distribution for  $-2\log L$  for variances and  $\chi^2_1$  distribution for covariances (Stram & Lee, 1994).

Tests of variances in univariate analyses compared models including and excluding the relevant effect. Where the effect had another effect nested within it, the comparison excluded the nested effect. Tests of covariances (and hence correlations) compared the full model with one in which the covariance was constrained to be zero.

Individual narrow-sense heritabilities ( $h^2$ ) were calculated as

$$h^2 = \frac{\sigma_{\text{add}}^2}{\sigma_{\text{add}}^2 + \sigma_{\text{SCA}}^2 + \sigma_{\text{e}}^2},$$

where  $\sigma_{\text{add}}^2$ ,  $\sigma_{\text{SCA}}^2$  and  $\sigma_{\text{e}}^2$  are the additive within cross type, SCA within cross type and residual variances from the univariate individual tree model, respectively. These were compared with estimates of individual narrow-sense heritabilities made from the open-pollinated progenies as

$$h_{\text{OP}}^2 = \frac{\sigma_{\text{fam(ctype)}}^2}{r(\sigma_{\text{fam(ctype)}}^2 + \sigma_{\text{e}}^2)},$$

where  $\sigma_{\text{fam(ctype)}}^2$  is the among-family within-cross-type variance and  $r$  is the coefficient of relatedness.  $r$  is assumed to be 0.4, which is equivalent to an outcrossing rate of 70% and is a common assumption in analyses of open-pollinated eucalypt families (Griffin & Cotterill 1988; Potts & Jordan 1994). These estimates assume that nonadditive variances are small. Heritabilities were assumed to be significant if the additive or, for open-pollinated data, the family within-locality variances were significantly greater than zero.

The dominance ratio,  $d^2$ , an estimate of the relative significance of dominance assuming no higher-order gene interactions, such as epistasis (Becker, 1985), was calculated from the variances estimated from the univariate individual tree model as

$$d^2 = \frac{4\sigma_{\text{SCA}}^2}{\sigma_{\text{add}}^2 + \sigma_{\text{SCA}}^2 + \sigma_{\text{e}}^2}.$$

Genetic correlations were calculated from variance and covariance components according to the general formula

$$r_{s(1,2)} = \frac{\sigma_{s(1,2)}}{\sqrt{\sigma_{s(1)}^2 \sigma_{s(2)}^2}},$$

where  $r_{s(1,2)}$  is the correlation between traits 1 and 2 at level  $s$  (e.g. the additive genetic effects),  $\sigma_{s(1,2)}$  is the covariance between the traits at that level, and  $\sigma_{s(1)}^2$  and  $\sigma_{s(2)}^2$  are the variance components for each trait at that level. Correlations between open-pollinated female parents and controlled-cross male parents were calculated

**Table 2** Genetic parameters in *Eucalyptus globulus* ssp. *globulus* from the West Ridgley factorial trial

Trait	$\sigma_{\text{add}}^2$	$\sigma_{\text{SCA}}^2$	$\sigma_{\text{add(OP)}}^2$	$h^2$	$d^2$	$h_{\text{OP}}^2$	$r_g$
Height to phase change	0.046 ± 0.013	0.0020 ± 0.0007	0.070 ± 0.028	0.67 ± 0.13	0.11 ± 0.05	0.74 ± 0.22	0.95 ± 0.06
Reproductive juvenility	0.28 ± 0.06	0.04 ± 0.02	1.58 ± 0.65	0.21 ± 0.04	0.12 ± 0.07	0.93 ± 0.38	0.86 ± 0.11

The narrow-sense heritabilities ( $h^2$ ), additive variances ( $\sigma_{\text{add}}^2$ ), nonadditive variances ( $\sigma_{\text{SCA}}^2$ ) and dominance ratios ( $d^2$ ) estimated from the controlled-cross data are given as well as the narrow-sense heritabilities ( $h_{\text{OP}}^2$ ) and additive variances ( $\sigma_{\text{add(OP)}}^2$ ) estimated from the open-pollinated data, and the genetic correlations between parental effects under controlled-crossing and open-pollination ( $r_g$ ). Heritabilities and correlations are all highly significantly greater than zero ( $P < 0.01$ ). Dominance ratios and open-pollinated heritability estimates are all significantly greater than zero ( $P < 0.05$ ).

from variances and covariances estimated with the univariate parent model. These genetic correlations are based on genetic effects nested within cross type and indicate the degree to which additive genetic effects are reflected in the genetic effects for open-pollinated families. Thus, a very strong correlation would imply that genetic differences between open-pollinated families would reflect additive genetic differences and, hence, be available for natural selection. Correlations between vegetative phase change and flowering traits were calculated from the additive variances and covariances estimated with the bivariate analyses of controlled-pollinated data. These correlations indicate the degree of additive genetic dependence of the traits.

*Range-wide, open-pollinated family trials* Univariate analyses, bivariate analyses between traits within the Massy Greene trial and bivariate analyses between traits in different trials were performed on these data using ASREML. The analyses followed parent models with random genetic effects for race, locality within races and family within localities and a random experimental effect for incomplete blocks within replicates. The only fixed effect was replicate. Univariate analyses of binary traits used binomial models with a probit link function. Within-site bivariate analyses allowed unconstrained covariances, but residual covariances were constrained to a small value in between-site analyses. The significance of variances and covariances was tested as described above.

Narrow-sense heritabilities were calculated from the variances estimated in the univariate analyses as

$$h_{\text{OP}}^2 = \frac{\sigma_{\text{fam(loc)}}^2}{r(\sigma_{\text{fam(loc)}}^2 + \sigma_{\text{plot}}^2 + \sigma_{\epsilon}^2)},$$

where  $\sigma_{\text{fam(loc)}}^2$  is the family-within-locality variance.

Genetic correlations were calculated at the race and family within localities levels based on the variances and covariances estimated in the bivariate analyses according to the formula described above.

## Results

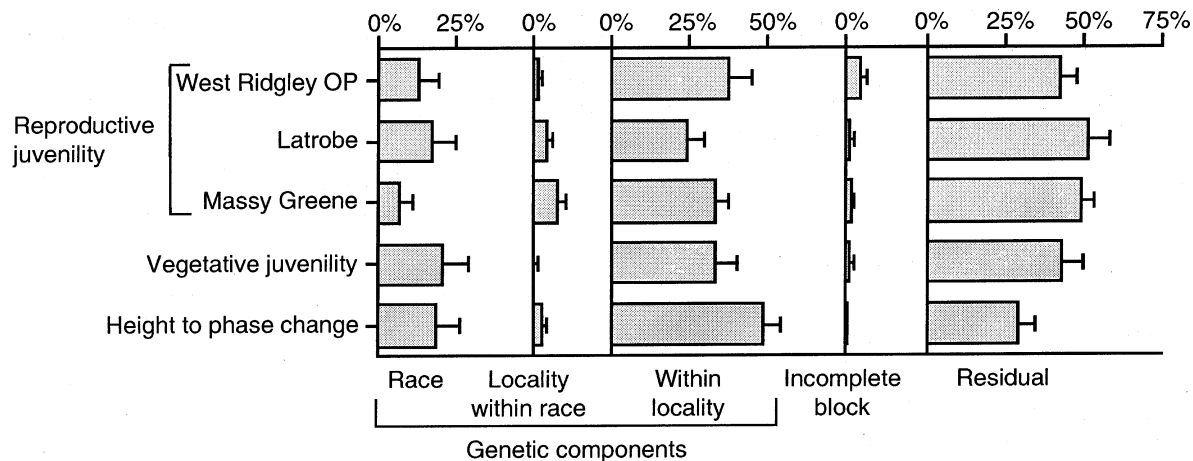
### *Additive vs. nonadditive genetic variation*

Reproductive juvenility was moderately heritable and the height of vegetative phase change highly heritable in the West Ridgley factorial trial ( $h^2 = 0.21$  and  $0.67$ , respectively; Table 2). There was very little nonadditive genetic variation for these traits ( $d^2 = 0.12$  and  $0.11$ , respectively; Table 2). The timing of vegetative phase change and of first flowering was therefore under moderate to strong additive genetic control in this trial, with little nonadditive genetic variation.

For height to phase change, the estimates of narrow-sense heritability from open-pollinated data and from controlled-cross data were similar but, for reproductive juvenility, the estimate from open-pollinated data was higher than that from controlled-cross data. However, for both traits, the genetic correlations between the controlled-cross and open-pollinated genetic effects from the same parents were very high ( $r_g > 0.85$ ; Table 2). This implies that genetic analysis of open-pollinated data closely reflects the additive genetic control for the timing of vegetative phase change, and quite closely for first flowering.

### *Variation in range-wide, open-pollinated family trials*

In the range-wide, open-pollinated trials, large proportions (46–70%) of the phenotypic variation for the vegetative phase change and flowering traits were attributable to genetic variation (Fig. 2). The greatest proportion of genetic variation occurred within localities (24–48%), but high proportions also occurred among races (7–21%). There was little variation among localities within races except for reproductive juvenility at Massy Greene (7.6%). Variation among incomplete blocks (within replicates) was small in all cases (< 5.2% of the total variation), implying that mesoenvironmental effects are small, because these components are



**Fig. 2** Percentages of the total variance attributable to each effect for vegetative phase change and reproductive traits in the open-pollinated trials of *Eucalyptus globulus* ssp. *globulus*. All the genetic variances are highly significantly greater than zero ( $P < 0.001$ ), and the other variances are all significant ( $P < 0.05$ ). The within-locality variance is 2.5 times the family-within-locality variance to allow for the open-pollinated origin. The residual variance has been reduced by the amount added to the family-within-locality variance.

independent of genotype. There was a large amount of genetic variation for each trait. Races ranged in mean height to phase change from 3.3 m to 4.9 m, and in the proportion flowering at 4 years from 48% to 84%. The genetic variation in the absolute time to flowering and vegetative phase change are difficult to determine accurately, but the phenotypic variation was large (vegetative phase change ranged from 1 year to more than 5 years, and first flowering ranged from 2 years to more than 5 years in the Massy Greene trial). This, combined with the high proportion of genetic variation for the binary surrogates, implies that the absolute genetic variation was large.

The height to phase change, vegetative juvenility and reproductive juvenility were all highly heritable within these trials ( $h^2 > 0.32$ ; Table 3; see also Chambers

*et al.*, 1997). These heritabilities were slightly lower than, but comparable with, those in the West Ridgley factorial trial (Tables 2 and 3).

The family-within-locality and race genetic correlations between the two measures of the timing of vegetative phase change were very high ( $r = 0.7 \pm 0.05$  and  $0.91 \pm 0.07$ , respectively) and highly significantly greater than zero ( $P < 0.0001$ ), indicating that these traits primarily measured the same underlying biological trait. The lack of a perfect among-family correlation between these traits was probably caused by their opposite responses to growth rates. Nonadditive genetic effects may also have contributed, because the among-family additive genetic correlations of the corresponding traits in the West Ridgley factorial trial were very strong ( $r = 0.98 \pm 0.04$ ). The strong genetic correlations

**Table 3** Estimated heritabilities ( $h_{OP}^2$ ) with standard errors, total within-replicate variance ( $\sigma_{phen}^2$ ) and within-locality genetic variance ( $\sigma_{add}^2 = 2.5 \times \sigma_{family\ within\ locality}^2$ ) for vegetative phase change traits and reproductive juvenility in the range-wide, open-pollinated family trials of *Eucalyptus globulus* ssp. *globulus*

	Number of families	Number of trees	$\sigma_{phen}^2$	$\sigma_{add}^2$	$h_{OP}^2$
Vegetative phase change traits at Massy Greene					
Height to phase change	565	3934	0.0685	$0.33 \pm 0.02$	$0.62 \pm 0.05$
Vegetative juvenility	565	4298	1.5713	$0.52 \pm 0.10$	$0.43 \pm 0.07$
Reproductive juvenility					
Massy Greene	565	4298	1.2619	$0.48 \pm 0.07$	$0.40 \pm 0.05$
Latrobe	544	5009	1.4988	$0.37 \pm 0.07$	$0.32 \pm 0.06$
West Ridgley	429	3819	1.5296	$0.57 \pm 0.11$	$0.46 \pm 0.07$

All heritabilities are highly significantly greater than zero ( $P < 0.001$ ).

among the measured traits suggest that they are measuring the same biological trait and, hence, are adequate surrogates for the underlying determinant of the timing of vegetative phase change.

**Correlations between vegetative phase change and first flowering**

The additive genetic correlation in the West Ridgley factorial trial between height to phase change and reproductive juvenility was small and not significantly greater than zero ( $0.17 \pm 0.20$ ;  $P > 0.05$ ). Genetic correlations within localities of reproductive juvenility with both height to phase change and vegetative juvenility at Massy Greene were also small and not significant ( $0 < r < 0.03$ ; Table 4). The time and height of vegetative phase change are therefore genetically independent of the time of first flowering in two trials containing unrelated genetic material. This independence no doubt applies to the underlying control of vegetative phase change and first flowering, because it occurs for both height to phase change and vegetative juvenility, which respond in opposite ways to variation in growth rates. The correlations across sites (Massy Greene with Latrobe and West Ridgley open-pollinated) also support this conclusion ( $0 < r < 0.16$ ; Table 4). Although genetic correlations of first flowering are high (Chambers *et al.*, 1997), the possibility that site-by-genotype interaction for the timing of vegetative phase change may have affected these correlations cannot be excluded. The race-level correlations were not significant ( $P > 0.05$ ; Table 4), but races with early vegetative phase change also tended to flower early ( $r > 0.40$ ).

**Table 4** Genetic correlations of reproductive juvenility in *Eucalyptus globulus* ssp. *globulus* in the Massy Greene (MG), West Ridgley open-pollinated (WROP) and Latrobe (LA) trials with vegetative phase change traits in the Massy Greene trial

Stratum	Trial	Height to phase change	Vegetative juvenility
Family within locality	MG	$0.00 \pm 0.08$	$0.03 \pm 0.09$
	WROP	$0.16 \pm 0.08$	$0.06 \pm 0.10$
	LA	$0.13 \pm 0.08$	$0.01 \pm 0.09$
Race	MG	$0.70 \pm 0.27$	$0.55 \pm 0.30$
	WROP	$0.40 \pm 0.34$	$0.42 \pm 0.32$
	LA	$0.47 \pm 0.30$	$0.46 \pm 0.29$

None of the correlations is significantly greater than zero ( $P > 0.05$ ).

**Discussion**

**Genetic variation for first flowering and vegetative phase change**

The range-wide, open-pollinated trials and the controlled-cross factorial experiment both show strong genetic control ( $h^2 > 0.4$ ) of the timing of two developmental events critical to heterochronic evolution in ssp. *globulus*. The underlying determinant (presumably developmental age) of the timing of these events may be under even stronger genetic control, because the surrogates used here are affected by growth, which is less heritable (usually  $h^2 < 0.3$ ; Jordan *et al.*, 1998).

The quantitative genetic control of the timing of first flowering and vegetative phase change was shown to be largely additive in the factorial experiment, with trivial nonadditive genetic contributions. This is important, because the additive variation is the part of quantitative genetic variation that can be transferred between generations and, hence, is the chief determinant of the response of populations to natural selection (Falconer, 1989). The estimates of genetic variation and covariation from the open-pollinated trials assume random mating within populations, but ssp. *globulus* often shows high and variable levels of self-pollination (Borrhalho & Potts, 1996; Hardner *et al.*, 1998), which can increase the effects of dominance and result in variable inbreeding depression. However, the almost perfect genetic correlations between open-pollinated and controlled-cross measures of first flowering and vegetative phase change in the factorial trial imply that the open-pollinated families reflect essentially the same genetic variation among parents as estimated from the controlled-cross families. The heritabilities and correlations estimated from open-pollinated progeny should therefore primarily reflect additive genetic effects. This is in marked contrast to growth, where additive and nonadditive effects are weak and of similar size ( $h^2 < 0.1$ ,  $d^2 < 0.15$ ; Vaillancourt *et al.*, 1995), inbreeding depression is high (Hardner & Potts, 1995), and the genetic correlations between controlled-cross and open-pollinated progeny performance are virtually zero (Hodge *et al.*, 1996).

**Genetic independence of first flowering and vegetative phase change**

There is no quantitative genetic association between the timing of phase change and of first flowering. Even though flowering usually follows vegetative phase change, first flowering is unlikely to be conditional on the presence of vegetative phase change, because flowering occasionally occurs in *E. globulus* ssp. *globulus*

during the juvenile vegetative phase (for example in 17 out of 4298 healthy trees at Massy Greene), and flowering in the juvenile phase can be induced chemically with no effect on vegetative phase change beyond that expected from the growth effects of the treatment (Hasan & Reid, 1995).

Quantitative genetic independence of vegetative phase change and first flowering has now been shown in two species of *Eucalyptus* (present study; Wiltshire *et al.*, 1998) and in sweet corn (Abedon *et al.*, 1996), suggesting that it may be widespread in plants. In particular, the Wiltshire *et al.* (1998) study of the *Eucalyptus tenuiramis* complex showed that flowering is not conditional on the presence of adult foliage, because first flowering occurs in both the juvenile and the adult vegetative phases, depending primarily on population. This genetic independence is in marked contrast to the strong covariation in the timing of different ontogenetic changes found in studies of many animals (Klingenberg, 1998 and papers cited therein).

*Eucalyptus globulus* ssp. *globulus* is from a genus in which heterochrony appears to have been very significant. For example, there appear to have been at least 22 independent speciation events involving flowering shifting from the adult to the juvenile leaf phase within *Eucalyptus* (Potts & Wiltshire, 1997). These changes often involve delaying vegetative phase change, although it appears that the change is rarely lost completely. Considerable variation in the relative and absolute timing of vegetative phase change and first flowering also occurs within species. In the *E. tenuiramis* complex, for example, first flowering in a common environment experiment varied among families from  $\approx$ 18 months to at least 8 years (Wiltshire *et al.*, 1998). Vegetative phase change also ranged from  $\approx$ 1 year to more than 8 years in both early and late flowering types (Wiltshire *et al.*, 1998). The potential adaptive significance in the timing of vegetative phase change and of first flowering is the focus of ongoing study. Nevertheless, the high degree of genetic variation in, and the independence of, the duration of developmental stages shown in the present study clearly demonstrates the genetic potential for rapid heterochronic evolution of morphologically and ecologically disparate forms.

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