# ORIGINAL PAPER

João Costa e Silva · Nuno M. G. Borralho ·
Brad M. Potts

# Additive and non-additive genetic parameters from clonally replicated and seedling progenies of *Eucalyptus globulus*

**Abstract** The first estimates of the importance of epistatic effects within *Eucalyptus globulus* were obtained from analysis of clonally replicated full-sib progeny tests grown in Portugal. Parents comprised diverse selections from the Portuguese landrace. Variance components were estimated for 4-year-old diameter growth and pilodyn penetration, an indirect measure of wood density, both key traits in the pulpwood breeding objective. The experimental components of variance were used to estimate heritabilities and proportions of the phenotypic variance due to dominance and epistasis. The additive variance was the only significant genetic component affecting either diameter or pilodyn. Estimates of the additive, dominance and epistatic effects accounted for 8–10%, 0–4% and 0.4% of the phenotypic variance in diameter, and for 11–17%, 0% and 5% of the phenotypic variance in pilodyn, respectively. A comparison of residual coefficients of variation within seedling and cloned progenies indicated that C effects within clones were not a serious source of random variability. Despite the test sites encompassing a diverse range of locations, no important genotype by environment interaction was detected. The results suggested that an improvement

J. Costa e Silva (✉)
Centro de Estudos Florestais,
Departamento de Engenharia Florestal,
Instituto Superior de Agronomia,
Universidade Técnica de Lisboa,
Tapada da Ajuda, 1349-017 Lisboa Codex, Portugal
e-mail: jces@isa.utl.pt
Tel.: +351-21-3653380
Fax: +351-21-3645000

N. M. G. Borralho
RAIZ Forest and Paper Research Institute,
Herdade da Torre Bela, Apartado 15, 2065 Alcoentre, Portugal

B. M. Potts
Cooperative Research Centre for Sustainable Production Forestry,
School of Plant Science, University of Tasmania,
Private Bag 55, 7001 Hobart, Tasmania, Australia

strategy combining both recurrent selection for additive genetic merit and clonal testing may be adequate for optimizing genetic gains from this genetic base.

## Introduction

Genetic variance among individuals is usually partitioned into additive and non-additive components. Non-additive genetic variance can be further divided into dominance variance caused by intra-locus gene interactions, and epistatic variance due to interactions of genes at two or more loci (Falconer and Mackay 1996). Genetic evaluations in forest tree breeding have been largely based on additive genetic models in the form of family or individual tree models. Ignoring non-additive genetic effects in genetic evaluation models might bias predictions of breeding values, as well as estimators of variance components and additive genetic parameters (Mäki Tanila and Kennedy 1986; Van der Werf and de Boer 1989; Borralho 1994; Lu et al. 1999). Moreover, information on the relative importance of additive and non-additive genetic effects is required to properly evaluate the potential for genetic gain from various breeding and deployment options used in the genetic improvement of forest trees. Deployment programs based on clonal forestry or on specific combining ability of crosses exploit both additive and non-additive genetic effects, whereas strategies based on open-pollinated seed orchards concentrate on the utilization of additive effects only.

*Eucalyptus globulus* ssp. *globulus* (hereafter *E. globulus*) is still in an early stage of domestication, and thus most of the genetic parameters reported to date are based on open-pollinated progenies (Lopez et al. 2002). However, due to inbreeding depression from selfing and/or related mating, genetic parameters derived from open-pollinated eucalypt populations may be inaccurate. This appears to be the case for growth (Griffin and Cotterill 1988; Hodge et al. 1996; Hardner and Tibbits 1998; Volker 2002) but not for wood density (Hardner and Tibbits 1998; Volker 2002). Most *E. globulus* breeding

programs are now moving to control-pollinated assessment, which will allow more accurate estimation of genetic parameters and the separation of additive from non-additive genetic effects.

In some forest tree species, information on both clonal replicates of individual genotypes and family structure can be used to explore the components of genetic variance. Cloned progenies from controlled crosses can be used to estimate additive and non-additive genetic variances, and allow a partial separation of the later component into dominance and epistasis (Stonecypher and McCullough 1986; Foster and Shaw 1988; Mullin and Park 1992; Mullin et al. 1992; Wu 1996; Paul et al. 1997). So far, in *E. globulus*, there are no reports available on the use of clonally replicated progenies from controlled crosses for describing the structure of the genetic variance. In addition, there are no published estimates of the relative importance of epistasis in *E. globulus*, although few estimates of dominance effects are available (Hodge et al. 1996; Volker 2002).

This study aims to assess the relative importance of additive, dominance and epistatic effects for growth and wood density traits in *E. globulus*. Components of variance and genetic parameters were estimated from data obtained from 4-year-old seedling and clonally replicated progenies derived from controlled crosses, and tested over a range of sites in Portugal.

## Materials and methods

### Genetic material

The trials examined in this study comprise full-sib families consisting of seedling progenies (trial 1), clonally replicated progenies (trials 3, 4, 5 and 6) or both types of planting stock (trial 2) (Table 1). All base parents used in the crossings were from plus trees selected in commercial plantations in Portugal. The criteria for selection were overall good growth and form, compared with immediate neighbor trees. This material is commonly referred as belonging to the Portuguese land race, which is of an unknown

origin in Australia (original introduction of seed was unrecorded, and dated back to the early 19th century, as reported by Doughty 2000).

Controlled crosses were carried out between 1992 and 1997 in the seed orchards of RAIZ (a Portuguese forest and paper research institute). Limitations in successfully completing the crosses led to a sparse diallel mating scheme. No reciprocals and selfs were attempted. The seedling trial 1 comprised 36 parents, 75 full-sib families and 1126 genotypes within full-sib families. Trials 2–6, with cloned progenies (plus seedling progenies in trial 2 only), were combined as a single data set, and the total numbers for parents, full-sib families and genotypes within full-sib families were 43, 78 and 435 (i.e. 295 clones plus 140 seedlings in trial 2), respectively. In terms of connectedness among trials, the number of common parents, full-sib families and clones varied between 26 and 40, 28 and 53, and 41 and 139, respectively. There were some full-sib families represented by a single genotype, due to problems related to seed availability, rooting success of the cuttings and survival in the field. For trial 1, 70 full-sib families had at least two genotypes: within this subset of families, the average number of seedlings per family was 16. For the combined data from trials 2–6, 58 full-sib families had at least two genotypes: within this subset of families, the average numbers of clones and genotypes (i.e. clones plus seedlings) per family were 5 and 7, respectively. The average number of crosses per parent was 4 for both the seedling trial and the combined data from trials with cloned progenies.

### Production of planting stock

Seedling progeny from several full-sib families were raised in containers and then partially cloned. In these families, stem cuttings were harvested from a single seedling-origin mother plant, or from previously rooted cuttings growing in an outdoor irrigated multiplication yard. Cuttings (typically with one leaf pair and 10 cm long) were dipped into a hormone powder, and set in a medium composed of 60% peat and 40% Styrofoam. Cuttings were kept for 4 weeks in an environmentally controlled glasshouse, and then moved to an open nursery area until planting.

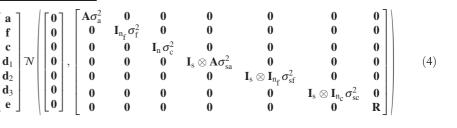### Sites, field test design and measurements

The field tests were established across a range of sites and conditions in Portugal (Table 1). Although sites differed in their soil and climate, silviculture was reasonably similar across the trials. All trials used randomized block designs, but with the number of replicates and the spacing between trees varying from trial to trial (Table 1). Each full-sib family was normally

**Table 1** Details of field trials

| Details | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Trial 6 |
|---|---|---|---|---|---|---|
| Type of planting stock | Seedlings | Seedlings and clones | Clones | Clones | Clones | Clones |
| Location and environment | | | | | | |
| Locality | Sever do Vouga | Alcácer do Sal | Penalva do Castelo | Odemira | Castelo de Paiva | Castelo Branco |
| Latitude (north) | 40° 42′ | 38° 22′ | 40° 42′ | 37° 25′ | 41° 00′ | 40° 00′ |
| Longitude (west) | 08° 23′ | 08° 20′ | 07° 39′ | 08° 39′ | 08° 23′ | 07° 35′ |
| Altitude (m) | 150 | 50 | 550 | 300 | 150 | 450 |
| Mean annual rainfall (mm) | 1200 | 600 | 1400 | 1000 | 1400 | 1200 |
| Mean annual temperature (°C) | 12.5 | 15 | 12.5 | 15 | 12.5 | 7.5 |
| Field layout | | | | | | |
| Number of replicates | 19 | 17 | 8 | 10 | 10 | 10 |
| Spacing (m×m) | 3.5×2.0 | 3.0×3.0 | 3.5×2.0 | 5.0×2.0 | 4.0×1.9 | 4.0×1.8 |
| Traits (means at 4 years) | | | | | | |
| DBH (cm) | 9.4 | 11.3 | 9.5 | 10.5 | 8.1 | 9.3 |
| PIL (mm) | 11.3 | 11.9[a] | 11.6 | n.a.[b] | n.a.[b] | n.a.[b] |

[a] Measured in clones only
[b] Not available

represented in each replicate and, in order to provide a more efficient sampling, the genotypes within full-sib families were randomly allocated to single-tree non-contiguous plots within replicates. Each clone within a full-sib family was represented in different replicates (with 1 ramet per clone and per replicate) of a trial, whereas each seedling within a full-sib family was present only once in a trial.

$$
\begin{bmatrix} \mathbf{a} \\ \mathbf{f} \\ \mathbf{c} \\ \mathbf{d}_1 \\ \mathbf{d}_2 \\ \mathbf{d}_3 \\ \mathbf{e} \end{bmatrix} \mathcal{N} \left( \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_{n_f}\sigma_f^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}_n\sigma_c^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_s \otimes \mathbf{A}\sigma_{sa}^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_s \otimes \mathbf{I}_{n_f}\sigma_{sf}^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_s \otimes \mathbf{I}_{n_c}\sigma_{sc}^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{R} \end{bmatrix} \right) \quad (4)
$$

All surviving trees in all field tests were measured for breast-height diameter growth (DBH, 1.3 m above ground) at age 4 years (Table 1). Wood density was indirectly evaluated by measuring pilodyn penetration (PIL). Four-year-old PIL measures were taken in trials 1, 2 (clonally replicated progenies only) and 3 (Table 1). A single PIL reading was made on each tree at 1.3 m above ground, using a 6 J pilodyn wood tester with a 2.5 mm striker pin. In *E. globulus*, Raymond and MacDonald (1998) reported high repeatability of PIL readings around the stem and also generally high correlations between mean PIL at breast height and whole-tree basic density, which suggests that PIL at this sampling position may be a feasible fast and non-destructive method for indirect assessment of wood density in this species.

Data analysis

For the seedling trial (i.e. trial 1), the analysis of variance components was undertaken according to the following general mixed linear model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{f} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is a vector of observations on a trait, $\mathbf{b}$ is a vector of fixed effects (i.e. site mean and replicates), $\mathbf{a}$ is a vector of random genetic effects of individual genotypes, $\mathbf{f}$ is a vector of random full-sib family effects, and $\mathbf{e}$ is a vector of random residual terms. $\mathbf{X}$, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are known incidence matrices relating the observations in $\mathbf{y}$ to effects in $\mathbf{b}$, $\mathbf{a}$ and $\mathbf{f}$, respectively. The random effects in the model defined in (1) were assumed to follow a multivariate normal distribution with means and variances defined by:

$$
\begin{bmatrix} \mathbf{a} \\ \mathbf{f} \\ \mathbf{e} \end{bmatrix} \sim N \left( \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{A}\sigma^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_f^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix} \right) \quad (2)
$$

where $\mathbf{0}$ is a null matrix; $\mathbf{A}$ is the numerator relationship matrix, which describes the additive genetic relationships among individual genotypes (Henderson 1984); $\mathbf{I}_{n_f}$ and $\mathbf{I}_n$ are identity matrices, with order equal to $n_f$ (the number of full-sib families) and $n$ (the number of trees), respectively; $\sigma_a^2$ is the genetic variance between individual genotypes, $\sigma_f^2$ is the variance between full-sib families, and $\sigma_e^2$ is the residual variance.

Trials with cloned progenies were combined into a single data set, and variance components were estimated according to a mixed linear model defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{f} + \mathbf{Z}_3\mathbf{c} + \mathbf{Z}_4\mathbf{d}_1 + \mathbf{Z}_5\mathbf{d}_2 + \mathbf{Z}_6\mathbf{d}_3 + \mathbf{e} \quad (3)$$

where $\mathbf{y}$, $\mathbf{b}$, $\mathbf{a}$, $\mathbf{f}$ and $\mathbf{e}$ are defined as above, $\mathbf{c}$ is a vector of random effects of clones within full-sib families, and $\mathbf{d}_1$, $\mathbf{d}_2$ and $\mathbf{d}_3$ correspond to vectors of random interactions between sites and effects in $\mathbf{a}$, $\mathbf{f}$ and $\mathbf{c}$, respectively. Under the model defined in (3),

the fixed effects in $\mathbf{b}$ included the overall mean, sites and replicates within sites. $\mathbf{X}$, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are defined as above, and $\mathbf{Z}_3$, $\mathbf{Z}_4$, $\mathbf{Z}_5$ and $\mathbf{Z}_6$ are known incidence matrices relating the observations in $\mathbf{y}$ to effects in $\mathbf{c}$, $\mathbf{d}_1$, $\mathbf{d}_2$ and $\mathbf{d}_3$, respectively. The random effects in the model defined in (3) were assumed to follow a multivariate normal distribution with means and variances defined by:

where $\mathbf{0}$, $\mathbf{I}_{n_f}$, $\sigma_a^2$ and $\sigma_f^2$ are defined as above; observations on different ramets of a clone were treated as repeated measurements on a single genotype, and thus $\mathbf{A}$ is the matrix of additive genetic relationships among individual genotypes as mentioned before; $\mathbf{I}_{n_c}$ and $\mathbf{I}_s$ are identity matrices, with order equal to $n_c$ (the number of clones within full-sib families) and $s$ (the number of sites), respectively; $\sigma_c^2$ is the variance among clones within full-sib families, and $\sigma_{sa}^2$, $\sigma_{sf}^2$ and $\sigma_{sc}^2$ are variances due to interactions between sites and effects in $\mathbf{a}$, $\mathbf{f}$ and $\mathbf{c}$, respectively; $\otimes$ denotes the Kronecker product operation. The across-site analysis combined trials 2–6 for DBH and 2–3 for PIL, and assumed homogeneity of variances and covariances between sites for effects in $\mathbf{a}$, $\mathbf{f}$ and $\mathbf{c}$. Heterogeneity of variance for effects in $\mathbf{e}$ was accounted for by defining $\mathbf{R}$ as $\bigoplus_{j=1}^{k} \mathbf{R}_j = \bigoplus_{j=1}^{k} \mathbf{I}_{n_j}\sigma_{e_j}^2$, where $k$ is either 6 (DBH) or 2 (PIL), and $\oplus$ denotes the direct sum operation. In the across-site analysis of DBH, $\mathbf{R}$ included five $\sigma_{e_j}^2$ terms for residual variances among ramets within clones (within full-sib families) plus an extra term in trial 2 to fit the residual variance among seedlings within full-sib families. The use of a single residual term in trial 2 would have biased the $\sigma_a^2$ estimate under the model defined in (3), as the residual variance for the seedling material includes both genetic and environmental effects, while the residual variance for the clonal material contains environmental effects only (assuming that the measurement error is negligible). The across-site analysis of PIL included only cloned progenies, and thus the $\sigma_{e_j}^2$ terms in $\mathbf{R}$ correspond to residual variances among ramets within clones.

The variance components were estimated by restricted maximum likelihood (REML, Patterson and Thompson 1971), using the average information REML algorithm (Gilmour et al. 1995) implemented in the ASREML program (Gilmour et al. 1999). As dominance relationships are present in full-sib families with at least two genotypes, the estimation of $\sigma_f^2$ has excluded the information from families with only one genotype. This was achieved by adding to the data a factor with two levels — 1 coding for family sizes =1; 2 coding for family sizes ≥2 — and using the ASREML model function at(factor,2) to define a dummy variable which is 1 if the factor has level 2 for the observation. A similar procedure was further applied in the across-site analysis of DBH to fit the model term $\mathbf{c}$ only for cloned progenies.

Likelihood ratio tests (LRT) were used to assess the statistical significance of the estimated variance components. Maternal effects attributed to a female were dropped from the linear model, after a fitted female term was found to be non-significant ($P > 0.05$) for all trials in previous single-site analyses. All interactions with replicates were pooled in the residual component to simplify the linear model. Previous single-site analyses indicated that the interaction between full-sib families and replicates was never

significant ($P>0.05$), and only in trial 3 was the interaction among parents and replicates significant at the 5% level for both traits.

Under the usual assumptions of a large, random mating parental population with diploid inheritance and near linkage equilibrium at gene loci affecting an observed trait (Comstock et al. 1958), the $\sigma_a^2$, $\sigma_f^2$ and $\sigma_c^2$ variance components estimated under the mixed linear models defined as above have the following genetic expectations:

$$\sigma_a^2 = 4\sigma_{GCA}^2 = V_A + 1/4V_{AA} + 1/16V_{AAA} + \dots \quad (5)$$

$$\sigma_f^2 = \sigma_{SCA}^2 = 1/4V_D + 1/8V_{AA} + 1/8V_{AD} + 1/16V_{DD}$$
$$+ 3/32V_{AAA} + 1/16V_{AAD} + 1/32V_{ADD} + 1/64V_{DDD} + \dots \quad (6)$$

$$\sigma_c^2 = \sigma_{C(FS)}^2 - 2\sigma_{GCA}^2 = 3/4V_D + 5/8V_{AA} + 7/8V_{AD} + 15/16V_{DD}$$
$$+ 27/32V_{AAA} + 15/16V_{AAD} + 31/32V_{ADD} + 63/64V_{DDD} + \dots \quad (7)$$

where $V_A$ is the additive genetic variance, $V_D$ is the dominance genetic variance, $V_{AA}$ and $V_{AAA}$ pertain to epistatic genetic variance due to interactions of additive effects at two and three loci, $V_{DD}$ and $V_{DDD}$ refer to epistatic genetic variance due to interactions of dominance effects at two and three loci, and $V_{AD}$, $V_{AAD}$ and $V_{ADD}$ correspond to epistatic genetic variance due to interactions of additive and dominance effects at two and three loci. $\sigma_{GCA}^2$, $\sigma_{SCA}^2$ and $\sigma_{C(FS)}^2$ are the variances due to general combining ability of the parents (GCA), specific combining ability of the crosses (SCA) and differences between clones within full-sib families, respectively, as estimated under Griffing's (Griffing 1956) model for diallel mating designs and extended to include clonal replication (e.g. Stonecypher and McCullough 1986; Mullin and Park 1992; Mullin et al. 1992).

Approximated estimates of genetic parameters were obtained from the across-site analysis as follows:

$$h^2 = \frac{V_A}{V_P} \approx \frac{\sigma_a^2}{\sigma_a^2 + \sigma_f^2 + \sigma_c^2 + \sigma_{sa}^2 + \sigma_{sf}^2 + \sigma_{sc}^2 + \sum_{j=1}^{5} \sigma_{e_j}^2 / 5} \quad (8)$$

$$d^2 = \frac{V_D}{V_P} \approx \frac{4\sigma_f^2}{\sigma_a^2 + \sigma_f^2 + \sigma_c^2 + \sigma_{sa}^2 + \sigma_{sf}^2 + \sigma_{sc}^2 + \sum_{j=1}^{5} \sigma_{e_j}^2 / 5} \quad (9)$$

$$i^2 = \frac{V_I}{V_P} \approx \frac{\sigma_c^2 - 3\sigma_f^2}{\sigma_a^2 + \sigma_f^2 + \sigma_c^2 + \sigma_{sa}^2 + \sigma_{sf}^2 + \sigma_{sc}^2 + \sum_{j=1}^{5} \sigma_{e_j}^2 / 5} \quad (10)$$

where $V_I$ is the epistatic genetic variance, $V_P$ is the total phenotypic variance, $h^2$ is the heritability, $d^2$ is the proportion of dominance variance, $i^2$ is the proportion of epistatic variance, and $\sum_{j=1}^{5} \sigma_{e_j}^2 / 5$ is the average environmental variance estimated from the residual variances among ramets within clones. Estimates of $h^2$ and $d^2$ were obtained in a similar manner from the analysis of the seedling data, but using $\sigma_a^2 + \sigma_f^2 + \sigma_e^2$ (where $\sigma_e^2$ is the residual variance for seedlings within full-sib families) as an estimator of $V_P$. The standard errors of the genetic parameter estimates were calculated according to the general expression for the variance of a ratio, based on an approximation using a first-order Taylor series expansion (Lynch and Walsh 1998).

As it can be seen from the relationships among experimental and causal variance components, $\sigma_a^2$ and $\sigma_f^2$ contain portions of epistasis with successively decreasing contributions of interactions involving larger groups of loci. Thus, unbiased estimation of $V_A$ and $V_D$ based on $\sigma_a^2$ and $4\sigma_f^2$, respectively, assumes that low-order interloci interactions represent a small portion of the total epistasis. Similarly, interactions involving groups of more than two or three loci are assumed when $V_I$ is calculated by $\sigma_c^2 - 3\sigma_f^2$, since this estimator contains only a fraction (i.e. $1/4 V_{AA} + 1/2 V_{AD} + 3/4 V_{DD} + 9/16 V_{AAA} + 3/4 V_{AAD} + 7/8 V_{ADD} + 15/16 V_{DDD} \dots$) of the total epistasis with a major contribution of high-order interactions. In addition, non-genetic effects introduced by cloning ("C effects", Libby and Jund 1962) were assumed to be negligible or absent.

## Results

Single-site and pooled estimates of variance components and genetic parameters, obtained from the analysis of the seedling trial and combined data from trials with cloned progenies, are presented in Table 2 for 4-year-old DBH and PIL measurements. $\sigma_a^2$ was significantly different from zero ($P \leq 0.05$) for both DBH and PIL. The pooled

**Table 2** Estimated variance components and genetic parameters from single-site (trial 1) and combined analyses (trials 2–6 for DBH, and 2–3 for PIL) of *Eucalyptus globulus* controlled crosses. The results are for 4-year-old diameter (*DBH*) and pilodyn (*PIL*) measurements at breast height. The *P* values are based on likelihood ratio tests, which were carried out to test the departure of the variance component estimates from zero. The approximate standard errors for the genetic parameters are given in parenthesis

| Traits | Trials | Variance components | | | | | | | Genetic parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\sigma_a^2$ | $\sigma_{sa}^2$ | $\sigma_f^2$ | $\sigma_{sf}^2$ | $\sigma_c^2$ | $\sigma_{sc}^2$ | $\sigma_{e_j}^2$ | $h^2$ | $d^2$ | $i^2$ |
| DBH (cm) | 1 | 0.32 P=0.006 | n.a.[d] | 0.0[a] | n.a.[d] | n.a.[d] | n.a.[d] | 3.87[b] | 0.08 (0.04) | 0.0 | n.a.[d] |
| | 2–6 | 0.43 P<0.001 | 0.068 P>0.05 | 0.037 P>0.05 | 0.025 P>0.05 | 0.13 P>0.05 | 0.099 P>0.05 | 2.61[b] 2.37[c] 3.34[c] 4.60[c] 2.47[c] 3.86[c] | 0.10 (0.04) | 0.04 (0.05) | 0.004 (0.04) |
| PIL (mm) | 1 | 0.35 P=0.012 | n.a.[d] | 0.0[a] | n.a.[d] | n.a.[d] | n.a.[d] | 2.92[b] | 0.11 (0.07) | 0.0 | n.a.[d] |
| | 2–3 | 0.55 P<0.001 | 0.0[a] | 0.0[a] | 0.0[a] | 0.17 P>0.05 | 0.23 P=0.036 | 1.65[c] 2.81[c] | 0.17 (0.07) | 0.0 | 0.05 (0.06) |

[a] Variance estimate constrained to be fixed at a very small positive value
[b] Seedlings
[c] Clones
[d] Not available

$h^2$ estimates (i.e. 0.10 and 0.17) from the combined data analysis approached those (i.e. 0.08 and 0.11) obtained under the analysis of the seedling progenies. The analyses carried out for DBH indicated very small or statistically non-significant ($P > 0.05$) $\sigma_f^2$ values, which resulted in zero or low (i.e. 0.04) $d^2$ estimates. $\sigma_f^2$ and $d^2$ were virtually zero for PIL, which may suggest very low levels of dominance effects for the indirect measure of wood density. $\sigma_c^2$ was not significant at the 5% level for any of the traits, and the pooled $i^2$ estimates were low (i.e. 0.004 for DBH, and 0.05 for PIL).

The variances due to interactions between sites and effects in **a** or **f** were low and not significant ($P > 0.05$) in the combined data analysis of DBH and PIL (Table 2). These results indicate a low level of genotype by environment interaction for DBH and PIL at the sampled sites. Previous bivariate analyses between pairs of trials with cloned progenies, which considered the performance in each environment as a separate trait, also showed high across-site correlation estimates for effects in **a**. For DBH, the estimated genetic correlations across sites ranged from 0.63 to 0.99 with an average of 0.91 and, for PIL, the correlation parameter across trials 2 and 3 was 0.99. For effects in **c**, however, $\sigma_{sc}^2$ was statistically significant ($P \leq 0.05$) for PIL (Table 2), and the across-site correlation estimated from bivariate analysis was accordingly low (i.e. 0.45). Nevertheless, such results are likely to be of little importance, as previous single-site analyses of PIL did not find significant $\sigma_c^2$ at the 5% level.

The residual component was the largest source of variation in all analyses, with the environmental variance (estimated directly from trials with cloned progenies) representing on average 81% and 67% of the total phenotypic variance for DBH and PIL, respectively (Table 2).

## Discussion

Epistasis is usually assumed to be absent when $V_A$ and $V_D$ are estimated from control-pollinated progenies with seedling material. Both clonal replicates and family structure allow the partial separation of the genetic variance into its $V_A$, $V_D$ and $V_I$ components. However, to obtain reasonable estimates for these types of gene action, the genetic model using clones assumes that, when present, epistasis reflects primarily interactions involving groups of more than two or three loci (for a detailed discussion, see Mullin and Park 1992; Wu 1996). If this is true, the estimators of $V_A$ and $V_D$ will only include a minute fraction of the total epistasis, and the estimator of $V_I$ will account for most of the total interaction variance. Both of the analyses carried out for PIL indicated very small $\sigma_f^2$ values, suggesting that the relatively higher $\sigma_c^2$ estimate (Table 2) is likely to contain small contributions of both dominance effects and low-order epistatic interactions. This suggests that $V_I$ is probably not underestimated and $V_A$ may not be biased upwards for PIL. For

DBH, $\sigma_f^2$ and $\sigma_c^2$ were both small and much lower than $\sigma_a^2$ (Table 2), suggesting that possible bias in $V_A$ estimates caused by interactions of additive effects at different loci is probably not large.

C effects were assumed to be absent or negligible in the genetic model. C effects among clones relate to non-genetic sources of covariance between ramets of the same clone, and may be due to factors such as the age or the environment of the original ortet. When present, C effects among clones may inflate estimates of between-clone variances (Libby and Jund 1962; Burdon and Shelbourne 1974), and thereby will cause estimates of $V_I$ to be upwardly biased. None of the examined trials allow a reliable separation between C effects and epistasis. Nevertheless, C effects among clones were likely to be unimportant, as $\sigma_c^2$ itself was generally non-significant and small (Table 2). Other effects associated with cloning may arise from inequalities among propagules within clones, due to ontogenic factors such as cutting position on the ortet or morphological factors such as cutting size. These propagation effects may inflate the within-clone variances (Foster et al. 1984), and the inflated residual variances may reduce our ability to detect significant genetic effects in the clonal trials. As it can be determined from the information given in Table 2 for effects in **e**, the coefficient of variation (CV) within clones for DBH (CV ranging from 13.6% to 21.1%) was lower than or similar to that within seedling full-sib families (i.e. CV=20.9% in trial 1; also note that, in trial 2, CV=13.6% within clones was lower than CV=14.3% within seedling families). A similar tendency was also observed for PIL (i.e. CV ranging from 10.8% to 14.4% within clones, and CV=15.1% within seedling full-sib families). These results suggest that C effects within clones were not a serious source of random variability at age 4 years.

When compared to previous forestry studies using diallel mating designs with cloned progenies (e.g. Stone-cypher and McCullough 1986; Mullin et al. 1992), the present study has more parents, and similar number of full-sib families and average number of clones per full-sib family. However, large data sets are required to obtain accurate estimates of non-additive genetic variances, especially due to epistatic effects (Chang et al. 1990), and thus standard errors for estimated non-additive variances are likely to be greater than standard errors for additive variances. This is clearly exemplified in our study where, by considering the approximate standard errors in relation to the magnitude of the associated parameter estimates, the precision involved in the estimation of $d^2$ and $i^2$ was lower than that for $h^2$ (Table 2). Therefore, the power of statistical tests carried out for $\sigma_f^2$ and $\sigma_c^2$ in our population may have not been high enough to detect true differences for effects in **f** and **c**. Nevertheless, the seedling trial had more full-sib families and genotypes per full-sib family than the combined data from trials with cloned progenies, and yet the results from the analyses of both DBH and PIL were consistent in that dominance was low compared with

additive genetic effects (Table 2). In addition, the genetic parameters estimated here for growth agreed fairly closely with results from previous studies using *E. globulus* clones or control-pollinated progenies. Clonal heritabilities of $0.08 \pm 0.06$ and $0.11 \pm 0.06$ were estimated on an individual tree basis for 3-year-old sectional area measured at two sites in Portugal (Borralho et al. 1992). These broad-sense heritability estimates are similar to the narrow-sense $h^2$ values obtained in our study for 4-year-old DBH, suggesting that non-additive effects are likely to be small relative to additive effects. In a combined analysis across five sites in Australia, Volker (2002) found a $h^2$ of $0.08 \pm 0.03$ and no significant dominance (i.e. $d^2 = 0.02 \pm 0.02$) for 4-year-old DBH, which is comparable to results obtained here (Table 2). Using the same crossing program as Volker, Hodge et al. (1996) reported a $h^2$ of $0.08 \pm 0.01$ and a $d^2$ of $0.05 \pm 0.03$ for 2-year-old volume from an across-site analysis of intra-provenance full-sib crosses. For PIL, the pooled $h^2$ reported here (Table 2) was similar to or lower than across-site estimates obtained from open-pollinated ($0.33 \pm 0.03$, MacDonald et al. 1997; $0.19 \pm 0.06$, Muneri and Raymond 2000) or control-pollinated ($0.25 \pm 0.07$, Volker 2002) *E. globulus* progenies evaluated after age 5 years in Australia. The present results of no dominance effects for PIL (Table 2) were consistent with previous studies in *Eucalyptus*, where PIL exhibited little or no dominance variation (Hardner and Tibbits 1998; Volker 2002). There are no published estimates of $i^2$ for diameter growth or wood density in *Eucalyptus*. For DBH in *Populus deltoides*, however, Foster and Shaw (1988) found that epistasis had a small contribution to the genetic variance at ages 3 and 4 years, but observed that the epistatic variance estimate was the most important component at age 8. Working with conifers, Stonecypher and McCullough (1986) and Paul et al. (1997) reported estimates of epistatic variances to be zero for DBH at ages 5 and 6 years. All of these insights into the genetic architecture of forest trees support the results obtained in our population for 4-year-old DBH and PIL, in that non-additive genetic effects tend to be low compared with additive effects.

The small $d^2$ estimated in this study indicate that, for both traits, the genetic value of a full-sib family may reasonably be predicted from the parents' additive genetic merit. PIL exhibited higher $i^2$ values than DBH; however, $i^2$ was much smaller than $h^2$ (Table 2). These results suggest that attempts to exploit non-additive variance (either fully, by selection and deployment of clones, or partially, by selection and multiplication of full-sib families) may not add substantial extra genetic gain for the production population derived from this genetic material, when compared with options capturing additive effects only (e.g. mating offspring individuals, or parents, selected on BLUPs of breeding value). It should be noted, however, that the trend in the distribution of the genetic variance may change in future generations of breeding (Snedden et al. 2000), and thus a strategy using non-additive effects may be required to optimize genetic gain.

Nevertheless, even when non-additive effects are absent, clonal selection in a cloned breeding population may lead to genetic gains per year exceeding those of other plant production methods, mainly due to the reduction in the time needed for deploying selected material (Matheson and Lindgren 1985; Shelbourne 1991; Rosvall et al. 1998). In addition, using clonal replicates in progeny tests can improve the accuracy of selection for additive genetic merit (Shaw and Hood 1985; Shelbourne 1991), and thus act to increase gain for both breeding and production populations.

## Conclusions

This study evaluated the relative importance of additive and non-additive genetic parameters for diameter growth and pilodyn penetration measured in a 4-year-old population of *E. globulus* comprising the Portuguese landrace. Both traits exhibited significant additive genetic variance estimates, although heritabilities were moderate to low. The estimated relative levels of dominance and epistatic variation tended to be low compared with additive effects, and were generally associated with non-significant variance components. The results suggested that an improvement strategy combining both recurrent selection for additive genetic merit and clonal testing may be adequate for optimizing genetic gains from this genetic base. Nevertheless, although the genetic parameters calculated here were comparable with estimates from other reports using *E. globulus*, additional studies using clonal testing should include efficient mating designs with more parents and crosses per parent, as well as substantial resources in terms of progeny sizes. This would be needed to improve the accuracy of variance components and increase the chances for detecting significant parameter estimates.

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