

Quantifying individual variation in reaction norms using random regression models fitted through Legendre polynomials: application in eucalyptus breeding

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ABSTRACT: An accurate, efficient and informative statistical method for analyses of genotype \times environment ($G \times E$) interactions is a key requirement for progress in any breeding program. Thus, the objective of this study was to quantify individual variation in reaction norms using random regression models fitted through Legendre polynomials in eucalyptus (*Eucalyptus spp.*) breeding. To this end, a data set with 215 eucalyptus clones of different species and hybrids evaluated in four environments for diameter at breast height (DBH) and Pilodyn penetration (PP) was used. Variance components were estimated by restricted maximum likelihood, and genetic values were predicted by best linear unbiased prediction. The best-fitted model for DBH and PP was indicated by the Akaike information criterion, and the significance of the genotype effects was tested using the likelihood ratio test. Genetic variability between eucalyptus clones and very high accuracies ($r_{gg} > 0.90$) were detected for both traits. Reaction norms and eigenfunctions generated genetic insights into $G \times E$ interactions. This is the first study that quantified individual variation in reaction norms using random regression models fitted through Legendre polynomials in eucalyptus breeding and demonstrated the great potential of this technique.

Key words: mixed model methodology, covariance functions, genotype \times environment interactions, genotypic plasticity, forest tree breeding.

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INTRODUCTION

Eucalyptus (*Eucalyptus spp.*) is widely cultivated in tropical and subtropical regions. Its cultivation is mainly intended to produce pulp, bio-oil, firewood, and charcoal. The international pulp trade and the intense search for alternative energy sources have increasingly motivated the establishment of eucalyptus plantations in several countries worldwide (Fonseca et al. 2010). In this sense, eucalyptus breeding programs have sought to identify more efficient selection techniques to increase yield and quality of traits of industrial interest.

The genotype \times environment ($G \times E$) interactions are characterized by the differential behavior of genotypes in relation to environmental variations (Resende 2015). These interactions can be expressed in various ways and with different intensities and can generate significant obstacles for genetic selection (Li et al. 2017; van Eeuwijk et al. 2016), including eucalyptus (Nunes et al. 2017; Resende et al. 2017; 2018). Thus, the use of accurate, efficient and informative statistical methods that capture the information present in this source of variation and advantageously exploit its effects is fundamental in any breeding program.



Recently, mixed model methodology has become very popular for analyses of $G \times E$ interactions (Li et al. 2017; van Eeuwijk et al. 2016). There are numerous reasons for their use, including the fact that they allow estimation of variance components and prediction of genetic values simultaneously, deal with unbalanced data, describe heterogeneity of genetic covariances and residual variances across environments, and modeling spatial trends (Resende 2016; van Eeuwijk et al. 2016).

Within the context of mixed model methodology, individual variation in reaction norms can be quantified using random regression models fitted through Legendre polynomials (Resende et al. 2014). Random regression models involve a parsimonious covariance structure that provides predicted genetic values in specific environments or as a trend over environments (Alves et al. 2020); and Legendre polynomials have been considered to have better convergence properties as the regression are orthogonal (Mrode 2014) and are the easiest to apply (Schaeffer 2016).

Random regression models are widely used in longitudinal data analyses (Mrode 2014; Resende et al. 2014). In perennials breeding, random regression models have been used to model growth over time (Resende et al. 2001; Rocha et al. 2018). Besides that, reaction norms have been commonly used for genomic and phenotypic selection in multi-environment trials (Jarquín et al. 2014; Morais Júnior et al. 2018; Alves et al. 2020).

However, no study considering random regression models fitted through Legendre polynomials has been described in forest tree breeding for analyses of $G \times E$ interactions. Thus, the objective of this study was to quantify individual variation in reaction norms using random regression models fitted through Legendre polynomials in eucalyptus breeding.

MATERIAL AND METHODS

Experimental data

The data used in this work come from evaluation of a clonal field test of different eucalyptus species (*E. grandis*, *E. urophylla*, *E. saligna*, and *E. pellita*) and hybrids [*E. grandis* × *E. urophylla*, *E. urophylla* × *E. maidenii*, *E. grandis* × *E. saligna*, *E. urophylla* × *E. saligna*, *E. urophylla* × *E. globulus*, *E. pellita* × *E. grandis*, *E. grandis* × *E. maidenii*, *E. grandis* × *E. dunnii*, *E. grandis* × *E. globulus*, *E. globulus* × *E. tereticornis*, *E. urophylla* × *E. deanei*, *E. urophylla* × *E. tereticornis*, *E. urophylla* × (*E. grandis* × *E. urophylla*), *E. globulus* × (*E. grandis* × *E. urophylla*), *E. grandis* × (*E. grandis* × *E. urophylla*), *E. urophylla* × (*E. camaldulensis* × *E. grandis*), *E. saligna* × (*E. grandis* × *E. urophylla*), *E. robusta* × (*E. grandis* × *E. urophylla*), *E. grandis* × (*E. dunnii* × *E. grandis*), *E. maidenii* × (*E. grandis* × *E. urophylla*), *E. saligna* × (*E. urophylla* × *E. grandis*), *E. urophylla* × (*E. grandis* × *E. globulus*), *E. urophylla* × (*E. tereticornis* × *E. saligna*), *E. urophylla* × (*E. urophylla* × *E. grandis*), (*E. grandis* × *E. kirtoniana*) × (*E. robusta* × *E. tereticornis*), and (*E. grandis* × *E. urophylla*) × (*E. urophylla* × *E. globulus*)], implemented in September 2007, in four experimental areas of the CMPC Company, which are located in the state of Rio Grande do Sul, Brazil. The geographic location and annual climatic conditions of each environment are presented in Table 1. In each environment, a field trial in a randomized block design was established, with 215 clones in single tree plots and 30 replications. Trees were planted at a spacing of 3.5 m between rows and 2.6 m between plants.

Table 1. Geographic location (GL) and annual climatic conditions (ACC) of each environment (E).

GL and ACC	E1	E2	E3	E4
Geographic coordinates	Latitude: 30°11'09" S	Latitude: 30°29'45" S	Latitude: 30°27'19" S	Latitude: 30°14'46" S
	Longitude: 52°00'10" W	Longitude: 52°19'35" W	Longitude: 52°39' 53" W	Longitude: 53°49'7" W
Altitude (m)	141	378	250	301
Average temperature (°C)	17.5	16	17	16.8
Absolute minimum temperature (°C)	-0.9	-1.7	-0.6	0.0
Absolute maximum temperature (°C)	32.3	30.7	33.3	34.7
Rainfall (mm)	1422	1564	1368	1133

Source: CMPC Company.

This work used data from the assessment of all surviving trees at three-years of age in the field tests (22,295 trees in total) for diameter at breast height (DBH) (cm) and Pilodyn penetration (PP) (mm). The DBH was measured using a diameter tape and the PP using a Pilodyn. According to Greaves et al. (1996), PP, which is an indirect method to determine the basic density of wood, has been effective to evaluate eucalyptus.

Statistical analyses

In order to use Legendre polynomials, phenotypic mean of each environment (μ_i) must be scaled to range from -1 to +1. The environmental gradient values (E_i) were obtained as follows (Eq. 1) (Schaeffer 2016):

$$E_i = -1 + 2[(\mu_i - \mu_{min})/(\mu_{max} - \mu_{min})] \quad (1)$$

Variance components were estimated by restricted maximum likelihood (REML) (Patterson and Thompson 1971) and genetic values were predicted by best linear unbiased prediction (BLUP) (Henderson 1975). Random regression models were fitted through Legendre polynomials for DBH and PP as follows (Eq. 2):

$$Y_{ijk} = \mu + S_j + R/S_{jk} + \sum_{d=0}^D \alpha_{id} \Phi_{ijd} + e_{ijk} \quad (2)$$

where Y_{ijk} is the i^{th} genotype ($i = 1, 2, \dots, 215$) in the j^{th} environment ($j = 1, 2, 3, 4$) in the k^{th} replication ($k = 1, 2, \dots, 30$); μ is the overall mean; S_j is the fixed effect of environment j ; R/S_{jk} is the fixed effect of replication k nested in environment j ; d is the polynomial degree, ranging from 0 to D ($D = \text{number of environments} - 1$); α_{id} is the random regression coefficient for the Legendre polynomial for the genotype effect; Φ_{ijd} is the d^{th} Legendre polynomial for the j^{th} environment for the i^{th} genotype; and e_{ijk} is the residual random effect associated with Y_{ijk} .

In the matrix notation, the above model is described as follows (Eq. 3):

$$y = X\beta + Z\alpha + e \quad (3)$$

where y is the vector of phenotypic data; β is the vector of the replication-environment combination that comprises the fixed effects of environment and replication within the environment, added to the overall mean; α is the vector of genotype effects (random); and e is the vector of residuals (random). Uppercase letters represent the incidence matrices for these effects. In addition, $\alpha \sim N(0, K_g \otimes I_{215})$ and $e \sim N(0, R)$, where I_{215} is an identity matrix of order 215, \otimes denotes the Kronecker product, K_g is the covariance matrix for the coefficients of genetic effects, and R represents the matrix of residual variances.

The polynomial order in random regression models were selected using the Akaike Information Criterion (AIC) (Akaike 1974) as follows (Eq. 4):

$$AIC = -2\text{Log}L + 2p \quad (4)$$

where $\text{Log}L$ is the logarithm of the maximum (L) of the restricted likelihood function, and p is the number of estimated parameters. Besides that, different residual variance structures (homogeneous and heterogeneous) were tested.

The significance of the genotype effects was tested using the likelihood ratio test (LRT) (Wilks 1938) as follows (Eq. 5):

$$LRT = -2(\text{Log}L - \text{Log}L_R) \quad (5)$$

where $\text{Log}L_R$ is the logarithm of the maximum (L_R) of the restricted likelihood function of the reduced model (without genotype effects).

Estimates of genetic variance ($\hat{\sigma}_g^2$) and predicted genetic values (\tilde{g}_{ij}), in the original scale, were obtained by Eqs. 6 and 7 (Kirkpatrick et al. 1990):

$$\hat{\sigma}_g^2 = \Phi_{ijd} \hat{K}_g \Phi'_{ijd} \quad (6)$$

$$\tilde{g}_{ij} = \sum_{d=0}^D \hat{\alpha}_{id} \Phi_{ijd} \quad (7)$$

Phenotypic variance ($\hat{\sigma}_p^2$), broad-sense individual heritability (h_g^2), and accuracy (r_{gg}) were estimated by Eqs. 8–10 (Resende et al. 2014):

$$\hat{\sigma}_p^2 = \hat{\sigma}_g^2 + \hat{\sigma}_e^2 \quad (8)$$

$$h_g^2 = \hat{\sigma}_g^2 / \hat{\sigma}_p^2 \text{ and} \quad (9)$$

$$r_{gg} = \sqrt{1 - \frac{\Phi_{ijd} PEV \Phi'_{ijd}}{\hat{\sigma}_g^2}} \quad (10)$$

where $\hat{\sigma}_e^2$ is the residual variance and *PEV* is the prediction error variance, obtained by the diagonal elements of the inverse of the coefficient matrix (information matrix) of the mixed model equations.

The eigenfunctions (ψ_f) of the genetic coefficient covariance matrix, aiming to study the $G \times E$ interactions, were obtained by Eq. 11 (Kirkpatrick et al. 1990):

$$\Psi_f = \sum_{d=0}^D (C\psi_f)_d \Phi_d \quad (11)$$

where $(C\psi_f)_d$ is the d^{th} element of the f^{th} eigenvector of \hat{K}_g , and Φ_d is the normalized value of the d^{th} Legendre polynomial.

The areas under the reaction norms (*A*), aiming to rank the clones, were obtained by Eq. 12:

$$A = \mu + \int_{-1}^1 \sum_{d=0}^D \hat{\alpha}_d \Phi_d x^d dx \quad (12)$$

where x^d is the environmental gradient.

Statistical analyses were performed using the ASReml 4.1 (Gilmour et al. 2015) and R (R Core Team 2018) software. The ASReml program files are available in supplementary material.

RESULTS

Selection of models

According to the AIC (Akaike 1974), the best model for DBH and PP is denoted by Leg.4.Rhe (Legendre polynomial of the three degree for genotype effects, with a heterogeneous residual variance structure) (Table 2), since the lower AIC value reflect a better overall fit. Thus, this model was adopted to estimate the variance components and to predict the genetic values along the environmental gradients. According to the LRT, genetic variability ($p < 0.01$) was detected for DBH and PP (Table 2).

Variance components and genetic parameters

For DBH, genetic variance was not stable over the environmental gradient, ranging from 0.9073 (E2) to 2.5426 (E4); and residual and phenotypic variances increased over the environmental gradient (Table 3). Heritability estimates were not

Table 2. Akaike information criterion (AIC) and likelihood ratio test (LRT) for the genetic effects for diameter at breast height (DBH) and Pilodyn penetration (PP) in eucalyptus.

Model ^a	P ^b	DBH		PP	
		AIC	LRT	AIC	LRT
Leg.1.Rho	2	18922.90	16259.36**	15358.70	7289.60**
Leg.2.Rho	4	18497.30	15829.72**	15024.10	6950.98**
Leg.3.Rho	7	17967.90	15294.28**	14889.50	6810.44**
Leg.4.Rho	11	17044.90	14363.30**	14058.50	5971.36**
Leg.1.Rhe	5	-2005.80	-3653.17**	-5577.20	-13339.02**
Leg.2.Rhe	7	-2360.00	-4011.37**	-6088.10	-13853.96**
Leg.3.Rhe	10	-3084.60	-4741.95**	-6274.50	-14046.32**
Leg.4.Rhe	14	-4000.00	-5665.31**	-6929.40	-14709.24**

^a: models tested are referred to as *Leg.O.R_λ*, where *O* represent the Legendre polynomials orders fitted for the genetic random effects, and *R_λ* may assume a homogeneous (*Rho*) or heterogeneous (*Rhe*) residual variance structure; ^b: number of parameters; and **: significant at 0.01 probability of error type I by the chi-square test.

Table 3. Estimates of variance components and genetic parameters for diameter at breast height (DBH) and Pilodyn penetration (PP) in eucalyptus, in each environment (E).

Component / parameter	DBH				PP			
	-1.00 (E2)	-0.44 (E3)	0.52 (E1)	1.00 (E4)	-1.00 (E1)	-0.34 (E2)	-0.05 (E4)	1.00 (E3)
$\hat{\sigma}_g^2$	0.9073	2.2010	1.3649	2.5426	3.6123	4.6763	4.2168	4.2046
$\hat{\sigma}_e^2$	2.8782	3.4388	4.9018	6.5113	3.9234	5.7182	3.5848	2.4856
$\hat{\sigma}_p^2$	3.7855	5.6399	6.2667	9.0539	7.5357	10.3945	7.8017	6.6901
h_g^2	0.24	0.39	0.22	0.28	0.48	0.45	0.54	0.63
\bar{r}_{gg}	0.95	0.97	0.94	0.95	0.98	0.98	0.98	0.99

$\hat{\sigma}_g^2$: genetic variance, $\hat{\sigma}_e^2$: residual variance, $\hat{\sigma}_p^2$: phenotypic variance, h_g^2 : broad-sense individual heritability, and \bar{r}_{gg} : mean accuracy.

stable over the environmental gradient, ranging from 0.22 (E1) to 0.39 (E3); and mean accuracies were higher than 0.90 in all environments (Table 3).

For PP, genetic, residual, and phenotypic variances were not stable over the environmental gradient (Table 3). Genetic variance ranged from 3.6123 (E1) to 4.6763 (E2); and residual and phenotypic variances reached a peak in E2 (Table 3). Heritability estimates were not stable over the environmental gradient, ranging from 0.45 (E2) to 0.63 (E3); and mean accuracies were higher than 0.95 in all environments (Table 3).

Reaction norms

Genotypic plasticity and $G \times E$ interactions (nonlinear) were observed for DBH (Fig. 1a) and PP (Fig. 1b), since the reaction norms intersected, diverged, or converged (van Eeuwijk et al. 2016).

Eigenfunctions

For DBH, first, second, third and fourth eigenfunctions explained, respectively, 78.26, 15.54, 4.68 and 1.51% of the genetic variation (Fig. 2a). For PP, first, second, third, and fourth eigenfunctions explained, respectively, 66.80, 32.48, 0.56, and 0.15% of the genetic variation (Fig. 2b).

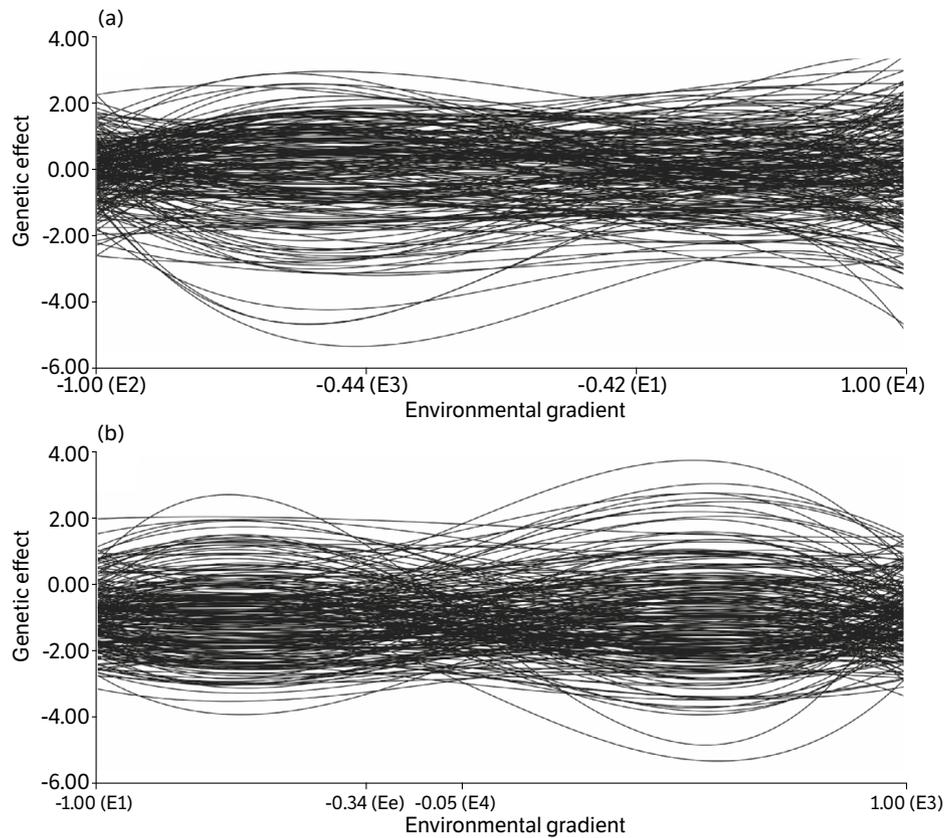


Figure 1. Reaction norms for diameter at breast height (DBH) (a) and Pilodyn penetration (PP) (b) over environmental gradient. Each curve represents one eucalyptus clone.

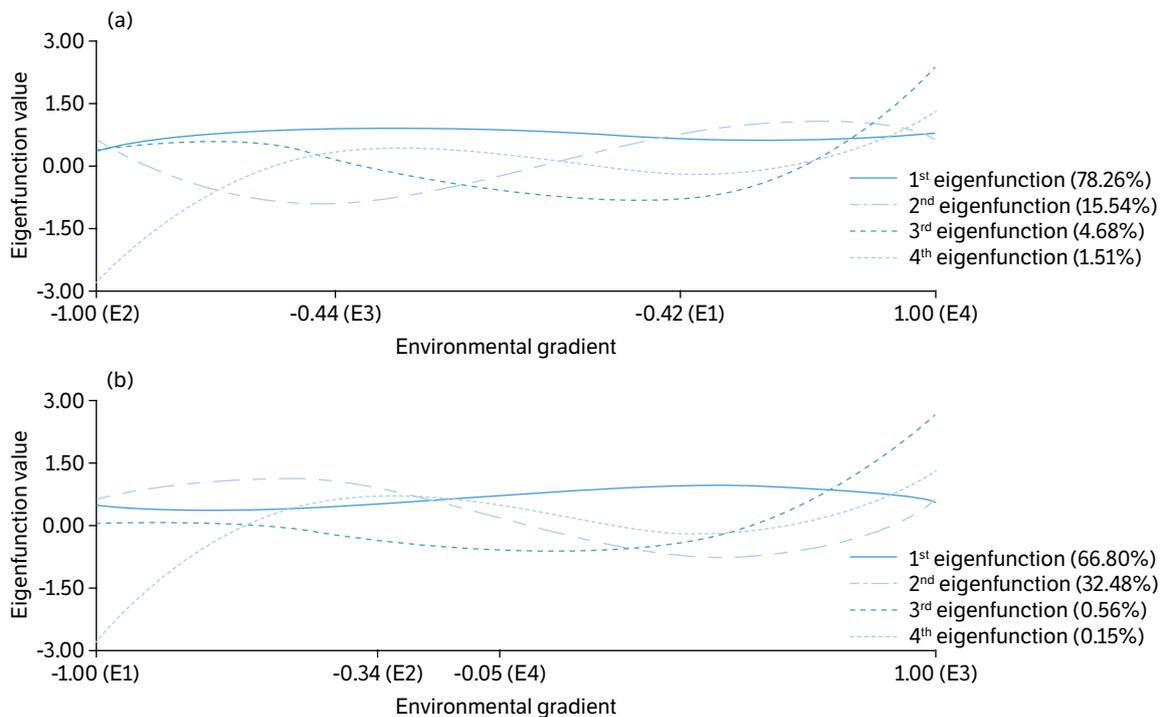


Figure 2. Estimates of the eigenfunctions for diameter at breast height (DBH) (a) and Pilodyn penetration (PP) (b) over environmental gradient. Their proportional eigenvalues for the genetic covariance function are in parentheses.

Area under the reaction norms

The areas under the reaction norms ranged from 6.14 (clone 204) to 18.12 (clone 24) cm² for DBH (supplementary material – Table S1), and from 9.95 (clone 91) to 29.79 (clone 43) mm² for PP (Supplementary material – Table S1).

DISCUSSION

Random regression and model selection

The covariance functions developed by Kirkpatrick and Heckman (1989) that uses orthogonal base functions, such as Legendre polynomials, allows the fit of virtually any shape of growth curves or reaction norms (Calus et al. 2004). Reaction norms model the trajectory of genetic values along the environmental gradient, i.e., as a deviation from other fixed and random effects included in the model (Resende et al. 2014). Kirkpatrick et al. (1990) demonstrated the equivalence between random regression and covariance functions.

Among the various criteria for selection of models, the AIC is prominent (Cavanaugh and Neath 2019). The selected model fits heterogeneous residuals (i.e., one residual variance for each environment), and the genetic effects was modeled by Legendre polynomials of degree three for DBH and PP. This implies the estimation of 14 parameters of covariance. Heterogeneous residuals were also reported by Resende et al. (2017) evaluating clonal trials of eucalyptus.

The random regression model can be considered a reduced and simplified multiple-trait model, which allows the same parameters of interest (heritability and genetic correlation among all pairs of environments) to be estimated, but with lower parameterization and with less computational effort (Resende et al. 2001). These models directly define covariance functions, and since there are reliable estimates of variance components, they allow the prediction of genetic values of a genotype in different environments, based on evaluation in only one environment (Alves et al. 2020).

Variance components and genetic parameters

The estimation of variance components and prediction of genetic values are essential procedures in any breeding program. Currently, REML/BLUP is the standard procedure for estimation of variance components and optimal selection in plant breeding (Resende 2016). Knowledge of genetic parameters is of great importance in plant breeding, since the breeding strategy to be used depends on the information obtained from these parameters (Resende 2002).

According to Resende (2015), DBH has heritabilities of moderate magnitude ($0.15 < h_g^2 < 0.50$), and PP has heritabilities of moderate ($0.15 < h_g^2 < 0.50$) to high ($h_g^2 > 0.50$) magnitudes. Moderate heritability ($h_g^2 = 0.18$, for DBH) were also reported by Nunes et al. (2017), who used the compound symmetry model for genetic evaluation of eucalyptus clones.

One of the most relevant parameters for evaluation of the effectiveness of the inference about the predicted genetic value of a genotype is selective accuracy (Resende and Duarte 2007). This parameter indicates the correct arrangement of the genotypes for selection and recommendation purposes. This parameter does not only depend on the magnitude of the residual variation and the number of replications, but also on the ratio between the genetic and residual variations associated with the traits under evaluation (Resende and Duarte 2007). In this study, very high accuracies ($r_{\hat{g}g} \geq 0.90$) were estimated for DBH and PP in all environments, indicating high reliability and a favorable scenario for recommendation of superior clones since high accuracy allows correct ranking of the genotypes.

Reaction norms

The presence of $G \times E$ interactions is very clear since the reaction norms are non-constant, the genotypes show genotypic plasticity; and when the reaction norms intersect, a complex $G \times E$ interaction occurs (van Eeuwijk et al. 2016). This type of $G \times E$ interactions has more severe consequences for breeders as it changes the rank of genotypes in accordance with

environmental conditions, i.e., it indicates that the superior genotype in one environment will not normally perform as well in another environment (Resende 2015).

Genotypic plasticity is essential for genotype performance in changing environments (Rocha et al. 2018). Reaction norms shows that the evaluated clones present various forms of genotypic plasticity. In this context, genotypic plasticity can be considered as favorable or unfavorable changes for genotype adaptedness (van Eeuwijk et al. 2016).

Resende et al. (2018) investigated the environmental uniformity, site quality and tree competition interact to determine stand productivity of clonal eucalyptus and showed the importance of adopting environmental gradient-based approaches in tree genetic testing and clone recommendation as a way to more accurately match genotypes to specific sites. Marchal et al. (2019) investigated the role of genotypic plasticity on construction of hybrid larch (*Larix decidua* × *Larix kaempferi*) heterosis and on expression of its quantitative genetic parameters. They used random regression models fitted through Legendre polynomials to model reaction norms of ring width and wood density with respect to water availability and concluded that hybrid larch appeared to be the most plastic taxon as its superiority over its parental species increased with increasing water availability.

Eigenfunctions

The estimation of covariances between the random regression coefficients produces estimates of covariance functions (Kirkpatrick et al. 1990), which refer to a continuous description of the covariance structure of the trait along the environmental gradient. The analyses of the eigenfunctions are given by the total variance decomposition considering the principal components analyses (Arnal et al. 2019). This approach is similar to genetic correlations among the environments (Van der Werf et al. 1998).

According to Kirkpatrick et al. (1990), the first eigenfunction clustered general adaptability genes that was equally expressed in all environments. This can be interpreted as the genetic correlation that exists among the environments. The second eigenfunction clustered specific adaptability genes that expressed themselves depending on environmental differences. This can be interpreted as a lack of genetic correlation among the environments. The third and fourth eigenfunctions showed small eigenvalues and represent deformations for which there is little (or no) genetic variation.

Area under the reaction norms

In plant breeding, $G \times E$ interactions can reduce heritability and genetic gain with the selection. Li et al. (2017) comment that breeders have been adopting two selection strategies in the presence of significant $G \times E$ interactions: selecting stable genotypes that are not sensitive to environmental changes or selecting genotypes for specific environments in order to maximize genetic gain with the selection at that environment. In context of the present work, both selection strategies can be applied.

The genotype ranking was performed based on the areas under the reaction norms. The advantage of this strategy is that selection response can be predicted not only in genotypic expression in any environment but also in quantifying the environmental sensitivity of the trait through the reaction norm (robustness or responsiveness to changes in the environment), and it can be used for any number of environments. It is important to highlight that in this study, random regression models were used to fit realistic reaction norms, allowing investigation of changes in genetic covariances along the environmental gradient, as suggested recently by several authors (Marcatti et al. 2017; Li et al. 2017; Marchal et al. 2019).

Spatial genotypic plasticity is generally what interests breeders most because of the operational implications for deployment of varieties (Marchal et al. 2019). For DBH, clones with larger area under the reaction norms are desirable as they relate directly to the volume of the tree (Alves et al. 2018). However, for PP, which is related to the basic density of wood, the direction of the selection depends of the purpose of raw material (pulp, bio-oil, firewood, charcoal, among others) (Fonseca et al. 2010).

CONCLUSION

The results showed that random regression models fitted through Legendre polynomials are a powerful technique to quantifying individual variation in reaction norms and therefore can be efficiently applied in the genetic evaluation of eucalyptus. Besides that, for untested environments, the genetic selection can be made based on the areas under the reaction norms.

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AUTHOR'S CONTRIBUTION

Conceptualization: Alves R. S. and Resende M. D. V.; Methodology: Alves R. S., Resende M. D. V., Rocha J. R. A. S. C. and Silva F. F.; Investigation: Alves R. S., Rocha J. R. A. S. C., Peixoto M. A. and Teodoro P. E.; Writing – Original Draft: Alves R. S., Rocha J. R. A. S. C., Peixoto M. A. and Teodoro P. E.; Writing – Review and Editing: Alves R. S.; Funding Acquisition: Santos G. A.; Resources: Bhering L. L. and Santos G. A.; Supervision: Resende M. D. V.

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SUPPLEMENTARY MATERIAL

```

ASReml program file - DBH
!WORKSPACE !RENAME !ARGS 1 2 // !DOPART $1
Title: RRM_dbh.
environment * !A
clone * !I
rep *
grad *
dbh !M 0
data.dbh.txt !SKIP 1 !MAXITER 1000
!PART 1 # residue homogeneous
!CYCLE 0 1 2 3
dbh ~ mu environment.rep !r leg(grad,$I).clone !f mv
0 0 1
leg(grad,$I).clone 2
leg(grad,$I) 0 US !GP
((SI^2+3*SI+2)/2)*0
clone
!PART 2 # residue heterogeneous
!CYCLE 0 1 2 3
dbh ~ mu environment.rep !r leg(grad,$I).clone !f mv
4 1 1
6352 0 ID
6422 0 ID
6416 0 ID
6419 0 ID
leg(grad,$I).clone 2
leg(grad,$I) 0 US !GP
((SI^2+3*SI+2)/2)*0
clone
ASReml program file - PP
!WORKSPACE !RENAME !ARGS 1 2 // !DOPART $1
Title: RRM_PP.
environment * !A
clone * !I
rep *
grad *
pp !M 0
data.pp.txt !SKIP 1 !MAXITER 1000
!PART 1 # residue homogeneous
!CYCLE 0 1 2 3
pp ~ mu environment.rep !r leg(grad,$I).clone !f mv
0 0 1
leg(grad,$I).clone 2
leg(grad,$I) 0 US !GP
((SI^2+3*SI+2)/2)*0
clone
!PART 2 # residue heterogeneous
!CYCLE 0 1 2 3
pp ~ mu environment.rep !r leg(grad,$I).clone !f mv
4 1 1
6416 0 ID
6352 0 ID
6419 0 ID
6422 0 ID
leg(grad,$I).clone 2
leg(grad,$I) 0 US !GP
((SI^2+3*SI+2)/2)*0
clone

```

Table S1. Genotype ranking according to areas under the reaction norms (A) for diameter at breast height (DBH) and Pilodyn penetration (PP) in eucalyptus.

Rank	DBH		PP	
	Clone	A	Clone	A
1	24	18.12	43	29.79
2	154	17.58	57	29.53
3	50	17.50	90	29.13
4	206	17.18	139	28.03
5	152	17.07	44	27.84
6	70	17.00	101	27.65
7	170	16.96	126	27.10
8	43	16.90	40	27.07
9	195	16.72	78	26.66
10	18	16.72	131	26.59
11	149	16.66	33	26.13
12	199	16.60	51	25.56
13	54	16.51	148	25.47
14	95	16.48	89	25.42
15	131	16.44	93	25.28
16	159	16.36	19	24.95
17	105	16.28	130	24.58
18	60	16.23	97	24.52
19	10	16.19	144	24.27
20	23	16.18	196	24.19
21	215	16.17	128	24.18
22	182	16.11	23	24.09
23	192	16.11	192	24.06
24	94	16.06	203	24.06
25	114	16.03	200	23.96
26	141	16.03	208	23.87
27	210	16.01	121	23.73
28	96	15.98	215	23.70
29	46	15.95	9	23.67
30	11	15.91	157	23.54
31	119	15.90	58	23.35
32	71	15.90	179	23.11
33	65	15.88	47	23.00
34	3	15.87	188	22.49
35	111	15.79	37	22.37
36	133	15.61	49	22.25
37	32	15.52	36	22.19
38	150	15.41	201	22.16
39	130	15.39	104	22.05
40	101	15.38	178	21.91
41	27	15.37	99	21.76
42	136	15.37	110	21.74
43	122	15.36	204	21.62
44	97	15.36	100	21.62
45	61	15.33	20	21.44

Continue...

Continuation...

Rank	DBH		PP	
	Clone	A	Clone	A
46	44	15.29	185	21.37
47	41	15.29	81	21.29
48	156	15.25	187	21.24
49	126	15.24	42	21.17
50	171	15.23	17	21.15
51	68	15.23	45	21.14
52	140	15.21	32	21.06
53	129	15.08	146	21.05
54	4	15.06	31	21.03
55	25	15.05	213	21.00
56	81	15.03	143	20.95
57	158	14.98	75	20.87
58	161	14.97	122	20.72
59	189	14.96	214	20.66
60	145	14.95	132	20.44
61	100	14.94	186	20.37
62	89	14.92	65	20.35
63	178	14.86	160	20.35
64	62	14.84	52	20.34
65	93	14.84	27	20.29
66	33	14.83	124	20.25
67	90	14.80	191	20.24
68	213	14.80	106	20.07
69	153	14.79	135	20.05
70	83	14.79	190	20.02
71	184	14.78	120	20.01
72	99	14.73	96	19.84
73	36	14.72	145	19.80
74	127	14.71	88	19.71
75	40	14.70	176	19.69
76	193	14.69	35	19.64
77	45	14.64	77	19.60
78	2	14.61	71	19.56
79	69	14.60	107	19.51
80	74	14.59	182	19.49
81	183	14.52	153	19.39
82	21	14.50	62	19.38
83	107	14.49	69	19.36
84	106	14.45	169	19.35
85	137	14.43	129	19.32
86	214	14.43	61	19.30
87	17	14.41	41	19.28
88	115	14.37	212	19.15
89	121	14.36	74	19.13
90	202	14.36	189	19.09

Continue...

Continuation...

Rank	DBH		PP	
	Clone	A	Clone	A
91	196	14.36	60	19.08
92	169	14.34	103	19.05
93	29	14.32	118	18.82
94	162	14.26	166	18.79
95	35	14.24	123	18.78
96	139	14.17	73	18.66
97	172	14.16	72	18.62
98	128	14.14	80	18.61
99	160	14.13	117	18.58
100	38	14.12	29	18.54
101	148	14.08	183	18.52
102	124	14.07	195	18.49
103	39	14.06	173	18.47
104	190	14.05	133	18.45
105	103	13.98	111	18.36
106	52	13.96	151	18.34
107	72	13.95	141	18.19
108	163	13.90	68	18.18
109	66	13.87	210	18.07
110	203	13.87	30	18.06
111	176	13.85	50	18.01
112	30	13.78	10	17.99
113	164	13.77	26	17.98
114	143	13.76	165	17.98
115	194	13.75	115	17.93
116	120	13.75	116	17.77
117	9	13.69	125	17.67
118	167	13.63	87	17.67
119	82	13.61	34	17.64
120	22	13.58	11	17.62
121	112	13.58	59	17.55
122	125	13.51	109	17.54
123	177	13.49	114	17.49
124	20	13.48	211	17.45
125	134	13.45	98	17.43
126	19	13.41	46	17.40
127	109	13.39	66	17.38
128	173	13.38	54	17.36
129	168	13.36	7	17.35
130	51	13.31	171	17.31
131	16	13.27	161	17.29
132	34	13.27	207	17.21
133	179	13.26	53	17.12
134	56	13.24	92	17.00
135	48	13.23	112	17.00

Continue...

Continuation...

Rank	DBH		PP	
	Clone	A	Clone	A
136	157	13.22	28	16.93
137	108	13.21	149	16.90
138	80	13.11	158	16.90
139	49	13.08	174	16.89
140	208	12.96	76	16.88
141	185	12.94	24	16.87
142	180	12.91	206	16.82
143	187	12.89	180	16.80
144	37	12.81	198	16.79
145	147	12.78	83	16.76
146	86	12.77	55	16.72
147	207	12.74	86	16.71
148	58	12.65	205	16.64
149	87	12.62	155	16.60
150	26	12.61	95	16.41
151	197	12.58	8	16.35
152	132	12.52	199	16.32
153	53	12.50	175	16.31
154	135	12.49	172	16.29
155	181	12.46	94	16.25
156	166	12.42	82	16.20
157	174	12.38	2	16.20
158	12	12.35	147	16.20
159	64	12.32	39	16.19
160	138	12.28	184	16.16
161	198	12.25	127	16.10
162	116	12.24	21	15.95
163	13	12.22	140	15.94
164	88	12.22	113	15.93
165	14	12.13	167	15.93
166	212	12.10	152	15.90
167	5	12.07	137	15.87
168	117	12.06	16	15.77
169	67	12.03	13	15.77
170	191	11.96	150	15.76
171	77	11.93	159	15.64
172	144	11.90	38	15.56
173	47	11.87	105	15.55
174	151	11.85	119	15.53
175	188	11.83	85	15.49
176	165	11.81	63	15.31
177	118	11.79	64	15.29
178	7	11.60	209	15.28
179	78	11.46	162	15.22
180	146	11.39	154	15.10

Continue...

Continuation...

Rank	DBH		PP	
	Clone	A	Clone	A
181	155	11.36	164	14.92
182	92	11.31	170	14.92
183	110	11.29	134	14.83
184	57	11.21	18	14.76
185	31	11.11	108	14.61
186	28	11.04	12	14.46
187	59	10.99	138	14.44
188	55	10.98	84	14.43
189	84	10.94	193	14.40
190	142	10.90	56	14.35
191	42	10.85	70	14.28
192	113	10.84	14	14.26
193	186	10.78	6	14.23
194	73	10.71	25	14.23
195	15	10.68	102	14.07
196	123	10.42	5	14.06
197	98	10.41	202	13.84
198	79	10.40	194	13.77
199	102	10.38	136	13.35
200	205	10.33	142	13.15
201	91	10.13	156	13.08
202	211	10.06	3	13.07
203	8	9.81	48	13.00
204	6	9.72	181	12.99
205	209	9.68	177	12.66
206	76	9.52	168	12.54
207	1	9.25	22	12.24
208	201	8.68	67	12.12
209	200	8.56	79	11.95
210	75	8.41	163	11.60
211	63	8.39	15	11.50
212	85	7.95	4	11.23
213	175	7.72	1	11.05
214	104	6.75	197	10.84
215	204	6.14	91	9.95

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