

1 Evaluating the accuracy of genomic prediction of growth and wood  
2 traits in two *Eucalyptus* species and their F<sub>1</sub> hybrids

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18 **Abstract**

19 **Background:** Genomic prediction is a genomics assisted breeding methodology that can  
20 increase genetic gains by accelerating the breeding cycle and potentially improving the  
21 accuracy of breeding values. In this study, we used 41,304 informative SNPs genotyped in a  
22 *Eucalyptus* breeding population involving 90 *E.grandis* and 78 *E.urophylla* parents and their

23 949 F<sub>1</sub> hybrids to develop genomic prediction models for eight phenotypic traits - basic  
24 density and pulp yield, circumference at breast height and height and tree volume scored at  
25 age three and six years. Based on different genomic prediction methods we assessed the  
26 impact of the composition and size of the training/validation sets and the number and  
27 genomic location of SNPs on the predictive ability (PA).

28 **Results:** Heritabilities estimated using the realized genomic relationship matrix (GRM) were  
29 considerably higher than estimates based on the expected pedigree, mainly due to  
30 inconsistencies in the expected pedigree that were readily corrected by the GRM. Moreover,  
31 GRM more precisely capture Mendelian sampling among related individuals, such that the  
32 genetic covariance was based on the actual proportion of the genome shared between  
33 individuals. PA improved considerably when increasing the size of the training set and by  
34 enhancing relatedness to the validation set. Prediction models trained on pure species parents  
35 could not predict well in F<sub>1</sub> hybrids, indicating that model training has to be carried out in  
36 hybrid populations if one is to predict in hybrid selection candidates. The different genomic  
37 prediction methods provided similar results for all traits, therefore GBLUP or rrBLUP  
38 represents better compromises between computational time and prediction efficiency. Only  
39 slight improvement was observed in PA when more than 5,000 SNPs were used for all traits.  
40 Using SNPs in intergenic regions provided slightly better PA than using SNPs sampled  
41 exclusively in genic regions.

42 **Conclusions:** Effects of training set size and composition and number of SNPs used are the  
43 most important factors for model prediction rather than prediction method and the genomic  
44 location of SNPs. Furthermore, training the prediction model on pure parental species  
45 provide limited ability to predict traits in interspecific hybrids. Our results provide additional  
46 promising perspectives for the implementation of genomic prediction in *Eucalyptus* breeding  
47 programs.

48

## 49 **Keywords**

50 Genomic relationship, genomic heritability, two-generation, genome annotation, high-density

51 SNP-chip, Bayesian LASSO, GBLUP, rrBLUP

## 52 **Background**

53 *Eucalyptus* species and their hybrids are the most widely planted hardwoods in tropical,  
54 subtropical and temperate regions, due to their fast growth, short rotation, wide  
55 environmental adaptability and suitability for commercial pulp and paper production [1, 2].

56 Interspecific hybrids of *E.grandis* and *E.urophylla*, in particular, are generally superior to  
57 their parents in growth, wood quality and biotic and abiotic stresses resistance, by inheriting  
58 both the fast growth and good rooting abilities of *E.grandis* and the disease tolerance and  
59 wide adaptability of *E.urophylla* [3]. A conventional breeding cycle toward clonal selection  
60 in hybrid populations involves mating, progeny trial, a small-scale clonal trial and a second  
61 expanded clonal trial, that together typically take between 12 and 18 years [1, 4]. To  
62 accelerate the genetic gain per unit time, new methods that can help shorten the breeding  
63 cycles are greatly needed.

64 Genomic prediction or genomic selection (GS) is one of the most recent developments in  
65 genomics-assisted methods that are aimed at improving breeding efficiency and genetic gains.  
66 Genomic prediction provides a genome-wide paradigm for marker-assisted selection  
67 (MAS)[5, 6]. In GS all markers are fitted simultaneously in a model that relies on the  
68 principle of linkage disequilibrium (LD) to capture most of the relevant variation throughout  
69 the genome, whereas MAS focuses on discrete quantitative trait loci (QTLs) that had  
70 previously been detected, usually in underpowered experiments and thus leaving most of the  
71 variation unaccounted for [7]. GS are generally performed in three steps: (1) genotyping and  
72 phenotyping a ‘reference’ or ‘training population’ and developing genomic prediction models

73 that allow for prediction of phenotypes from genotypes; (2) validation of the predictive  
74 models in a ‘validation population’, i.e. a set of individuals that did not participate in model  
75 training; (3) application of the models to predict the genomic estimated breeding values  
76 (GEBVs) of unphenotyped individuals which are then selected according to their GEBVs [6].  
77 GS has been successfully implemented in the breeding of livestock [7, 8] and crops [9, 10]  
78 and several recent papers suggest that has great potential also in forest trees [11, 12].

79 The accuracy of genomic prediction models can vary depending on the statistical method  
80 employed. Several methods have been developed for GS, including ridge-regression best  
81 linear unbiased prediction (rrBLUP), genomic best linear unbiased prediction (GBLUP),  
82 BayesA, BayesB, Bayesian LASSO, BayesR and reproducing kernel Hilbert space (RKHS)  
83 regression [7, 13]. These methods vary in the assumptions of the distribution and variances  
84 of marker effects. rrBLUP assumes that marker effects follow a normal distribution where all  
85 effects are shrunk to a similar and small size, while Bayesian methods (BayesA, BayesB,  
86 Bayesian LASSO and BayesR) assume that genetic variances specific to the marker effects  
87 and including a priori data on the probability distributions of marker effects. The GBLUP  
88 method computes the additive genetic merits from a genomic relationship matrix and is  
89 equivalent to rrBLUP under conditions that are generally met in practice [14]. The RKHS  
90 regression model is a linear combination of the basic function provided by the reproducing  
91 kernel [15]. Recent studies have indicated that the selection of suitable statistical methods  
92 depends on the actual data at hand and the pattern of phenotypic variation in the traits of  
93 interest and with reference population used [9, 16].

94 Besides statistical methods, other factors are known to influence the accuracy of genomic  
95 prediction models, such as the size of the training population, number of markers employed,  
96 and relatedness between the training and validation population and, by extension, to the  
97 future selection candidates. Hayes et al. [17] found that for a given effective population size

98 ( $N_e$ ), increasing the size of the reference population leads to improved accuracy of GS based  
99 predictions. Closer relationship between training population and selection candidates has  
100 been reported to lead to a higher accuracy of genomic predictions, while enlarge genetic  
101 diversity of the training population resulted in lower accuracy [18]. A number of simulation  
102 and empirical studies have shown that increasing the number of markers may improve the  
103 predictive accuracy as the  $N_e$  also increases [9, 19-21]. However, increasing the number of  
104 markers in small  $N_e$  populations has little or no improvement on predictive accuracy [22, 23].

105 Going one step further from previous studies in forest trees, where individuals of the same  
106 breeding generation were allocated to training and validation sets for the evaluation of  
107 genomic prediction models, in this study we used both the parental and progeny generations  
108 of *E. grandis*, *E. urophylla* and their F<sub>1</sub> hybrids to build prediction models using different  
109 subsets of parents and progeny for training and validation. A multi-species single-nucleotide  
110 polymorphism (SNP) chip containing 60,904 SNPs [24] were used to provide high-density  
111 genotyping of the two generations. Based on these data, we developed genomic prediction  
112 models for height, circumference at breast height (CBH), volume, wood basic density and  
113 pulp yield, using a number of statistical methods and compared their performance to the  
114 traditional pedigree-based prediction. Furthermore, we evaluated the impact of varying the  
115 number of SNPs and the training set/validation set composition and size on the predictive  
116 ability (PA) of genomic prediction.

## 117 **Methods**

### 118 **Breeding population**

119 The breeding population in this study was established by controlled crossings of 86 *E.*  
120 *urophylla* and 95 *E. grandis* trees (G0 population) following a incomplete diallel mating  
121 design, resulting in 16,660 progeny individuals (G1 population) comprising 476 full-sib  
122 families with 35 individuals per family. In 2009, the progenies were deployed in a field trial

123 in a randomized complete block design with single-tree plots and 35 reps per family in  
124 Belmonte (Brazil, 39.19W, 16.06 S, 210 m above the sea level) at Veracel Celulose S.A.  
125 (Eunápolis, BA, Brazil). Our experimental population consists of 168 parents (78 of  
126 *E.urophylla* and 90 of *E.grandis*) (G0), as not all parents were still alive at the time of study,  
127 and 958 progeny individuals (G1) sampled across 338 full-sib families by avoiding low  
128 performing trees. The number of individuals in each full-sib family ranged from one to 13  
129 with an average of 2.8 individuals per family.

### 130 **Phenotyping**

131 For the 958 G1 samples, height, volume, and circumference at breast height (CBH) were  
132 measured at age three and six years, respectively, and the wood traits (basic density and pulp  
133 yield) were measured at age five years. For the 168 G0 parents, the same traits had been  
134 measured at age seven years for *E. grandis* and at age five years for *E. urophylla*. Briefly,  
135 height was measured using a Suunto hypsometer/height meter (PM-5/1520 series) and CBH  
136 was measured with a centimetre tape at 130 cm above ground. Wood properties were  
137 estimated by employing near-infrared reflectance spectra of sawdust samples collected at  
138 breast height using a FOSS NIRSystem 5000-M and applying calibration models developed  
139 earlier by Veracel S.A..

140 A mixed linear model was applied to minimize the impacts of environmental and age  
141 differences on each trait.

$$Y = X\beta + Zu + Wb + e$$

142 where  $Y$  is a vector of trait;  $\beta$  is a vector of fixed effects, including overall mean,  
143 experimental sites and age differences;  $u$  is a vector of random additive genetic effect of  
144 individuals with a normal distribution,  $u \sim N(0, A\sigma_u^2)$ ,  $A$  is a matrix of additive genetic  
145 relationships among individuals;  $b$  is a vector of random incomplete block effect nested in  
146 each experimental site; and  $e$  is a heterogeneous random residual effect in each experimental

147 site.  $\mathbf{X}$ ,  $\mathbf{Z}$  and  $\mathbf{W}$  are incidence matrices for  $\boldsymbol{\beta}$ ,  $\mathbf{u}$  and  $\mathbf{b}$ , respectively. The phenotypes of each  
148 trait were then corrected by subtracting variation of sites, ages and blocks effects for all  
149 individuals, and are referred to as adjusted phenotypes. The adjusted phenotypic traits were  
150 used for calculating the heritability of traits and for building genomic prediction models.

### 151 **Genotyping and quality control**

152 The 168 G0 and 958 G1 populations were genotyped using the Illumina Infinium  
153 EuCHIP60K [24] that contains probes for 60,904 SNPs. EUChip60K intensity data (.idat files)  
154 were obtained through GENESEEEK (Lincoln, NE, USA). SNP genotypes were called using  
155 GenomeStudio (Illumina Inc., San Diego, CA, USA) following standard genotyping and  
156 quality control procedures with no manual editing of clusters as described earlier [24].  
157 Further quality control of the genotyped samples was performed using PLINK [25]. Nine G1  
158 individuals were removed due to low sample call rate (<70%) or high inbreeding coefficient  
159 ( $F > 1$ ). 10,240 SNPs were excluded due to low call rate (<70%), 9,243 SNPs were filtered out  
160 due to monomorphism or minor allele frequency (MAF) < 0.01, and 117 SNPs were removed  
161 due strong deviations from Hardy-Weinberg equilibrium (p-value <  $1 \times 10^{-6}$ ).

162 After quality control, missing genotypes of the remaining individuals were filled in by  
163 imputation. We first tested the accuracy of imputation methods across a range of missing data  
164 (2% - 30%) by artificial removing SNPs from a fraction of our genotypes. Among the  
165 available family-based and population based methods we assessed the following programs for  
166 imputation accuracy: BEAGLE [26], fastPHASE [27], MENDEL [28], random forest, SVD  
167 Impute, k-nearest neighbors [29], BLUP A matrix, Bayesian PCA, NIPALS, Probabilistic  
168 PCA [30]. BEAGLE provided the best accuracy for all missing data percentages, with  
169 accuracies exceeding 95% in all cases (Additional file 1). We therefore used BEAGLE to  
170 impute missing genotypes at the retained 41,304 SNPs across the 168 G0 and 949 G1  
171 individuals. The imputed genotypic data was subsequently used in all genomic prediction

172 analyses. LD between SNP pairs was measured using the squared correlation coefficient ( $r^2$ )  
173 for SNPs located on the same chromosome. The decay of LD versus physical distance was  
174 then modelled using the nonlinear regression method described in Remington et al. [31].

175 We further studied the population structure and pairwise genomic relationship among the  
176 1117 individuals by performing principal components analysis (PCA) [32] and kinship  
177 analysis [33] using 10,213 independent SNPs (LD-pruned) ( $r^2 < 0.2$ ) calculated in PLINK  
178 [25]. Pedigree-based genetic relationship was estimated from ABLUP (see below for further  
179 information).

### 180 **Statistical methods for genomic prediction**

181 Four statistical methods were assessed to estimate the parameters in equation (1) and for  
182 predicting GEBVs, including genomic best linear unbiased predictor (GBLUP) [5], ridge  
183 regression BLUP (rrBLUP) [6], Bayesian LASSO (BL) [34], and reproducing kernel Hilbert  
184 space (RKHS) regression [15]. The performance of the four genomic prediction methods was  
185 compared with that of the commonly used pedigree-based BLUP (ABLUP) [35].

186 The GEBVs were estimated using the following mixed linear model:

$$187 \quad \mathbf{y} = \mathbf{1}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{e} \quad (1)$$

188 where  $\mathbf{y}$  is the vector of adjusted phenotypes of single trait,  $\boldsymbol{\beta}$  is the vector of overall mean  
189 fitted as a fixed effect,  $\mathbf{a}$  is the vector of random effects, and  $\mathbf{e}$  is the vector of random  
190 residual effects.  $\mathbf{1}$  and  $\mathbf{Z}$  are incident matrix of  $\boldsymbol{\beta}$  and  $\mathbf{a}$ , respectively.

191 **ABLUP.** ABLUP is the standard method for predicting breeding values using the expected  
192 relatedness among individuals based on pedigree information [35]. For ABLUP, the vector of  
193 random additive effects ( $\mathbf{a}$ ) in the equation (1) is assumed to follow a normal distribution,  
194  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2)$ , where  $\mathbf{A}$  is the additive numerator relationship matrix estimated from  
195 pedigree information and the  $\sigma_a^2$  is the additive genetic variance. The residual vector  $\mathbf{e}$  is

196 assumed as  $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$ , where  $\mathbf{I}$  is the identity matrix. Under these assumptions, equation  
 197 (1) can be re-written as:

$$198 \quad \begin{bmatrix} \mathbf{X}^T \mathbf{X} & \mathbf{X}^T \mathbf{Z} \\ \mathbf{Z}^T \mathbf{X} & \mathbf{Z}^T \mathbf{Z} + \mathbf{A}^{-1} \frac{\sigma_e^2}{\sigma_a^2} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{a}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}^T \mathbf{y} \\ \mathbf{Z}^T \mathbf{y} \end{bmatrix} \quad (2)$$

199 where  $\frac{\sigma_e^2}{\sigma_a^2}$  is estimated using a restricted maximum likelihood method. The estimated breeding  
 200 values ( $\hat{\mathbf{a}}$ ) can be calculated directly from equation (2). ABLUP calculations were performed  
 201 using ASReml 3.0 [36].

202 **GBLUP.** The GBLUP method is derived from ABLUP, but differs in that the matrix  $\mathbf{A}$  in  
 203 equation (2) is replaced with the genomic relationship matrix ( $\mathbf{G}$ ) that is calculated from the  
 204 genotypic data as  $\mathbf{G} = \frac{(\mathbf{M}-\mathbf{P})(\mathbf{M}-\mathbf{P})^T}{2 \sum_{j=1}^p p_j(1-p_j)}$ , where  $\mathbf{M}$  is the matrix of samples and their  
 205 corresponding SNPs denoted as 0, 1, 2,  $\mathbf{P}$  is the matrix of allele frequencies with the  $j$ -th  
 206 column given by  $2(p_j - 0.5)$ , where  $p_j$  is the observed allele frequency of the samples [5]. In  
 207 GBLUP, the random additive effects ( $\mathbf{a}$ ) in the equation (1) is assumed to follow  
 208  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{G}\sigma_g^2)$ , where  $\sigma_g^2$  is the genetic variance and GEBVs are again calculated from  
 209 equation (2) but with  $\mathbf{A}^{-1}$  replaced by  $\mathbf{G}^{-1}$  and  $\sigma_a^2$  replaced by  $\sigma_g^2$ . The GBLUP calculations  
 210 were performed using ASReml 3.0 [36].

211 **rrBLUP.** As opposed to the previous two methods rrBLUP alters the notations of  
 212 parameters  $\mathbf{a}$  and  $\mathbf{Z}$  in the equation (1), where  $\mathbf{Z}$  now refers to a design matrix for SNP  
 213 effects, rather than incident matrix and  $\mathbf{a}$  refers to SNP effects that are assumed to follow  
 214  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{I}\sigma_m^2)$ , where  $\sigma_m^2$  denotes the proportion of the genetic variance contributed by each  
 215 SNP [6]. With these alterations, equation (2) becomes:

$$216 \quad \begin{bmatrix} \mathbf{X}^T \mathbf{X} & \mathbf{X}^T \mathbf{Z} \\ \mathbf{Z}^T \mathbf{X} & \mathbf{Z}^T \mathbf{Z} + \mathbf{I}\lambda \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{a}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}^T \mathbf{y} \\ \mathbf{Z}^T \mathbf{y} \end{bmatrix} \quad (3)$$

217 where  $\lambda = \sigma_e^2 / \sigma_u^2$  is the ratio between the residual and marker variances. A prediction for  
218 the GEBV for each individual is calculated as  $\hat{g}_i = \mathbf{Z}_i^T \hat{\mathbf{a}}$  from equation (3), where  $\mathbf{Z}_i^T$  is the  
219 SNP vector for individual  $i$  and  $\hat{\mathbf{a}}$  is the vector of estimated SNP effects. All calculations  
220 were performed using the rrBLUP package in the R environment [33].

221 **Bayesian LASSO.** The Bayesian LASSO (BL) method is the Bayesian treatment of  
222 LASSO regression proposed by Legarra et al. [34]. In BL the vector of SNP effects  $\mathbf{a}$  in  
223 equation (1) is assumed to follow a hierarchical prior distribution with  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{T}\sigma_m^2)$ , where  
224  $\mathbf{T} = \text{diag}(\tau_1^2, \dots, \tau_p^2)$ .  $\tau_j^2$  is assigned as  $\tau_j^2 \sim \text{Exp}(\lambda^2)$ ,  $j=1, \dots, p$ .  $\lambda^2$  is assigned as  
225  $\lambda^2 \sim \text{Gamma}(r, \delta)$ . The residual variance  $\sigma_e^2$  is assigned as  $\sigma_e^2 \sim \chi^{-2}(df_e, S_e)$ .

226 We implemented the BL method using the BLR package in R [37]. Here a Monte Carlo  
227 Markov Chains sampler was applied and prior parameters ( $df_e, S_e, r, \delta$ , and  $\lambda^2$ ) were defined  
228 following the guidelines proposed by de los Campos *et al.* [38]. The chain length was 20,000  
229 iterations, with the first 2,000 excluded as burn-in and with a subsequent thinning interval of  
230 100.

231 **RKHS.** RKHS assumes that the random additive effects in equation (1) are  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{K}\sigma_g^2)$ ,  
232 where  $\mathbf{K}$  is computed by means of a Gaussian kernel that is given by  $K_{ij} = \exp(-hd_{ij})$  [15].  
233  $h$  is a semi-parameter that controls how fast the prior covariance function declines as genetic  
234 distance increase and  $d_{ij}$  is the genetic distance between two samples computed as  $d_{ij} =$   
235  $\sum_{k=1}^p (x_{ik} - x_{jk})^2$ , where  $x_{ik}$  and  $x_{jk}$  are  $k$ th SNPs ( $k=1, \dots, p$ ) for the  $i$ th and  $j$ th samples,  
236 respectively. We implemented the RKHS method through the BGLR package in R [39],  
237 which uses a Gibbs sampler for the Bayesian framework and assigns the prior distribution of  
238  $\sigma_g^2$  and  $\sigma_e^2$  as  $\sigma_g^2 \sim \chi^{-2}(df_g, S_g)$  and  $\sigma_e^2 \sim \chi^{-2}(df_e, S_e)$ , respectively. Here we chose a multi-  
239 kernel model suggested by Perez [39], where three  $h$  were defined as  $h_1 = 2/(5 * \bar{d})$ ,

240  $h_2 = 2/\bar{d}$ ,  $h_3 = 2 * 5/\bar{d}$ ,  $\bar{d}$  was the median of  $d_{ij}$ . The Gibbs chain length was 20,000  
241 iterations with the first 2000 iterations discarded as burn-in and a thinning interval set to 100.

## 242 **Heritability estimation**

243 We estimated the pedigree-based narrow-sense heritability ( $h_a^2$ ) using the relationship  
244 matrix from the ABLUP method, and the narrow-sense genomic heritability ( $h_g^2$ ) using the  
245 genomic relationship matrix from GBLUP [40]. The respective heritabilities were calculated  
246 as:

$$247 \quad h_a^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{ea}^2} \quad h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{eg}^2}$$

248 where  $\sigma_a^2$  is the additive genetic variance and  $\sigma_{ea}^2$  is the residual variance estimated with  
249 ABLUP, while  $\sigma_g^2$  is the genetic variance and  $\sigma_{eg}^2$  is the residual variance estimated with  
250 GBLUP.

## 251 **Size and genetic composition of the training and validation sets**

252 We simultaneously assessed the impact of the size and genetic participation of G0 and G1  
253 individuals in the training set (TS) and validation set (VS) of the genomic prediction models.  
254 Regarding TS/Vs sizes, we divided all 1,117 (G0 and G1) individuals into five different size  
255 groups with a ratio of TS to VS of 1:1, 2:1, 3:1, 4:1 and 9:1. The corresponding sizes of the  
256 TS/Vs were respectively 558/559, 743/374, 836/281, 892/225 and 1003/114. Within these  
257 pre-established size compositions, four scenarios of the participation of G0 and G1  
258 individuals were evaluated to assess the impact of varying the degrees of relationship and  
259 diversity between TS and VS. In the first scenario (CV<sub>1</sub>) assignment of individuals to either  
260 TS or VS was random. For the second scenario (CV<sub>2</sub>) all G0 parents were assigned to the TS  
261 and complemented with G1 individuals up to the required number in the set, while the VS  
262 was composed exclusively of G1 individuals. The third and fourth scenarios were built based  
263 on minimizing and maximizing relatedness between TS and VS. The relatedness-based  
264 assignment of individuals was determined using the procedure described in Spindel *et al.* [9].

265 Briefly, 1,117 individuals were assigned to 182 clusters based on genotypes using the k-  
266 means clustering algorithm, a method that attempts to minimize the distance between  
267 individuals in a cluster and the centre of that cluster. Using the relatedness estimates, CV<sub>3</sub>  
268 was then built by assigning individuals to TS and VS based on dissimilarity, such that  
269 individuals from the same cluster were not allowed to be both in the same TS or VS. For CV<sub>4</sub>  
270 individuals from same cluster were forced to be either in the TS or VS [9].

### 271 **Genomic prediction models**

272 We evaluated the effects of the five statistical methods (GBLUP, rrBLUP, BL, RKHS and  
273 ABLUP), five TS/VS sizes and four TS/VS composition scenarios (5\*5\*4 = 100 models in  
274 total) on the predictive ability (PA) of genomic prediction. For each of the 100 models, 200  
275 replicate runs were carried out for each trait and the performance of the models were  
276 evaluated in terms of their PA ( $r_y, \hat{g}$ ), which is defined as the Pearson correlation between the  
277 adjusted phenotypes and the GEBVs of the samples in the VS. ANOVA was performed on 80  
278 out of 100 models tested (20 ABLUP models excluded) to partition the variance into different  
279 sources, with all effects declared as fixed, comparing all the sources of variation (genomic  
280 prediction method, TS/VS size and genetic composition). Significant differences found were  
281 further assessed by means of a paired t tests ( $\alpha = 5\%$ ), adjusted by a Bonferroni correction.  
282 The 80 models as described above were used for assessing the impact of TS/VS composition  
283 and TS/VS size, while all 100 models were used to evaluate the statistical methods against  
284 ABLUP. All available SNPs were used in all the analyses of these models.

### 285 **Numbers and genomic location of SNPs subsets**

286 We finally assessed the impact of the number of SNPs and their locations (gene vs.  
287 intergenic region) on the PA of genomic prediction models. 12 subsets with different  
288 numbers of SNPs were generated by randomly selecting 10, 20, 50, 100, 200, 500, 1,000,  
289 2,000, 5,000, 10,000, 20,000 and 41,304 SNPs from all the available SNPs. For SNP location,

290 SNPs subsets located in different regions of the genome were established by including SNPs  
291 located in four different regions: (i) coding sequences (CDS) only (11,786 SNPs); (ii) entire  
292 genic regions including CDS, UTRs, introns, and sequences 2kb up and downstream of the  
293 gene (30,405 SNPs); (iii) intergenic regions (10,899 SNPs), and (iv) all 41,304 SNPs. The  
294 location of each SNP was obtained by mapping SNPs onto *E.grandis* genome database using  
295 SnpEff [41]. Genomic prediction models were built for all four TS/VS compositions using  
296 only the two statistical methods (GBLUP and RKHS) that showed optimal predictive  
297 performance in the previous analyses, and the TS/VS size ratio of 4:1 (892/224) were used on  
298 the PA evaluations.

## 299 **Results**

### 300 **Phenotypic trait correlations**

301 Growth (height, volume, and CBH) and wood properties (basic density and pulp yield)  
302 were measured for all 168 G0 and 949 G1 individuals. The raw phenotypic data were  
303 adjusted using a mixed linear model to minimize the impacts of environment and age  
304 differences. The pairwise correlations between the adjusted traits were described by  
305 calculating Pearson correlation coefficients (Figure 1). Growth traits were correlated with  
306 each other. Interestingly, however, while CBH and volume at age three and six years were  
307 highly correlated ( $r = 0.92$  and  $0.95$  respectively), height at age three was only weakly  
308 correlated with height at age 6 ( $r = 0.36$ ). For wood properties traits, basic density was  
309 negatively correlated with pulp yield, although weakly ( $r = -0.28$ ). Growth traits showed no  
310 correlations with wood traits ( $r = -0.1$  to  $0.1$ ).

### 311 **Breeding population structure and relatedness**

312 Population structure across G0 and G1 individuals was assessed by PCA based on 10,213  
313 LD-pruned, independent SNPs ( $r^2 < 0.2$ ). The first two PCs explained 6.07% and 3.8% of the  
314 total genetic variance (Figure 2a) and clearly separated the G0 individuals of the two species,

315 *E.grandis* and *E.urophylla*, with the *E.grandis* individuals further subdivided into two  
 316 subgroups likely representing the two main provenances used in breeding programs in Brazil.  
 317 The G1 individuals were generally projected into the space defined by their parents, but with  
 318 a few outliers. The expected pedigree-based and realized genomic-based genomic  
 319 relationships among G0 and G1 individuals were visualized in heatmaps (blue and red in  
 320 Figure 2b, respectively). The result of the genomic relationship analysis corroborated the  
 321 PCA result, in which *E. urophylla* was clustered into a single group, whereas *E. grandis*  
 322 formed two subgroups. The average values of the realized genomic relationships among what  
 323 were considered to be full-sibs, half-sibs and unrelated individuals from the pedigree data  
 324 were generally lower than the expected relationships values (0.309 vs. 0.5, 0.131 vs. 0.25  
 325 and .0056 vs. 0, respectively) (Table 1). This result suggests that pedigree errors were likely  
 326 present in this population. These putative pedigree errors in turn negatively affected the  
 327 pedigree-based trait heritability, which were considerably lower than those estimated using  
 328 genomic-based realized genomic relationships (Table 2).

329 **Table 1.** Pairwise expected pedigree-based and realized genomic-based relationships in the  
 330 different family types.

	Full-sib families (961) <sup>a</sup>			Half-sib families (12718)			Unrelated individuals (434252)		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Pedigree-expected relationship	0.5	0.5	0.5	0.25	0.25	0.25	0	0	0
Genomic-realized relationship	-0.274	0.309	0.933	-0.464	0.131	0.908	-0.467	-0.056	0.891

331 <sup>a</sup>Number in parentheses indicate the number of pairwise estimates

332 **Table 2.** Pedigree-based and genomic heritabilities for each trait

	CBH (3) <sup>a</sup>	Height (3)	Volume (3)	CBH (6)	Height (6)	Volume (6)	Basic density	Pulp yield
$h_a^2$ <sup>b</sup>	0.051(0.03)	0.074(0.04)	0.057(0.03)	0.085(0.04)	0.097(0.05)	0.068(0.04)	0.23(0.04)	0.27(0.05)
$h_g^2$	0.113(0.04)	0.171(0.05)	0.162(0.04)	0.184(0.04)	0.193(0.05)	0.196(0.04)	0.35(0.05)	0.46(0.05)

333 <sup>a</sup> Number in parentheses correspond the age at measurement;

334 <sup>b</sup>  $h_a^2$  and  $h_g^2$  correspond to the pedigree and genomic narrow-sense heritability, respectively,

335 with their standard deviation in parenthesis.

336

### 337 **Predictive abilities with different statistical methods**

338 Estimates of PAs were obtained using different statistical methods, compositions and sizes  
339 of TS/VS for each trait (Additional file 2). An ANOVA showed that all these factors had a  
340 significant effect on the PA (P-value < 0.005) (Additional file 3). Across the four genomic  
341 prediction methods used (GBLUP, rrBLUP, BL, and RKHS) the average PA varied from  
342 0.27 to 0.274 (Additional file 4). All the four methods outperformed the pedigree-based  
343 ABLUP prediction (mean PA = 0.121) by an average of 80%-200% across the eight traits  
344 (Figure 3). RKHS yielded a slightly better PAs for six out of eight traits and this method was  
345 particularly suitable for predicting traits that displayed a lower heritability such as CBH and  
346 height. The other three methods generally gave similar results across all traits, although with  
347 a slightly better performance than RKHS for pulp yield (Figure 3).

### 348 **Impact of TS/VS compositions and relative sizes on predictive ability**

349 The average PAs differed significantly for the different TS/VS composition tested varying  
350 from 0.253 to 0.286 (Additional file 5). The genomic prediction model built with CV<sub>2</sub> (all G0  
351 parents in the TS) showed the highest PAs for all traits except pulp yield, whereas models  
352 based on CV<sub>3</sub> (minimum relatedness between TS and VS) gave the worst predictions. The  
353 models based on CV<sub>1</sub> (random assignment) and CV<sub>4</sub> (maximum relatedness between TS and  
354 VS) showed no significant differences in PA (Figure 4, Additional file 5). The average PA  
355 was significantly improved from 0.251 to 0.285, as the TS/VS ratio increased from 1:1  
356 (558/559) to 9:1 (1003/113) (Additional file 6), irrespective of the prediction method (Figure  
357 3) or the genetic composition of TS/VS used (Figure 4), clearly showing the importance of an  
358 adequate size of the training set used to build prediction models. Furthermore, there was a  
359 steeper increase in PA when TS/VS ratio increased from 1:1 (558/559) to 2:1 (743/374) than  
360 from 2:1 (743/374) to 9:1 (1003/114) for all traits (Figure 3 and 4).

361 **Impact of the number of SNPs and their genomic location on predictive ability**

362 Estimates of PA using different numbers of SNPs (Additional file 7) and sets of SNPs in  
363 different genomic locations (Additional file 8) were obtained with two prediction methods for  
364 all the different TS/VS compositions. An ANOVA showed that both the number of  
365 genotyped SNPs and their genomic location significantly affect the PA for both prediction  
366 methods (GBLUP and RKHS) (P-value < 0.005), and that the number of SNPs has a larger  
367 impact than their genomic location (Additional file 9). The average PAs across all traits  
368 decreased from 0.278 to 0.113 when the number of SNPs used in the prediction models  
369 dropped from 41,304 to only 10, and the reduction was especially strong when the number of  
370 SNPs went below 5,000 (Additional file 10). On the other hand, no significant improvement  
371 was generally seen in the average of PA when more than 5,000 SNPs were used (Additional  
372 file 10, Figure 5). The results obtained for the different traits suggest that traits with lower  
373 heritability are more sensitive to the reduction in the number of SNPs (Figure 5). For instance,  
374 PA for basic density ( $h^2 = 0.35$ ) went from 0.47 to 0.24 (a 50% decrease) when the number of  
375 SNPs dropped from 40,000 to 10, whereas CBH of age three ( $h^2 = 0.113$ ) decreased from  
376 0.128 to 0.03 (a 77% decrease). Overall, few and only slight significant differences were seen  
377 in PAs by using SNP sets located in different genomic regions (Figure 6), the average PAs  
378 range from 0.270 to 0.284 (Additional file 11). Predictions using SNPs located in intergenic  
379 regions were marginally better than using SNPs in genic regions or all SNPs, except for pulp  
380 yield that could be better predicted based on models using SNPs from coding and gene  
381 regions (Figure 6). When comparing the PA of models using SNPs in coding versus entire  
382 gene regions, the latter had a slightly better performance, most likely due to the larger  
383 number of SNPs used (30,504 vs. 11,786) and not to any specific effect of genomic location.  
384 When we assessed the pairwise LD ( $r^2$ ) amongst the SNPs in the four regions tested, the

385 extent of LD differed among them, with LD showing the most rapid decay in coding regions  
386 and the slowest one in intergenic regions (Additional file 12).

## 387 **Discussion**

388 This study presents the results of an empirical evaluation of the accuracy of genomic  
389 prediction of growth and wood quality traits in *Eucalyptus* using data from a high-density  
390 SNP array. Our results are based on data from a two generations breeding population and  
391 provide additional encouraging results on the prospects of using genomic prediction to  
392 accelerate breeding. We have assessed a range of factors, including the statistical methods  
393 used to estimate predictive ability, the size and composition of the training and validation sets  
394 as well as the number and genomic locations of SNPs used in the prediction model.  
395 Hereafter we will discuss how these factors influenced the prediction accuracy.

### 396 **Genomic data corrected pedigree inconsistencies**

397 All four genomic prediction methods performed significantly better than the pedigree-  
398 based evaluations for all complex traits assessed (Figure 3). While similar results have been  
399 reported for animals [16, 42] and crop species [9, 35] across a number of traits, in forest trees  
400 prediction accuracies using genomic data have generally been similar or up to 10-30% lower  
401 than accuracies obtained using pedigree-estimated breeding values, including *Eucalyptus* [4],  
402 loblolly pine (*Pinus taeda*) [43], white spruce (*Picea glauca*) [44, 45], interior spruce (*Picea*  
403 *engelmannii* × *glauca*) [46, 47] and maritime pine (*Pinus pinaster*) [48]. Genomic predictions  
404 with lower accuracies than pedigree-based predictions could arise from insufficient marker  
405 density, such that not all casual variants are captured in the genomic estimate [40], or an  
406 overestimate of the pedigree-based prediction due to its inability of ascertaining the true  
407 genetic relationships in half-sib families [46]. Our result however differ from previous studies  
408 in forest trees due to the fact that the average pairwise estimates of genetic relationship  
409 among individuals were substantially lower using SNP data than expectations based on

410 pedigree information (Table 1), clearly suggesting that the expected pedigrees, and  
411 consequently the pairwise relationships, had considerable inconsistencies that were corrected  
412 by the SNP data. We speculate that these inconsistencies likely derived from pollen  
413 contamination and mislabelling in the process of generating the full and half-sib families.  
414 Besides correcting potential pedigree errors, the relatively dense SNP data used in our study  
415 also was able to accurately capture the Mendelian sampling variation within families so that  
416 genetic variances estimates were based on the actual proportion of the genome that is identity  
417 by descent (IBD) or state (IBS) among half- or full-sib individuals, resulting in improved  
418 estimates of trait heritability (Table 2).

#### 419 **Genomic predictions show that traits adequately fit the infinitesimal model**

420 Overall, the different genomic prediction methods provided similar results for the traits  
421 evaluated with only a slight advantage for RKHS showing better PAs for growth traits that  
422 had lower heritability (Figure 3) although for pulp yield, RKHS instead was the worst  
423 performing method. It is possible that the definition of a kernel simply was not suitable for  
424 this particular trait [15]. Our results corroborate previous reports both in crops and animals  
425 [16, 49, 50], as well as in forest tree studies. In loblolly pine, for example, the performance of  
426 rrBLUP and three Bayesian methods was only marginally different when compared across 17  
427 traits with distinct heritabilities, with a small improvement using BayesA only for fusiform  
428 rust resistance where loci of relatively large effect have been described [43]. Similar results  
429 were obtained for growth and wood traits in other forest trees studies showing no  
430 performance difference between rrBLUP and Bayesian methods [45, 47, 48]. This occurs  
431 despite simulation studies suggesting that Bayesian methods, like BL, should outperform  
432 univariate methods such as rrBLUP and GBLUP [6, 51, 52]. One possible reason for the  
433 apparent disagreement between simulations and empirical data sets is that the true QTL  
434 effects for most of traits are relatively small and the distribution is less extreme than

435 simulated data [53]. Our results therefore support the proposal that either rrBLUP or GBLUP  
436 are effective methods in providing the best compromise between computation time and  
437 prediction efficiency [54] and that the quantitative traits assessed in our study adequately fit  
438 the assumption of the infinitesimal model.

#### 439 **Training set size, composition and relatedness strongly affect predictive ability**

440 Our results show that the size and the variable TS/VS compositions in terms of relatedness  
441 between training and validation sets had the largest impact on the PA irrespective of the  
442 analytical method used (Figure 4). The average PA rapidly increased with increasing sizes of  
443 the TS and did not show any sign of plateauing. Earlier simulations of *Eucalyptus* breeding  
444 scenarios had in fact shown that with up to N= 1,000 individuals in the TS, the accuracy  
445 would rapidly increase, and additional gains would be seen up to N= 2,000 individuals for  
446 lower heritability traits, larger numbers of QTLs involved and larger effective population size  
447 ( $N_e$ ). After N= 2,000 the predictive accuracy would tend to plateau irrespective of the  $N_e$  and  
448 genotyping density [20]. Later simulations mirroring a eucalypt breeding scheme also  
449 showed a considerable improvement of genomic predictions with increasing training  
450 population sizes by consolidating phenotypic and genotypic data of individuals from previous  
451 breeding cycles [55]. Simulations [19, 56] and proof-of-concept studies [57] in crop species  
452 also show improved PA with larger TS sizes. Larger training populations alleviate the  
453 probability of losing rare favourable alleles from the breeding population as generations of  
454 selection advance. Additionally by sampling more individuals for training, a larger diversity  
455 is captured and better estimates of the marker effects are obtained which in turn positively  
456 impact predictions in cross-validations and future genomic selection candidates.

457 As expected, relatedness between TS and VS had a large impact on PAs for all traits.  
458 Prediction models built under scenario CV<sub>3</sub> (minimized relatedness between TS and VS)  
459 resulted in significantly worse predictions than in scenario CV<sub>4</sub> when relatedness was

460 maximized. Increasing the genetic relationships between training and selection candidates  
461 effectively has the same consequence as reducing the  $N_e$  such that the stronger the  
462 relationship, the higher in the predictive accuracy. Our results are in line with previous  
463 reports in forest trees showing that models developed for one population had limited or no  
464 ability of predicting phenotypes in an unrelated one in white spruce [44, 45] and *Eucalyptus*  
465 [4], indicating that prediction models will be population specific. With lower relationship  
466 between TS and VS, the extent of LD is shorter and not stable across distantly related  
467 populations and the predictive ability of genomic prediction model is reduced. Recent  
468 simulations show that the accuracy of genomic prediction models decline approximately  
469 linearly with increasing genetic distance between training and prediction populations [58].  
470 Increased relatedness reduce the number of independently segregating chromosome segments  
471 and therefore increase the probability that chromosome segments IBD sampled in the training  
472 population are also found in the selection candidates. Our results provide additional  
473 experimental evidence that for successful implementation of GS the selection candidates have  
474 to show a close genetic relationship to the training population.

475 PAs were considerably higher when all the G0 parents were kept in the TS (scenario CV<sub>2</sub>).  
476 This result could be due to two reasons. On one hand, by keeping all G0 parents in TS, we  
477 had a large diversity available for training, which could explain the positive impact of G0  
478 inclusion on predictions. On the other hand, it is possible that by allocating all G0 individuals  
479 to the TS the positive effect we observe could strictly not be due to increased predictive  
480 power of including G0 individuals but rather a way to avoid the potentially negative impact  
481 of having pure species parents in the validation set in combination with G1 progeny that were  
482 largely F<sub>1</sub> hybrids. In order to evaluate this, we estimated PA of genomic prediction models  
483 by using GBLUP and RKHS, having only the 168 G0 parents for TS and randomly selected  
484 168 G1 individuals in VS. To control for the effect of the strongly reduced TS size, we

485 compared this setup with random assignment of individuals to TS or VS but keeping the size  
486 of each at N=168. The results showed considerably lower PAs (even zero or negative) when  
487 using only pure species parents to predict G1 hybrid progeny phenotypes (Additional file 13).  
488 This observation, together with the fact that PAs with scenario CV<sub>4</sub> (maximum relatedness  
489 between TS and VS) were also generally lower than CV<sub>2</sub>, suggesting that the higher PAs we  
490 observe for scenario CV<sub>2</sub> is mostly due to avoiding the negative effect of having pure species  
491 parents in the VS.

492 The issue of genomic prediction in hybrid breeding has been investigated so far only within  
493 species and only for domestic animals, more specifically for bovine and pig breeding in  
494 which selection is carried out in pure breeds but the aim is to improve crossbred performance  
495 [42, 59]. Results from simulations show that training on crossbred data provides good PAs by  
496 selecting purebred individuals for crossbred performance, although PAs drop with increasing  
497 distances between breeds [60]. When crossbred data is not available, separate purebred  
498 training populations can be used either separately or combined depending on the correlation  
499 of LD phase between the pure lines [61], which in turn is in part determined by the time of  
500 divergence between the populations. Compared to bovine breeds that belong to the same  
501 species and have diverged relatively recently (<300KYA) [62], the estimated divergence time  
502 between the two *Eucalyptus* species used in our study is much older, estimated at 2-5 MYA  
503 [63]. Therefore, no correlation of LD phase between these two species is expected and it is  
504 not surprising that training on the combined pure species sets to validate on the F<sub>1</sub> hybrids  
505 resulted in poor PA. To the best of our knowledge, our results are the first ones to provide an  
506 initial look at the issue of genomic prediction from pure species to interspecific hybrids  
507 indicating that, consistent with expectations, models have to be trained in hybrids if one is to  
508 predict phenotypes in hybrid selection candidates.

509 **Number of SNPs is more important than SNP genomic location**

510 Across all traits, no major improvement was detected in PA when more than 5,000 SNPs  
511 were used (Additional file 10, Figure 5), although a slight increase were observed for height  
512 of age three, basic density and pulp yield when using GBLUP based on 20,000 SNPs. Several  
513 studies have also shown that considerably lower numbers of SNPs provided PAs equivalent  
514 to those observed using all SNPs available [22, 64]. The necessary number of SNPs needed  
515 for genomic prediction model depends on the extent of LD, which strictly related to  $N_e$ . Our  
516 results, where we achieve equivalent PAs using either all of 12-20% of the genotyped  
517 markers suggests that it represents a closed breeding population with a relatively limited  $N_e$ .  
518 This has been a common approach in domestic animals with the intent of developing low-  
519 density genotyping chips to reduce genotyping costs [8]. The main advantage of using  
520 reduced SNP panels is cost-effectiveness, although it is expected that using a higher density  
521 of markers will be necessary to mitigate the decay of PAs over generations due to the  
522 combined effect of recombination and selection on the patterns of LD [65]. It is also  
523 questionable whether it will be more cost effective to have targeted low-density SNP chips  
524 for specific populations or a full SNP chip that can be used across breeding populations of  
525 several organizations. By having a SNP chip that will accommodate several populations the  
526 cost-effectiveness and economy of scale of amassing many more samples to be genotyped  
527 with the same chip will likely be much larger than the cost reduction observed by using a  
528 smaller number of SNPs on each specific population.

529 SNP location also contributed to the predictive ability of genomic prediction model  
530 although the effects were rather modest. PAs using SNPs in intergenic regions were slightly  
531 better than using SNPs in genic regions or using all SNPs, except for pulp yield that could be  
532 somewhat better predicted with SNPs in coding and gene regions (Figure 6). This likely  
533 represents a random sampling effect and not any specific enrichment for functional variants  
534 for this trait. However, the decline of LD was slower for SNPs in intergenic regions when

535 compared to SNPs in gene and coding regions (Additional file 12) and the slightly longer  
536 range of LD might help explain why using SNPs in intergenic regions provided better PAs.  
537 With slower LD decay, SNPs in intergenic regions might better capture QTLs across longer  
538 genomic segments than SNPs in coding regions where LD decays more rapidly.

## 539 **Conclusions**

540 Our experimental results provide further promising perspectives for the implementation of  
541 genomic prediction in *Eucalyptus* breeding programs. Genomic predictions largely  
542 outperformed the pedigree-based ones in our experiment, mainly due to the fact that our  
543 expected pedigree had major inconsistencies, such that all pedigree-based estimates were  
544 grossly underestimated. This unexpected result illustrated an additional advantage of using  
545 SNP data and genomic prediction in breeding programs. While the main advantage of  
546 genomic prediction in eucalypt breeding will likely be the reduction of the breeding cycle  
547 length [4], the use of a genomic relationship matrix allowed us to obtain precise estimates of  
548 genetic relationship and heritability that we would otherwise not have had access to.  
549 Furthermore our results corroborated the key role of relatedness as a driver of PA, the  
550 potential of using lower density SNP panels, and the fact that growth and wood traits  
551 adequately fit the infinitesimal model such that GBLUP or rrBLUP represent a good  
552 compromise between computation time and prediction efficiency. In contrast to previous  
553 studies in *Eucalyptus*, we had access to both the pure species parents (*E. grandis* and *E.*  
554 *urophylla*) and their F<sub>1</sub> progeny. We show that models trained on pure species parents do not  
555 allow for accurate prediction in F<sub>1</sub> hybrids, likely due to the strong genetic divergence  
556 between the two species and lack of consistent patterns of LD between the two species and  
557 their hybrids.

558 Several issues remain to be investigated for the operational adoption of genomic prediction  
559 in eucalypt breeding. First, how does the accuracy of genomic prediction decline over

560 successive generations of selection due to subsequent recombination? Second, how stable are  
561 genomic prediction models across multiple environments and how important is it to consider  
562 genotype by environment interactions in the models? Finally, we have only considered  
563 additive genetic variance for building genomic prediction models in our population, but it is  
564 possible and perhaps even likely that non-additive genetic effects will play an important role  
565 in many breeding populations and specifically in populations consisting of early generation  
566 hybrids.

567

568

## 569 **List of abbreviations**

570 BL: Bayesian LASSO; CBH: circumference at breast height; CDS: coding sequences;  
571 GBLUP: genomic best linear unbiased predictor; GEBV: genomic estimated breeding values;  
572 GRM: genomic relationship matrix; GS: genomic selection; IBD: identity by descent; IBS:  
573 identity by state; LD: linkage disequilibrium; MAS: marker-assisted selection;  $N_e$ : effective  
574 population size; PA: predictive ability; PCA: principal components analysis; QTLs:  
575 quantitative trait loci; RKHS: reproducing kernel Hilbert space; rrBLUP: ridge-regression  
576 best linear unbiased prediction; SNP: single-nucleotide polymorphism; TS: training set; VS:  
577 validation set.

## 578 **Declarations**

### 579 **Ethics approval and consent to participate**

580 Not applicable

### 581 **Consent for publication**

582 Not applicable

### 583 **Data availability**

584 The data that support the findings of this study are available from Veracel but restrictions  
585 apply to the availability of these data, which were used under license for the current study,  
586 and so are not publicly available. Data are available from the authors upon reasonable request  
587 and with permission of Veracel.

#### 588 **Competing interests**

589 The authors declare that they have no competing interests.

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#### 594 **Authors' contributions**

595 BT, BS and PKI conceived and designed the experiment; GSM phenotyped data; GSM and  
596 KZF collected samples for genotyping; DG prepared the DNA for genotyping; BT analysed  
597 the data and drafted the first version of the manuscript; DG and PKI provided guidance  
598 during data analyses; BT, DG, BS and PKI critically contributed to the final version of the  
599 manuscript. All authors read and approved the final manuscript.

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781

## 782 **Figures**

783 **Figure 1 Correlation and distribution of phenotypes.** Scatter plots (lower off-diagonal)  
784 and correlations with probability values (upper off-diagonal;  $H_0: r=0$ ) for adjusted phenotypes  
785 between pairs of traits. Color key on the right indicates the strength of the correlations.  
786 Diagonal: histograms of the distribution of adjusted phenotypes values.

787 **Figure 2 Genetic structure and relatedness in the breeding population.** (a) First two  
788 principal components of a PCA revealing population structure. Dots represent *E.grandis*  
789 (blue), *E.urophylla* (red) and their  $F_1$  (green) individuals. (b) Heatmaps of the pairwise  
790 pedigree-expected relationships (blue, upper off-diagonal) and genomic-realized relationship  
791 (red, lower off-diagonal) of the 1117 individuals assigned to *E.grandis* (G), *E.urophylla* (U)  
792 and their hybrid progenies (H).

793 **Figure 3 Predictive abilities with different methods and increasing sizes of training sets.**

794 Predictive ability (y axis) estimated using five methods across five training set/validation set  
795 sizes in numbers of individuals (x axis) 558/559, 743/374, 836/281, 892/225 and 1003/114.  
796 Red and blue dashed lines indicate the pedigree-based ( $h_a^2$ ) and genomic-realized ( $h_g^2$ )  
797 narrow-sense heritability respectively.

798 **Figure 4 Predictive abilities with variable levels of relatedness between training and**  
799 **validation sets.** CV<sub>1</sub>: random assignment of individuals to either training set (TS) or  
800 validation set (VS); CV<sub>2</sub>: all the G0 pure species parents assigned to the TS; CV<sub>3</sub>: minimum  
801 relatedness between TS and VS individuals; CV<sub>4</sub>: maximum relatedness between TS and VS  
802 individuals. Estimates were obtained using GBLUP and RKHS across five TS/VS sizes in  
803 numbers of individuals (x axis): 558/559, 743/374, 836/281, 892/225 and 1003/114.

804 **Figure 5 Predictive abilities with increasing numbers of SNPs.** Predictive ability  
805 estimated with GBLUP and RKHS with increasingly larger sets of SNP sampled at random  
806 from the total of 41,304 SNPs. Outliers are indicated by black dots. Letters indicate  
807 significant difference between the different models after Bonferroni adjustment ( $P < 0.05$ ).

808 **Figure 6 Predictive abilities using SNPs located in different genomic regions.** Predictive  
809 ability estimated with GBLUP and RKHS using 11,786 SNPs in coding DNA, 30,405 SNPs  
810 in genic regions (CDS, UTR, intron, and within 2kb upstream and downstream of genes),  
811 10,899 SNPs in intergenic regions and all 41,304 SNPs. Letters indicate significant difference  
812 between the different models after Bonferroni adjustment ( $P < 0.05$ ).

813

#### 814 **Additional files**

815 **Additional file 1:** Average accuracy of SNP imputation methods with increasing proportions  
816 of missing data. SNPs on chromosomes 6 and 8 were randomly removed from the dataset to

817 generate specific missing data proportions. Accuracy between imputed and true SNP  
818 genotypes were subsequently calculated with the different methods. (DOCX 1.8Mb)

819 **Additional file 2:** Predictive abilities on genomic selection model that comprises of  
820 statistical methods, genetic compositions and relative sizes of Training Set/Validation Set for  
821 each trait. (XLSX 17 kb)

822 **Additional file 3:** ANOVA analysis of sources of variation affecting the predictive ability.  
823 (DOCX 50 kb)

824 **Additional file 4:** Mean and standard deviation of predictive ability with the five prediction  
825 methods for the eight traits. (DOCX 99kb)

826 **Additional file 5:** Mean and standard deviation of predictive ability estimated with the four  
827 Training Set/Validation Set compositions. (DOCX 87kb)

828 **Additional file 6:** Mean and standard deviation of predictive ability estimated with the five  
829 relative sizes of Training Set/Validation Set expressed in proportions and numbers of  
830 individuals. (DOCX 91kb)

831 **Additional file 7:** Mean and standard deviation of predictive ability across increasing  
832 numbers of SNPs, statistical methods (RKHS and GBLUP), four Training Set/Validation Set  
833 compositions for each of eight traits (XLSX 62kb)

834 **Additional file 8:** Mean and standard deviation of predictive ability estimated with SNPs in  
835 four genomic locations, with two statistical methods (RKHS and GBLUP), four Training  
836 Set/Validation Set compositions for each of eight traits (XLSX 59kb)

837 **Additional file 9:** ANOVA of predictive ability with SNP genomic location and SNP number  
838 as sources of variation. (DOCX 63kb)

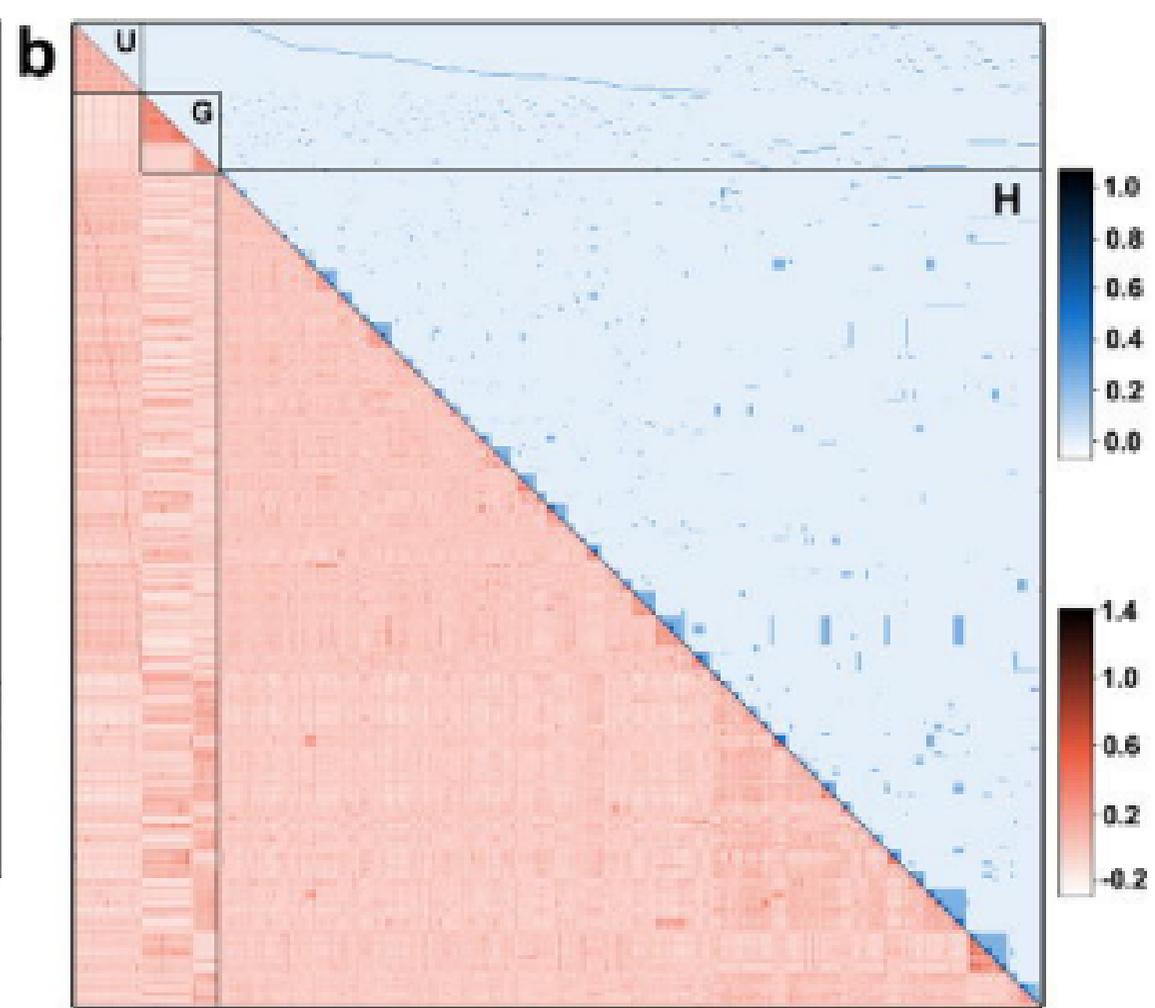
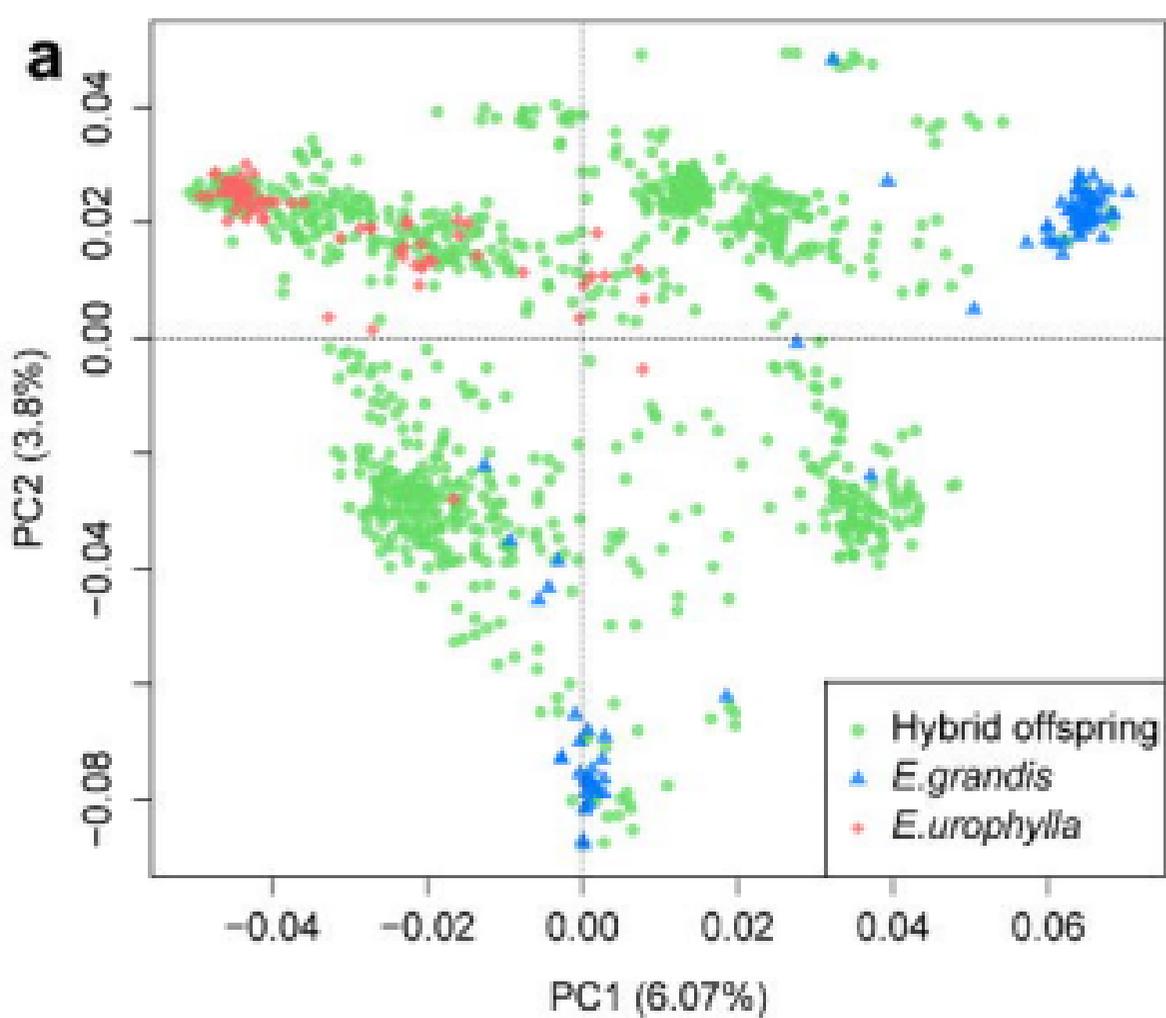
839 **Additional file 10:** Average predictive ability estimated with different numbers of SNPs  
840 fitted into the model. (DOCX 138kb)

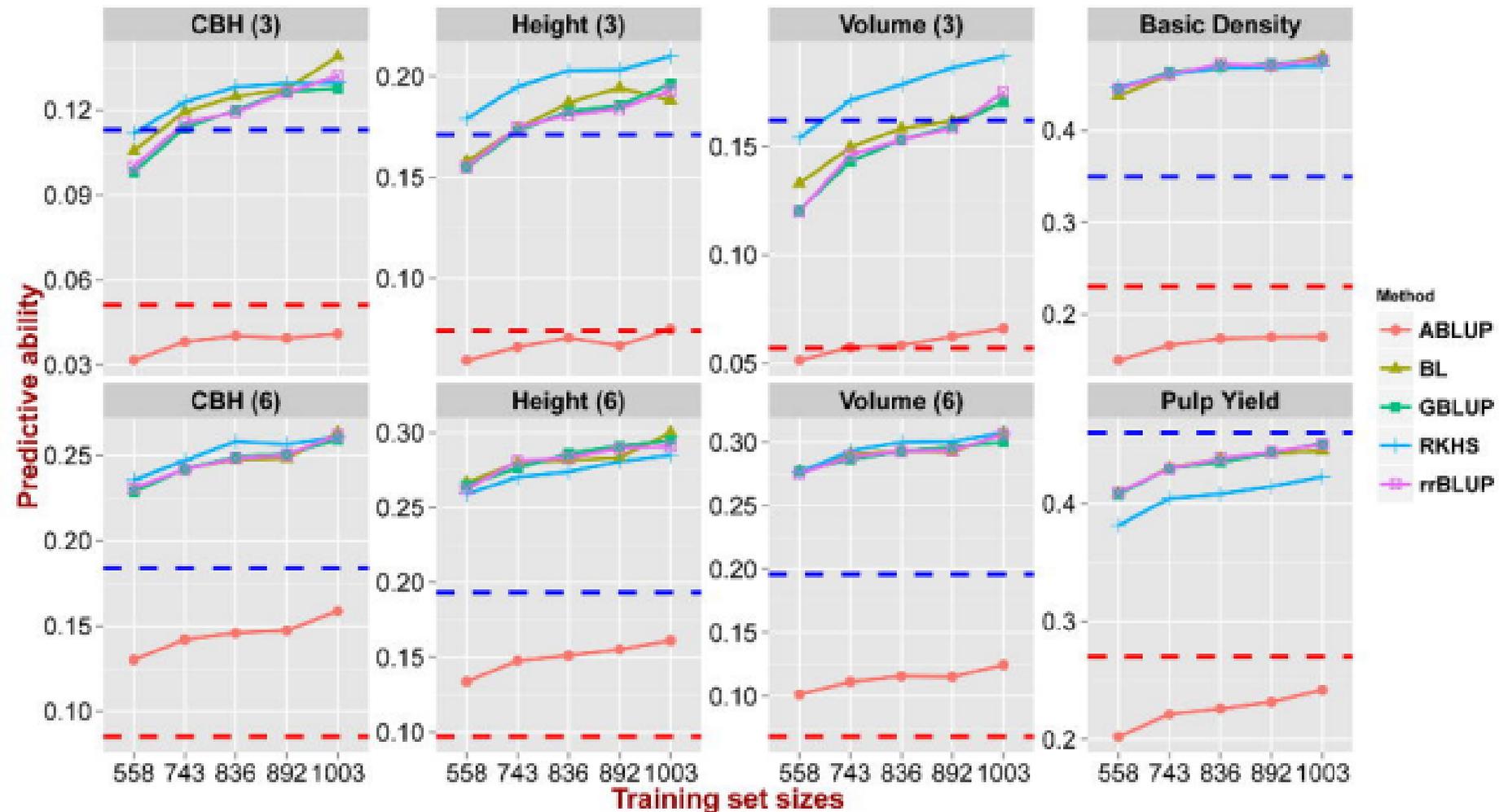
841 **Additional file 11:** Average predictive abilities estimated using SNP sets located in different  
842 genomic regions. (DOCX 83kb)

843 **Additional file 12:** Decay of linkage disequilibrium (LD) with physical distance estimated  
844 with SNPs in different genomic locations. (a) A comparison of the decay of LD with physical  
845 distance in four classes of SNPs located with coding, genic, intergenic and all regions,  
846 respectively. Dots of pairwise LD versus physical distance and the LD decay for SNPs  
847 located in all regions (b), coding region (c), genic region (d) and intergenic region (e),  
848 respectively. (DOCX 1.4Mb)

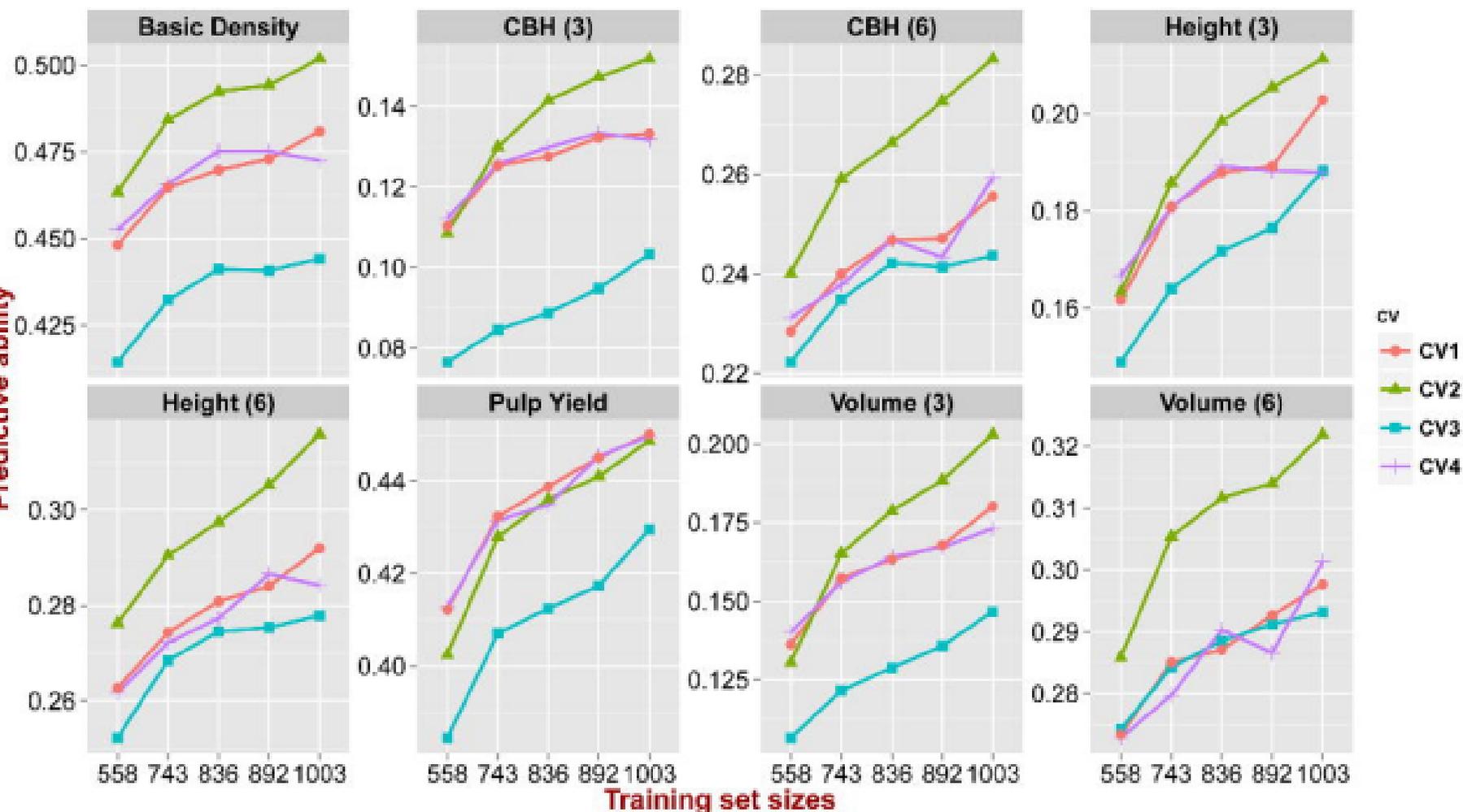
849 **Additional file 13:** Predictive abilities by training in pure species eucalypt parents and  
850 predicting in their F<sub>1</sub> hybrids. Predictive ability estimated under three training/validation sets  
851 (TS/VS) scenarios with two methods (GBLUP and RKHS) for each trait. PO168 (red boxes):  
852 all 168 *E. grandis* and *E. urophylla* pure species G<sub>0</sub> parents used for training and 168 G<sub>1</sub>  
853 random selected hybrid progeny for validation; random168 (green): randomly selected 168  
854 individuals from all 1,117 for TS and 168 randomly also for VS; random558 (blue):  
855 randomly divided all 1,117 individuals into TS and VS of same size (558/558). Outlier  
856 estimates are indicated by black dots. (DOCX 179kb)



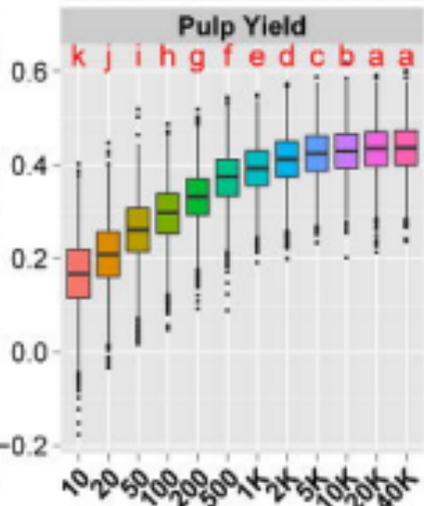
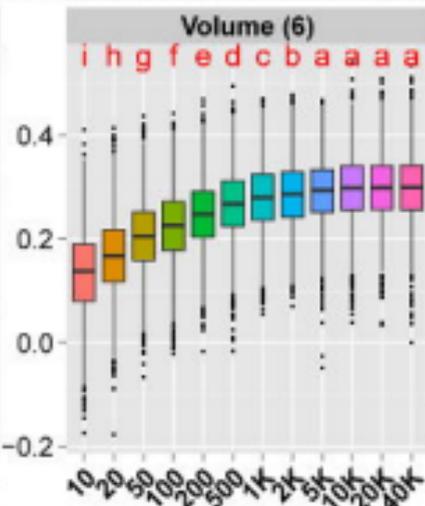
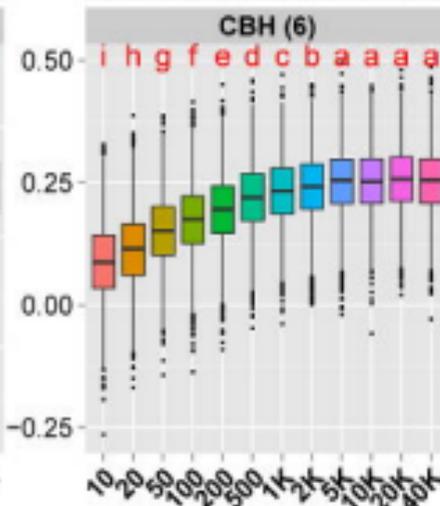
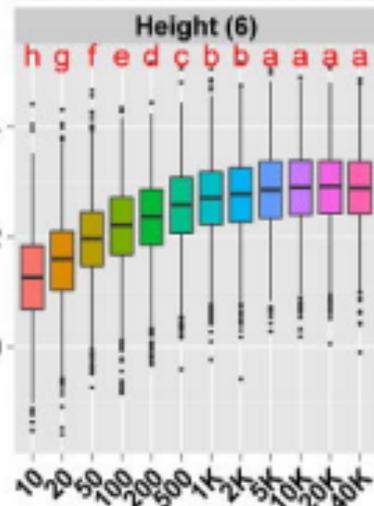
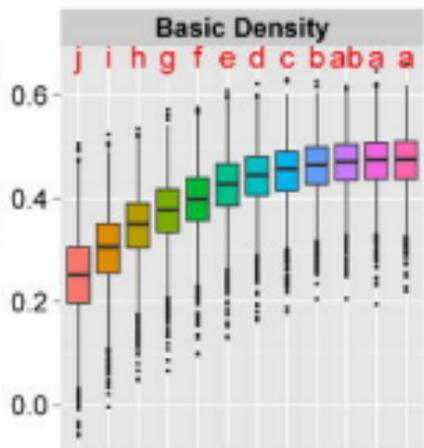
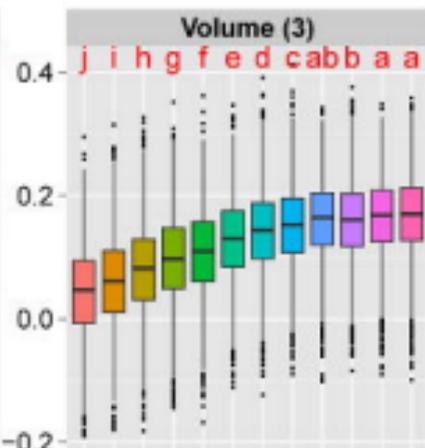
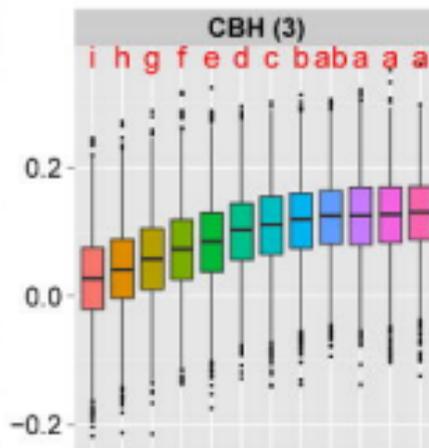
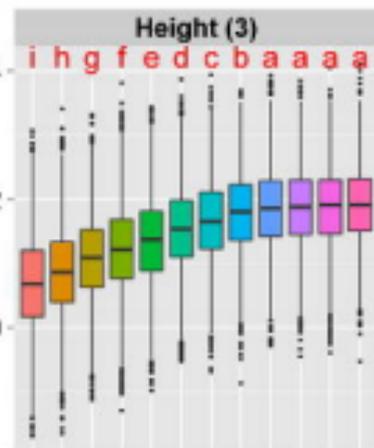




Predictive ability



Predictive ability



Sizes

Predictive ability

