

Genetic Diversity and Genomic Selection in *Eucalyptus Benthamii*

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1 **Genetic diversity and genomic selection in *Eucalyptus benthamii***

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ABSTRACT

32 The study investigates the genetic diversity and the ability of genomic-wide selection to predict breeding
33 genomic values of an *E. benthamii* trial. All individuals (115) of the breeding population were genotyped with
34 13 microsatellites loci. The diameter at breast height and total height were measured. The data analysis was
35 carried using the softwares: Structure, Popgene, GDA, SPAGeDi1.5 and R. Predictive ability, heritability and
36 standard errors markers were estimated using the RRblup method. The average number of alleles per locus
37 was nine, and the polymorphism level for each locus varied from 3 to 17. The average expected
38 heterozygosity ($H_e=0.655$) was very similar to observed heterozygosity and the estimated inbreeding ($F =$
39 0.02) was very low. These results corroborate that this population is in Hardy-Weinberg equilibrium for the
40 most loci. The trial genetic diversity is considered high, once the trial sampling demonstrated similar values to
41 the natural populations. The group coancestry (0.085) demonstrate that the trees, in general, related at the
42 half-sib level in this population. By using the Evanno's method it is inferred that the individuals came from
43 two original populations. The genetic distance calculated among the two groups was low ($D=0.21$). The
44 heritability estimated from genomic selection for phenotypic traits was very low; however, the heritability
45 estimated using the kinship coefficients was higher. The marker-based heritability using kinship coefficients
46 probably is the more accurate than the one estimated using genomic selection, showing that the population
47 samples can be used to establish breeding populations, hybrids and enriching the species germplasm bank.

48 Keywords: clustering method; coancestry; frost-tolerant; genome-wide selection; heritability

49 1. Introduction

50 Some *Eucalyptus* species are frost tolerant, showing good performance on subtropical areas with high
51 probability of frost. This aspect and their performance in wood productivity have aroused the interest of the
52 forestry sector in many countries. Currently, one of the major studied species which combines the frost
53 tolerance ability with high wood productivity is *Eucalyptus benthamii* (Bush et al. 2016). Some companies
54 have used this species to develop interspecific hybrids. International initiatives have verified a relation
55 between cold tolerance established from field trials plantations in southeast Texas with frost-prone regions of
56 southern Brazil (Hart et al. 2016). The species also has showed a good performance across different sites in
57 China (Xie et al. 2017). Kellyson et al. (2013) noticed that the species is appropriate to be managed by
58 breeding process, arguing that it shows quick progress through genetic selection. According to the authors

59 genetic gains can be obtained as much as in growth, as well in wood quality and in cold tolerance. In Brazil,
60 *E. benthamii* is the most adapted eucalypt species to south region, where intense frosts usually occur
61 (Santarosa et al. 2014). *E. benthamii* wood main use is in energetic products, such as charcoal and firewood.
62 One of the limiting factors to development of the species use is lack of information about the genetic material
63 introduced in the country hampering the progress of breeding programs.

64 The species natural occurrence is narrow being found only in a restricted area southwest of Sydney in
65 Australia. Therefore, it makes the species vulnerable to extinction (Butcher et al. 2005). Currently, the efforts
66 in the region have been to preserve and study the forest remnants (Baccarin et al. 2015). Small populations of
67 tree species can have their genetic variability reduced. The low number of individual can lead to a loss of
68 alleles, decreasing the adaptation capacity of the population. In the future, the high level of relatedness
69 between the breeding individuals can increase inbreeding (Maxted et al. 2013). This situation can be worsen
70 in advanced-generation of breeding. In this case it can hamper the genetic gains for the next generations. The
71 genetic variability of a population can be estimated by parameters such as the number of alleles per locus (A)
72 and genetic diversity estimated by expected heterozygosity (H_e) (Dungey et al 2018). Microsatellites markers
73 or Single Sequence Repeats (SSR) have shown an efficient capacity to capture this information. The use of
74 SSR markers has been frequent in tree population's studies (Mora et al. 2017; Kaur et al. 2018; Liu et al.
75 2018; Miranda et al. 2019; Chen et al. 2020; Lv et al. 2020)

76 The use of SSR allows the access of high-quality genetic information in a reduced period of time and
77 can also be applied to drive the selection of individuals. On this path, a lot of works have been successful
78 achieving this goal through genome-wide selection (SGW). The Genomic-wide Selection proposed by
79 Meuwissen et al. (2001) is based on the analysis of a large number of markers wide distributed in the whole
80 genome. The effects of the markers are estimated based on phenotypic data of an individual group called
81 training population. This strategy can reduce the time between the selection cycles (Crossa et al., 2011), and
82 makes it possible to select the best genotypes without the need of phenotyping at an early age. Several studies
83 infer that the use of GWS can increase the genetic gains in eucalyptus cultures and other crops (Hayes et al.
84 2013; Desta and Ortiz 2014; Isik et al 2014, Liu et al 2015; Gois et al. 2016; Torres-Dini et al. 2016; Müller
85 et al. 2017; Resende et al 2017; Laviola et al 2017)

86 Although, GSW it is still a relatively new technology, a deeper understanding of its mechanisms is required.
87 The major questionings about the factors that influence its predictive quality are: minimum number of
88 individuals in the training population, type and quantity of molecular markers that can be used and the
89 importance level of relatedness among the individuals (Desta and Ortiz 2014; Crossa et al. 2017; Azodi et al.
90 2019; Sawitri et al. 2020). The objective of this study is to investigate the genetic diversity of an *E. benthamii*
91 seed production area and to apply GWS to this population to estimate genomic breeding values and genetic
92 gains.

93 **2. Methods**

94 *2.1. Study population*

95 The studied stand was the first known germplasm bank of *E. benthamii* introduced in Brazil. It was
96 established by Embrapa Florestas in 1988 at the municipality of Colombo in state of Paraná, southern Brazil.
97 The area has been managed as a seed production area, and has provided cuttings and seeds to be used in
98 commercial planting. The seeds were provided by CSIRO¹(Australia), and were originated from a mix of 10
99 trees located at Wentworth Falls (NSW) (Graça et al. 1999). The initial number of individuals in the stand
100 was 443 trees and this number was reduced to 199 by a selective thinning in 1995. Currently there are 115
101 remaining individuals occupying a total area of 0.5 ha.

102 The stand is located at an elevation of 1,027 m above the sea level in a climate Cfb climate, fully humid
103 with warm temperature, according to Köppen. The average annual rainfall is 1,638 mm. The average
104 maximum temperatures on the warmer and colder months are 28° C and 19° C, respectively. There is natural
105 occurrence of frost in the colder days of the year. On the last 20 years temperatures around or below 0°C were
106 registered in more than 30 days. (INMET 2018).

107 *2.2. Plant material and genomic DNA extraction*

108 All individuals of the referred stand had their diameter at breast height (dbh) and height measured in
109 2017. For the genetic analyses, cambium samples were collected from all the individuals. Genomic DNA was
110 extracted using a CTAB-sorbitol based method (Inglis et al., 2018). Samples were quantified using Nanodrop
111 (Thermo Fisher Scientific) and diluted to a final concentration of 5.0 ng.µl⁻¹ to run PCR reactions for

¹ The Commonwealth Scientific and Industrial Research Organization (CSIRO) is an independent agency of the Australian Federal Government responsible for scientific research in Australia

112 genotyping 13 microsatellite loci using primers previously reported (Butcher et al. 2005). PCR were carried
113 out using 5.0 ng of DNA, 1 unit of Taq polymerase, buffer 1x, 0.25 mg bovine serum albumin, 0.28 μ M of
114 each primer in 8.0 μ l reaction. PCR products were multiplexed in duplexes and triplexes according to
115 fluorochrome labelling and size range for injection in 3730 DNA Analyzer (Thermo Fisher Scientific). Allele
116 call was conducted using Gene Mapper software (Thermo Fisher Scientific).

117 2.3. Genetic analysis

118 Analyses of obtained data were performed using the GDA software (Lewis and Zaykin 2001). The
119 following parameters were estimated: allele frequency (A), expected (H_e) and observed heterozygosity (H_o)
120 and fixation index (H_f). The expected allele frequencies were calculated based on the observed frequencies,
121 considering the Hardy-Weinberg equilibrium model. The observed heterozygosity was calculated based on
122 Brown and Weir (1983): $H_o = 1 - \sum p_{ii}$, where p_{ii} is the observed frequency of homozygotes on the i allele.
123 The expected heterozygosity considering the Hardy-Weinberg equilibrium was estimated according to Nei
124 (1978). The fixation index was estimated using the expected and the observed heterozygosity (Wright 1965).

125 The population structure was inferred by using a Bayesian model-based clustering method with
126 software STRUCTURE version 2.3 (Pritchard et al. 2000). STRUCTURE uses genotypic data to determine
127 the number of distinct genetic clusters (K) among the sample locations, and estimates individual assignment
128 probability to each cluster. Twenty replicate runs (100,000 Markov Chain Monte Carlo step burn-in plus an
129 additional 100,000 runs) were performed for each value of K . Results were summarized using STRUCTURE
130 Harvester version 0.6.6 (Earl and vonHoldt 2012), which generated a plot of the mean value of $L(K)$ (ln
131 likelihood of data) at each K . With STRUCTURE Harvester analysis it was possible to infer the most likely
132 number of clusters by identifying the highest $L(K)$ value with relatively small variance

133 Later, further genetic analyses were performed using the software POPGENE 1.32 (Young et al. 2000),
134 considering separately each group within the population. Allele frequency, expected and observed
135 heterozygosity and fixation index were estimated for each group. The genetic distance between the groups
136 was also calculated through the Nei's standard genetic Distance (D) (Nei, 1972).

137 Coancestry coefficient (θ_{xy}) was calculated by the estimate of pairwise kinship coefficient describe by
138 Loiselle et al. (1995). The estimates were calculated using Jackknife resampling among loci using SPAGeDi
139 1.5 software (Hardy and Vekemans 2015). The pairwise relatedness was also used to calculate individual

140 heritability (\hat{h}_2) using a marker-based method proposed by Ritland (1996). The calculation of \hat{h}_2 was given
 141 by the equation: $\hat{h}_2 = \frac{C_{ZR}}{2V_r}$, where, V_r is the actual variance of relatedness among all pairs i and R is the
 142 sample covariance between phenotypic similarity (Z_i) and estimated relatedness (R_i). Among related
 143 individuals the phenotypic similarity is written as: $Z_i = \frac{(Y_i - U)(Y'_i - U)}{V}$. For i pair, Y_i is the value of trait in the
 144 first individual, Y'_i the value of trait in the second individual, U is the mean of the trait and V the population
 145 variance (Ritland 1996).

146 2.4. Genome-wide selection

147 To analyze the phenotype data the mixed linear model used was BLUP/RELM. The Predicted additive
 148 genetic values were deregressed and corrected for parents' effects to obtain the adjusted phenotypic values to
 149 be used for genomic predictions. To use information from the SSR markers in the GWS technique it was
 150 necessary to transform the genetic information into a binary code of 0 and 1. For each individual a "0" was
 151 added when it did not show the corresponding allele and one "1" when it presented the correspondent allele.
 152 This way each individual presents a code indicating the presence or absence of all observed alleles in the
 153 population. The markers had their effects estimated adjusting all the allelic effects simultaneously using the
 154 random regression best linear unbiased predictor (RR-BLUP). The method was performed using RR-BLUP
 155 package in R (Endelman 2011), as described previously (Resende et al. 2008; 2012). The RR-BLUP assumed
 156 that the markers effects were random. The variance parameters were assumed to be unknown and were
 157 estimated by restricted maximum likelihood (REML). The linear mixed model adjusted to estimate the effects
 158 of markers was: $y = X_b + W_m + e$. Where y is the vector of phenotypic data (deregressed additive genetic
 159 values), b is the vector of fixed effects, m is the vector of random effects of markers and e refers to the vector
 160 of random residues. X and W are the incidence matrices for b and m . The mixed model equations for
 161 genomic prediction of m via the RR-BLUP method is:

$$162 \begin{bmatrix} X'X & X'W \\ W'X & W'W + I \frac{\sigma_e^2}{(\sigma_g^2/n_Q)} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{m} \end{bmatrix} = \begin{bmatrix} X'y \\ W'y \end{bmatrix}$$

163 Where σ_g^2 is total genetic variation and n_Q is the number of loci. The σ_g^2 was estimated by REML from
 164 phenotype. The total genomic breeding value of individual j is given by $VGG = \hat{y}_j = \sum_i w_{ij} \hat{m}_i$, where W_i is
 165 equals to 0 corresponding to the genotype m , or 1 corresponding to the genotypes Mm and MM . The amount

166 of n_Q equals $n_Q = 2 \sum_i^n p_i (1 - p_i)$. The equations presented above assume a priori that all loci explain equal
167 amounts of the genetic variation.

168 Predictive accuracy, heritability and standards errors were estimated by averaging the Jack-Knife
169 (Quenouille 1949) cross-validation method. The approach used was the “leave-one-out”. A single individual
170 from the population was used as the validation set, and the remaining individuals as the estimation or training
171 set. This was repeated such that each individual in the sample was used once as the validation data. This
172 process was repeated 115 times, using each time a different set of individuals for estimation and one different
173 individual for validation having all individuals their phenotypes predicted and validated.

174

175 **3. Results**

176 Analysis of 13 SSR loci resulted in a total of 122 alleles. The mean of alleles per locus was nine,
177 ranging from three to 17 alleles (Table 1). The high variation in the numbers of alleles found per locus result
178 in a diverse expected heterozygosity (H_e) by locus. It did not demonstrate a disparity compared to the
179 observed one (H_o). The inbreeding levels estimated to this population were very low (Figure 1). The analysis
180 also evidenced that the population is in Hardy-Weinberg equilibrium for most of the locus.

181 The clustering method inferred the most likely number of clusters by identifying the highest L(K)
182 ($L'(K) = 343,11$) value with relatively small variance (Figure 2). It is possible to conclude that the stand
183 individuals came from two Australian’s original populations. Thus, the trees were gathered in two groups: one
184 with 54 and the other with 61 individuals (Figure 3). So, considering the existence of two groups in this
185 population, genetic values were performed considering separately the individual from each group (Table 2).
186 The genetic diversity levels calculated within both populations were very similar. The genetic distance among
187 them was 0.21.

188 This breeding population showed low prediction ability to dbh and height traits in GWS (Table 3).
189 This means that the number of markers and population sizes were not enough to validate this methodology.
190 The dbh trait indicates a high phenotypic variance.

191 The estimated pairwise kinship coefficient shows that the individuals in the population reveal a
192 considerable relatedness (Table 3). And the marker-based heritability estimated was very disparate from that
193 one found through GSW.

194 4. Discussion

195 The genetic diversity of the studied population was relatively elevated comparing the genetic parameters
196 to those natural population. In the restricted natural area of this species occurrence, the number of alleles (\bar{A} =
197 10.4) and the indexes of heterozygosity ($H_e = 0.739$ and $H_o=0.630$) are very similar to those found in the
198 stand (Butcher et al. 2005). Even though only ten adult trees were used as seed source for the population
199 setting, the stand is representative of a significant part of the total species diversity. The study's observations
200 address the high diversity levels for this population in relation to the gene flow of its original population. As
201 we noticed, both populations are in Hardy-Weinberg equilibrium and demonstrate very low endogamy
202 coefficients. This scenario suggests that this population was originated by random crossbreeding between
203 unrelated parents (Randall et al. 2016). The stand reveal heterozygosity level similar to other tree breeding
204 populations, like *E. meliodora* (Broadhurst 2013), *Acacia mangium* (Yuskiante and Isoda 2012), *Pinus taeda*
205 (H_E : 0.659; Ai-rabab'ah and Willians 2002), and *Araucaria cunninghamii* (H_E : 0.61, Peakall et al. 2003).
206 Costa et al. (2015) observed similar genetic diversity in populations of *Aracaucária Angustifolia* ($H_e = 0.674$,
207 $F = 0.062$). This indicates that crossbreeding with different pollen donors occurred in the reproductive events
208 that generate the studied population. Despite the existence of kinship with a high degree of relatedness (half-
209 brothers and full siblings), there is also the presence of crosses with low levels of kinship in the population.

210 On the other hand, the diversity indicators in this study were lower than *Eucalyptus* species with a more
211 widespread natural occurrence area. Breeding populations and seed orchards from *Eucalyptus* species such as
212 *E. dunnii* (Marcucci Poltri et al. 2003), *E. globulus* (Jones et al. 2006), *E. grandis* (Chaix et al. 2003) and *E.*
213 *urophylla* (Silva et al. 2018) showed higher expected heterozygosity. This occurs because the genetic
214 diversity is associated with the environmental variability. If the species distribution occurs only in narrow
215 area and similar environments, like *E. benthamii*, the genetic diversity tends not to be so elevated. Still, it is
216 noticeable that the estimated values are higher than those verified for some conifers such as *Pinus pinaster*
217 (Wshid et al. 2010), *Pinus tabulaeformis* (Di and Wang 2013), *Pinus resinosa* (H_E : 0.358; Ter-Mikaelian and
218 Bowling 2014) and *Pinus dabeshanensis* (Xang et al. 2015). It indicates that the typical genetic diversity of *E.*
219 *benthamii* is moderate among other tree species.

220 In general, Breeding stands composed of selected trees usually show a reduced genetic diversity. The
221 trend is that for each new crosses generation the genetic diversity will be reduced (Jones et al. 2006), since

222 favorable alleles concentration increase in selection processes. The stand under study exhibited a high value
223 of diversity compared to natural stands of the same species. This assures the capacity of this stand c contribute
224 with the species conservation, helping as collection of accesses to a germoplasm bank. After two selective
225 thinning the remaining trees represent selected elite regarding growth traits. Furthermore, the stand contains
226 a valuable material for breeding program aiming at frost tolerant fast growth trees, since more than 10 frosts
227 occurs annually.

228 The clustering method showed that seeds originated in this stand came from two original populations.
229 Due to low genetic distance among populations it is possible that the genetic pools of both populations are
230 very similar (Nei 1972). The parent's original populations must have could originally occupied areas with
231 similar environments. Furthermore, they must belong to the same provenance, since the genetic distance
232 between them is very small. Also, these populations probably are not completely isolated from each other,
233 likely, they exchanged gametes maintaining a gene certain flow level (Vekemans and Hardy 2004). The
234 presence existence of two groups of individuals in the stand can contribute significantly to breeding strategies
235 as well as in conservation strategies for the species. Individuals from different groups can be used in
236 controlled pollinations or can be crossed with closer individuals to get a purer pedigree. In breeding programs,
237 it is common to get a high reduction on genetic variability which hampers the estimate of genetic gains. By
238 knowing how genetic groups are distributed within population, it is easier to manage the maintenance of
239 genetic variability (Schwartz and Mckelvey 2009), and to develop strategies for collecting, conserving, and
240 using these germplasm resources.

241 GWS is an adequate method to direct the selection process in breeding population, but for this particular
242 *E. benthamii* population it will not work. This approach shows a low predictive capacity for this small
243 population. Other studies involving GWS in tree breeding populations usually show a higher selective
244 accuracy (\hat{r}_{yy}) and heritability. Grattapaglia et al. (2011) e Resende et al. (2012) found values for the \hat{r}_{yy} of
245 the 0.69 and 0.54 for dbh trait in eucalypt species. Duran et al. (2017) archived an even higher predict power
246 for volume with a clonal *E. globulus* (0.73). Tan et al. (2017) detected a more moderate average of 0.27 for a
247 hybrid generation of *E. grandis* x *E. urophylla*. For *Pinus taeda* Aguiar et al. (2015) estimated a \hat{r}_{yy} of 0.63
248 for dbh at age six years (14,000 markers). Considering crop species, such as grapes (*Vitis rupestris* × *Vitis*
249 *arizonica/girdiana*) and cassava (*Manihot esculenta*), respectively values of 0.67 and 0.50 were predicted for

250 growth traits (Viana et al. 2016; Silva et al. 2016). When we compare the cited studies and the present one,
251 apparently the number of individuals sampled on the population and marker density is the main difference.

252 By comparison with different studies it is possible to point out which variables cause more difference
253 at the GWS method results. Resende et al. (2012a) found lower accuracy in their results with a *Pinus taeda*
254 population using 4,953 SNPs markers and 941 individuals in the training population that Zapata-Venezuela et
255 al. (2012) using 3,406 SNPs and 526 individuals of the same species. On the other hand, a considerable
256 increase in the number of SNP markers provoked a big difference in the results for *Picea abies* (up to 6,932
257 SNPs) (Beaulieu et al. 2014) and *Picea engelmannii* (from 8,868 to 55,618 SNPs) (El-dien et al. 2015).
258 Considering *Eucalyptus grandis* x *Eucalyptus urophylla* hybrid Resende et al. (2012b) reached a good
259 predictive power using DArT(>3,000), a dominant marker, as well Müller et al (2019) using 46,000 SNPs, a
260 dominant/co-dominant marker, for the same hybrid. Marchal et al. (2016) using 313 SRR marker, similar as
261 applied in this work, obtained high levels of accuracy in the predict genetic values of 478 crosses in an
262 *Elaeis guineensis* (oil palm) population.

263 In comparing the cited studies to this one, the number of individuals sampled on the population and
264 marker density is the main difference is observed. In this case it is presumable that the number of individuals
265 in the training population and markers were insufficient to produce an accurate prediction. In consensus, the
266 method accuracy tends to get higher as the individual of the training population gets larger (Jannink 2010).
267 Torres-Dini et al. (2016), despite using a high number of markers (15,106 SNPs), obtained low accuracy
268 results with a small population (78 individuals) of eucalyptus hybrids. And regarding the number of markers,
269 papers show reliable results with trials using around eighty SRR markers (Fritsche-Neto et al. 2012).

270 Nevertheless, even in small populations with 100 or 200 samples, Grattapaglia & Resende (2011)
271 simulated that the GWS would have a better accuracy (about 0.7 for N = 100) using SNPs markers at density
272 of 2 per centiMorgans(cM). Muller (2017) employing GWS technique for *E. benthamii* breeding population
273 argues that the most influential factor in predictive power is the relatedness among individuals, followed by
274 the amount of genetic additive variance of population. It is also noticeable that this population has an
275 expressive phenotypic variance in relation to dbh trait. Due to the low accuracy of the estimation, the values
276 regarding to heritability are probably underestimated. The true value of population's additive genetic

277 variance should be higher than that estimated (Callus 2010). Due to the narrow origin of its genitors, besides
278 the low genetic variance, the individuals show an expressive relatedness as was demonstrated by the
279 population coancestry coefficient. The θ_{xy} of the population get closer to the half-sib number ($\theta_{xy}=0.125$)
280 (Cockerham 1967), this indicates that part of individuals in the population share at least one parental in
281 common. As the seeds that originated this population were collect from only 10 trees of the same provenance;
282 part of them probably share a common mother and different fathers.

283 The GWS and the marker-based procedures (using kinship coefficients) should estimate similar
284 values for heritability. On this particular study, the marker-based heritability must be the closest to real one.
285 The GWS methods for this test reveal low predict ability. That leads to inaccurate genetic values estimative
286 and, consequently, to one underrated heritability. Genetic markers provide information about relatedness
287 between individuals of unknown pedigree making it possible to estimate a kinship matrix based on average
288 values of relatedness (Ritland 1996). Da Silva et al. (2015) compared the results obtained for heritability
289 based on the kinship coefficients of Lynch and Ritland (1999), Queller and Goodnight (1989) and Wang
290 (2002) and conclude that the Ritland (1996) method is the most robust. The authors argued that the method
291 allows a greater adequacy of the data in the model used. However, the GWS procedure is more accurate than
292 that one based on relatedness, this because it effectively captures the actual kinship matrix performed and not
293 an average kinship matrix associated with the pedigree, like the second procedure (Arcia et al. 2011).
294 According to Munoz (2014), GWS is the method that best explores the mendelian sampling segregation
295 occurring during the gamete origin, once it directly evaluates the associated DNA at each locus of all
296 polygenic traits. Thereby, it captures the exactly kinship matrix and not a pedigree-matched relatedness
297 matrix. The GWS allows the obtaining of information that produces more accurate estimates of the genetic
298 values at the individual level. Although the trial had already submitted to two selective thinning, the
299 heritability estimated trough markers show a potential to achieve considerable genetic gain using short-term
300 selection methods.

301 **5. Conclusion**

302 The *E. benthamii* population presents considerable genetic diversity, and has potential to contribute to
303 species genetic breeding. The individuals with more genomic breeding values can be used to established seed

304 orchard aiming at breeding (frost tolerant stands, for hybrid production,) and conservation (enriching
305 germplasm bank of the species, as the only collection with replica in Brazil). Still, regarding the continuity of
306 future breeding or conservation programs using this population as base, it is indicated the introduction of
307 more individual to improve the germplasm collection. Considering the natural narrow occurrence of the
308 species, is proposed to cross the selected individuals with trees from the most distant provenances as possible
309 from this population genitors' origin region.

310 Genome-wide section method was not effective to provide high genetic gains through selection for this
311 particular population and marker density. A significant level of relatedness among the individuals was found,
312 being possible to assume that with a larger number of individuals the GWS could reach acceptable precision
313 level. It can be more efficient and less costly that the phenotypic selection. So, for further generations of this
314 population, as well for stands with similar genetic characteristics, the SGW could reach the aimed goals.

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317 team for the technical unconditional support to established, especially Roberto Carletto that help to collected
318 the data and the student Julio Soares.

319 **6. Data archiving statement**

320 The genotypes of the 115 individuals for 13 loci are available on Mendeley website, doi:
321 10.17632/yxjddg3cff.1 (Marchetti de Souza et al. 2020).

322 **7. Disclosure statement**

323 The authors declare no conflict of interest to disclosure with respect to this paper.

324 **8. References**

- 325 Ai-rabab'ah MA, Williams CG (2002) Population dynamics of *Pinus taeda* L. based on nuclear
326 microsatellites. *Forest Ecology Management* 163:263-27.
- 327 Aguiar AV, Teixeira-Freitas, DMA, Almeida Filho JE, Sousa VA, Resende MDV, Silva-Junior OB,
328 Grattapaglia D (2015) Genomic prediction of growth traits in *Pinus taeda* using genome-wide sequence-based
329 DArT-seq marker. IUFRO Tree Biotechnology 2015 Conference, Florence.

- 330 Arcia C, Lima BM, Almeida A, Resende MDV, Vencovsky R, Grattapaglia D (2011) Genome wide selection
331 for Eucalyptus improvement at international paper in Brazil. BMC Proceedings 5:44.
- 332 Azevedo CF, de Resende MDV, Silva FF, Viana JMS, Valente MSF, Resende Junior MFR, de Oliveira, EJ
333 (2016) New accuracy estimators for genomic selection with application in a cassava (*Manihot esculenta*)
334 breeding program. Genetics and molecular research 15(4).
- 335 Azodi CB, Bolger E, McCarren A, Roantree M, de los Campos G, Shiu SH (2019) Benchmarking Parametric
336 and Machine Learning Models for Genomic Prediction of Complex Traits. G3: Genes, Genomes,
337 Genetics 9(11):3691-3702.
- 338 Baccarin FJB, Brondani GE, de Almeida LV, Vieira IG, de Oliveira LS, de Almeida, M (2015) Vegetative
339 rescue and cloning of *Eucalyptus benthamii* selected adult trees. New Forests 46(4):465-483.
- 340 Beaulieu J, Doerksen TK, Mackay J, Rainville A, Bousquet J (2014) Genomic selection accuracies within and
341 between environments and small breeding groups in white spruce. BMC Genomics 15:1048
- 342 Broadhurst LM (2013) A genetic analysis of scattered Yellow Box trees (*Eucalyptus melliodora* A. Cunn. ex
343 Schauer, Myrtaceae) and their restored cohorts. Biological Conservation 161:48-57.
- 344 Brown AHD, Weir BS (1983) Measuring genetic variability in plant populations. Isozymes in plant genetics
345 and breeding, part A, p. 219-239, 1983.
- 346 Bush D, England N, Broadhurst L (2016) Genetic Diversity, Conservation and Breeding of *Eucalyptus*
347 *Benthamii*. Australasian Forest Genetics Conference March 2016, Rotorua, New Zealand.
- 348 Butcher PA, Skinner AK, Gardiner CA (2005) Increased inbreeding and inter-species gene flow in remnant
349 populations of the rare *Eucalyptus benthamii*. Conservation Genetics 6(2):213-226.
- 350 Callus MPL (2010) Genomic breeding value prediction: methods and procedures. Animal 4(1):157-164.
- 351 Chaix G, Gerber S, Razafimaharo V, Vignerón P, Verhaegen D, Hamon S (2003) Gene flow estimation with
352 microsatellites in a Malagasy seed orchard of *Eucalyptus grandis*. Theoretical and Applied Genetics 107(4):
353 705-712.
- 354 Chen S, Zhou C, He X, Weng Q, Li F, Li M, Gan S (2020) Enhanced correlations of EST-SSR-based genetic
355 distance with hybrid performance, specific hybridizing ability, and heterosis using effect-increasing and
356 effect-decreasing alleles: a case study in *Eucalyptus L'Hér.* Tree Genetics & Genomes 16(1):1-9.
- 357 Cockerham CC (1967) Group inbreeding and coancestry. Genetics 56(1):89.
- 358 Costa NCF, Vargas OF, Guidolin AF, Mantovani A (2015) Efeitos da paisagem de campo e florestamento
359 com *Pinus* na diversidade e estrutura genética de pequenas populações remanescentes de *Araucaria*
360 *angustifolia*. Scientia Forestalis 43(107):551-560
- 361 Crossa J, Pérez-Rodríguez P, Cuevas J, Montesinos-López O, Jarquín D, de los Campos G, Dreisigacker S
362 (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends in plant science
363 22(11):961-975.

- 364 Desta ZA, Ortiz R (2014) Genomic selection: genome-wide prediction in plant improvement. Trends in plant
365 science 19(9):592-601.
- 366 Di X, Wang M (2013) Genetic diversity and structure of natural *Pinus tabulaeformis* populations in North
367 China using amplified fragment length polymorphism (AFLP). Biochemical systematics and ecology 51:269-
368 275.
- 369 Dungey HS, Dash JP, Pont D, Clinton PW, Watt MS, Telfer EJ (2018) Phenotyping whole forests will help to
370 track genetic performance. Trends in plant science 23(10):854-864.
- 371 Earl DA (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output
372 and implementing the Evanno method. Conservation genetics resources 4(2):359-361.
- 373 Endelman JB: Ridge Regression and Other Kernels for Genomic Selection with R Package rrBLUP. Plant
374 Genome-Us, v. 4, n. 3, p. 250-255, 2011.
- 375 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software
376 STRUCTURE: a simulation study. Molecular ecology 14(8):2611-2620.
- 377 El-Dien OG, Ratcliffe B, Klápště J, Chen C, Porth I, El-Kassaby YA (2015) Prediction accuracies for growth
378 and wood attributes of interior spruce in space using genotyping-by-sequencing. BMC genomics 16(1):370.
- 379 Fritsche-Neto R, Do Vale JC, Lanes ECM, Resende MDV, Miranda GV (2012) Genome-Wide Selection for
380 tropical maize root traits under conditions of nitrogen and phosphorus stress. Acta Scientiarum.
381 Agronomy 34(4):389-395.
- 382 Glowatzki-Mullis ML, Muntwyler J, Gaillard C (2007) Cost-effective parentage verification with 17-plex
383 PCR for goats and 19-plex PCR for sheep. Animal genetics 38(1):86-88.
- 384 Gois IB, Borém A, Cristofani-Yaly M, de Resende, MDV, Azevedo CF, Bastianel M, Machado M A
385 (2016) Genetics and Molecular Research Genetics and Molecular Research 15(4):gmr15048863.
- 386 González-Martínez SC, Krutovsky KV, Neale DB (2006) Forest-tree population genomics and adaptive
387 evolution. New Phytologist 170(2):227-238.
- 388 Graça MEC, Shimizu JY, Tavares FR (1999) Capacidade de rebrota e de enraizamento de *Eucalyptus*
389 *benthamii*. Boletim de Pesquisa Florestal 39:135-138.
- 390 Grattapaglia D, Resende MDV, Resende MR, Sansaloni CP, Petroli CD, Missiaggia AA, Kilian A (2011)
391 Genomic Selection for growth traits in Eucalyptus: accuracy within and across breeding populations. BMC
392 proceedings 5(7):O16.
- 393 Grattapaglia D, Resende MDV (2011) Genomic selection in forest tree breeding. Tree Genetics & Genomes
394 7(2):241-255.
- 395 Hart PW, Johnson J, Paim R (2016) Status update on development of a eucalyptus plantation program in the
396 southeastern United States and higher elevations of southern Brazil. Tappi J 15:148-155.
- 397 Hayes BJ, Cogan NO, Pembleton LW, Goddard ME, Wang J, Spangenberg GC, Forster JW (2013) Prospects
398 for genomic selection in forage plant species. Plant Breeding, 132(2):133-143.

399 Inglis PW, Pappas MCR, Resende LV, Grattapaglia D (2018) Fast and inexpensive protocols for consistent
400 extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP
401 genotyping and sequencing applications. PLOS ONE 13(10):e0206085.

402 INMET - Brazilian National Institute of Meteorologia (2019). Available in: [http://www.inmet.gov.br/portal/
403 index.php?r=estacoes/estacoesAutomaticas](http://www.inmet.gov.br/portal/index.php?r=estacoes/estacoesAutomaticas)

404 Isik F. (2014). Genomic selection in forest tree breeding: the concept and an outlook to the future. New
405 Forests 45(3):379-401.

406 Ismail SA, Ghazoul J, Ravikanth G, Kushalappa CG, Shaanker RU, Kettle CJ (2014) Fragmentation genetics
407 of *Vateria indica*: implications for management of forest genetic resources of an endemic
408 dipterocarp. Conservation genetics 15(3):533-545.

409 Jannink JL, Lorenz AJ, Iwata H (2010) Genomic selection in plant breeding: from theory to
410 practice. Briefings in functional genomics 9(2):166-177.

411 Jones TH, Steane DA, Jones RC, Pilbeam D, Vaillancourt RE, Potts BM (2006) Effects of domestication on
412 genetic diversity in *Eucalyptus globulus*. Forest Ecology and Management 234(1-3):78-84.

413 Kaur H, Mittal A, Dhillon GPS, Gill RIS (2018) Molecular characterization and genetic diversity analysis of
414 Eucalyptus clones using SSR markers. Indian Journal of Agroforestry 20(1):45-52.

415 Kellison RC, Lea R, Marsh P (2013) Introduction of Eucalyptus spp. into the United States with special
416 emphasis on the southern United States. International Journal of Forestry Research 2013 article ID 189393.

417 Laviola BG, Rodrigues EV, Teodoro PE, de Azevedo Peixoto L, Bhering LL. 2017. Biometric and
418 biotechnology strategies in *Jatropha* genetic breeding for biodiesel production. Renewable and Sustainable
419 Energy Reviews 76:894-904.

420 Lewis PO, Zaykin D (2001) Genetic Data Analysis: Computer program for the analysis of allelic data.
421 Version 1(0):d16c.

422 Liu G, Arnold RJ, Xie YJ, Wu ZH (2018) Genetic relationships among 40 species of eucalyptus based on
423 simple sequence repeat markers. Journal of Tropical Forest Science 30(3):402-414.

424 Liu Q, Zhou Z, Wei Y, Shen D, Feng Z, Hong S (2015) Genome-wide identification of differentially
425 expressed genes associated with the high yielding of oleoresin in secondary xylem of Masson pine (*Pinus
426 massoniana* Lamb) by transcriptomic analysis. PloS one, 10(7).

427 Lv J, Li C, Zhou C, Chen J, Li F, Weng Q, Gan S (2020) Genetic diversity analysis of a breeding population
428 of *Eucalyptus cloeziana* F. Muell.(Myrtaceae) and extraction of a core germplasm collection using
429 microsatellite markers. Industrial Crops and Products 145:112157.

430 Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics
431 152(4):1753-1766.

432 Marchal A, Legarra A, Tisné S, Carasco-Lacombe C, Manez A, Suryana E, Bouvet JM (2016) Multivariate
433 genomic model improves analysis of oil palm (*Elaeis guineensis* Jacq.) progeny tests. Molecular breeding
434 36(1):2.

435 Marchetti de Souza B, Abreu LM, Pappas M, Azevedo VCR, de Sousa VA, dos Santos RF, de Aguiar AV
436 (2020), “Genetic diversity and genomic selection in *Eucalyptus benthamii*”, Mendeley Data, V1
437 <http://dx.doi.org/10.17632/yxjddg3cff.1>

438

439 Maxted N, Ford-Lloyd BV, Hawkes JG (2013) Plant genetic conservation: the in situ approach. Springer
440 Science & Business Media.

441 Meuwissen TH, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense
442 marker maps. *Genetics* 157:1819-1829.

443 Miranda AC, da Silva PH, Moraes ML, Lee DJ, Sebbenn AM (2019) Investigating the origin and genetic
444 diversity of improved *Eucalyptus grandis* populations in Brazil. *Forest Ecology and Management* 448:130-
445 138.

446 Mora F, Arriagada O, Ballesta P, Ruiz E (2017) Genetic diversity and population structure of a drought-
447 tolerant species of *Eucalyptus*, using microsatellite markers. *Journal of Plant Biochemistry and Biotechnology*
448 26(3):274-281.

449 Müller BS, Neves LG, de Almeida Filho JE, Resende MF, Muñoz PR, dos Santos PE, Grattapaglia D (2017).
450 Genomic prediction in contrast to a genome-wide association study in explaining heritable variation of
451 complex growth traits in breeding populations of *Eucalyptus*. *BMC genomics* 18(1):524.

452 Müller BS, de Almeida Filho JE, Lima BM, Garcia CC, Missiaggia A, Aguiar AM, Neves LG et al. (2019)
453 Independent and Joint-GWAS for growth traits in *Eucalyptus* by assembling genome-wide data for 3373
454 individuals across four breeding populations. *New Phytologist* 221(2):818-833.

455 Munoz PR, Resende MF, Huber DA, Quesada T, Resende MD, Neale DB, Peter GF et al. (2014) Genomic
456 relationship matrix for correcting pedigree errors in breeding populations: impact on genetic parameters and
457 genomic selection accuracy. *Crop Science* 54(3):1115-1123.

458 Nei M (1972) Genetic distance between populations. *The American Naturalist* 106(949):283-292.

459 Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of
460 individuals. *Genetics* 89(3):583-590.

461 Peakall R, Ebert D, Scott LJ, Meagher PF, Offord CA (2003) Comparative genetic study confirms
462 exceptionally low genetic variation in the ancient and endangered relictual conifer, *Wollemia nobilis*
463 (Araucariaceae). *Molecular Ecology* 12(9):2331-2343.

464 Poltri SM, Zelener N, Traverso JR, Gelid P, Hopp HE (2003) Selection of a seed orchard of *Eucalyptus*
465 *dunnii* based on genetic diversity criteria calculated using molecular markers. *Tree physiology* 23(9):625-632.

466 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype
467 data. *Genetics* 155(2):945-959.

468 Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43(2):258-275.

469 Quenouille, MH (1949) Approximate Tests of Correlation in Time-Series. *J Roy Stat Soc B* 11(1):68-84.

470 Randall BW, Walton DA, Lee DJ, Wallace HM (2016) The risk of pollen-mediated gene flow into a
471 vulnerable eucalypt species. *Forest ecology and management* 381:297-304.

472 Resende MF, Muñoz P, Resende MD, Garrick DJ, Fernando RL, Davis JM, Kirst M (2012a) Accuracy of
473 genomic selection methods in a standard data set of loblolly pine (*Pinus taeda* L.). *Genetics* 190(4):1503-
474 1510.

475 Resende MD, Resende MF, Sansaloni CP, Petroli CD, Missiaggia AA, Aguiar AM, Pappas GJ et al. (2012b)
476 Genomic selection for growth and wood quality in Eucalyptus: capturing the missing heritability and
477 accelerating breeding for complex traits in forest trees. *New Phytologist* 194(1):116-128.

478 Resende Jr MFR, Munoz P, Acosta JJ, Peter GF, Davis JM, Grattapaglia D, Kirst M et al. (2012) Accelerating
479 the domestication of trees using genomic selection: accuracy of prediction models across ages and
480 environments. *New Phytologist* 193(3):617-624.

481 Resende RT, Resende MDV, Silva FF, Azevedo CF, Takahashi EK, Silva-Junior OB, Grattapaglia D (2017)
482 Assessing the expected response to genomic selection of individuals and families in Eucalyptus breeding with
483 an additive-dominant model. *Heredity* 119(4):245.

484 Ritland K. A (1996) marker-based method for inferences about quantitative inheritance in natural
485 populations. *Evolution* 50(3):1062-1073

486 Santarosa E, Penteado Junior JF, Goulart I (2014) Transferência de tecnologia florestal: cultivo de Eucalipto
487 em propriedades rurais: diversificação da produção e renda, Embrapa Florestas, 138p.

488 Sawitri S, Tani N, Na'iem M, Widiyatno W, Indrioko S, Uchiyama K, Tsumura Y (2020) Potential of
489 Genome-Wide Association Studies and Genomic Selection to Improve Productivity and Quality of
490 Commercial Timber Species in Tropical Rainforest, a Case Study of *Shorea platyclados*. *Forests* 11(2):239.

491 Schwartz MK, Mckelvey KS (2009) Why sampling scheme matters: the effect of sampling scheme on
492 landscape genetic results. *Conservation Genetics* 10(2):441.

493 da Silva ECB, Kubota TYK, de Moraes MLT, Sebbenn AM (2015) Coefficients of herdability and relatedness
494 in a forest fragment of *Araucaria angustifolia* (Bertol.) Kuntze using genetic markers. *Scientia Forestalis*
495 43(105):147-153.

496 Silva PHM, Brune A, Pupin S, Moraes MLT, Sebbenn AM, de Paula RC (2018) Maintenance of genetic
497 diversity in *Eucalyptus urophylla* ST Blake populations with restriction of the number of trees per family.
498 *Silvae Genetica* 67(1):34-40.

499 Tan B, Grattapaglia D, Martins GS, Ferreira KZ, Sundberg B, Ingvarsson PK (2017) Evaluating the accuracy
500 of genomic prediction of growth and wood traits in two Eucalyptus species and their F 1 hybrids. *BMC plant*
501 *biology* 17(1):110.

502 Telfer E, Graham N, Stanbra L, Manley T, Wilcox P (2013) Extraction of high purity genomic DNA from
503 pine for use in a high-throughput Genotyping Platform. *New Zealand Journal of Forestry Science* 43(1):3.

504 Ter-Mikaelian M, Bowling C (2014) Effect of climatic conditions on height growth of red pine: Results of a
505 provenance test in northwestern Ontario. *The Forestry Chronicle* 90(6):794-800.

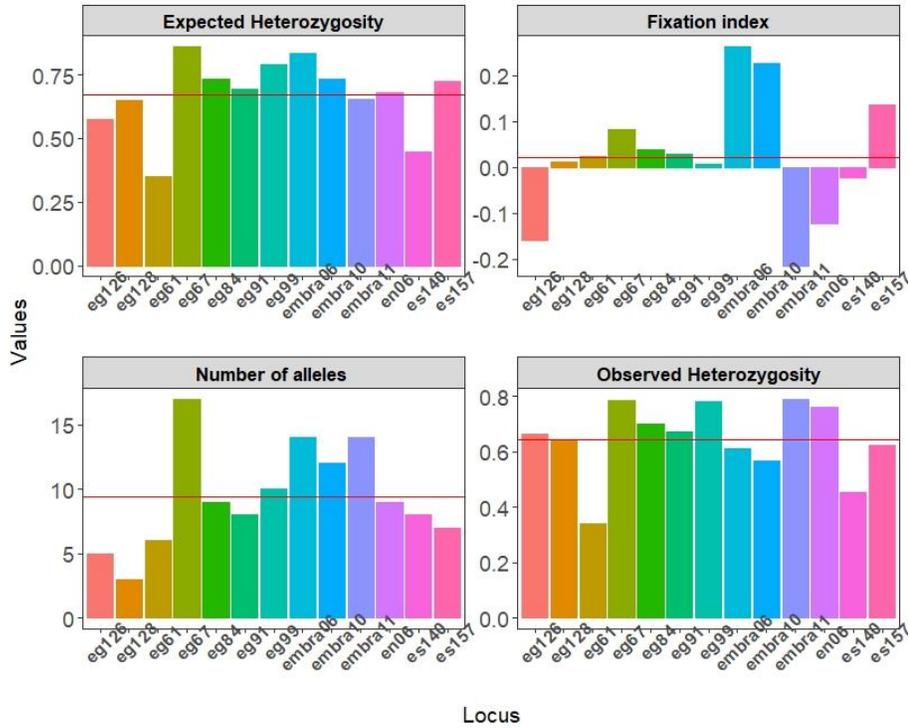
- 506 Torres-Dini D, Nunes ACP, Aguiar A, Nikichuk N, Centuri3n C, Cabrera M, Sebbenn AM (2016) Clonal
507 selection of *Eucalyptus grandis* x *Eucalyptus globulus* for productivity, adaptability, and stability, using SNP
508 markers. *Silvae Genetica* 65(2):30-38.
- 509 Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant
510 populations. *Molecular Ecology* 13:921-935.
- 511 Viana AP, Resende MDVD, Riaz S, Walker MA (2016) Genome selection in fruit breeding: application to
512 table grapes. *Scientia Agricola* 73(2):142-149.
- 513 Wahid N, Naydenov KD, Kamari S, Boulli A, Tremblay F (2010) Genetic structure of *Pinus pinaster* Ait.
514 populations in Morocco revealed by nuclear microsatellites. *Biochemical Systematics and Ecology* 38(1):73-
515 82.
- 516 Wang J (2002) An estimator for pairwise relatedness using molecular markers. *Genetics* 160(3):1203-1215.
- 517 Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of
518 mating. *Evolution*, 19(3):395-420.
- 519 Xiang XY, Zhang ZX, Duan RY, Zhang XP, Wu GL (2015) Genetic diversity and structure of *Pinus*
520 *dabeshanensis* revealed by expressed sequence tag-simple sequence repeat (EST-SSR) markers. *Biochemical*
521 *Systematics and Ecology* 61:70-77.
- 522 Xie Y, Arnold RJ, Wu Z, Chen S, Du A, Luo J (2017) Advances in eucalypt research in China. *Frontiers of*
523 *Agricultural Science and Engineering* 4(4):380-390.
- 524 Yeh FC, Yang RC, Boyle T (1999) POPGENE software package version 1.31 for population genetic
525 analysis. University of Alberta, Canada.
- 526 Yuskianti V, Isoda K (2012) Genetic diversity of *Acacia mangium* seed orchard in Wonogiri Indonesia using
527 microsatellite markers. *HAYATI Journal of Biosciences* 19(3):141-144.
- 528 Zapata-Valenzuela J, Isik F, Maltecca C, Wegrzyn J, Neale D, McKeand S, Whetten R (2012) SNP markers
529 trace familial linkages in a cloned population of *Pinus taeda* prospects for genomic selection. *Tree Genetics &*
530 *Genomes* 8(6):1307-1318.

531 Table 1- Descriptive genetics of the microsatellite loci used for individuals of *Eucalyptus benthamii* in
 532 Colombo, state of Paraná, Brazil. With number alleles (n), expected heterozygosity (H_e), observed
 533 heterozygosity (H_o) and Fixation index (F).
 534

Locus	n	H_o	H_e	F
eg61	6	0.342	0.352	0.023
embra06	14	0.613	0.834	0.262
embra10	12	0.567	0.734	0.225
embra11	14	0.790	0.653	-0.214
eg126	5	0.664	0.575	-0.159
eg67	17	0.785	0.859	0.082
es157	7	0.624	0.726	0.137
eg91	8	0.670	0.692	0.028
en06	9	0.761	0.681	-0.123
eg99	10	0.781	0.790	0.006
es140	8	0.455	0.448	-0.023
eg128	3	0.639	0.650	0.011
eg84	9	0.700	0.732	0.038
Mean	9.38	0.645	0.671	0.023

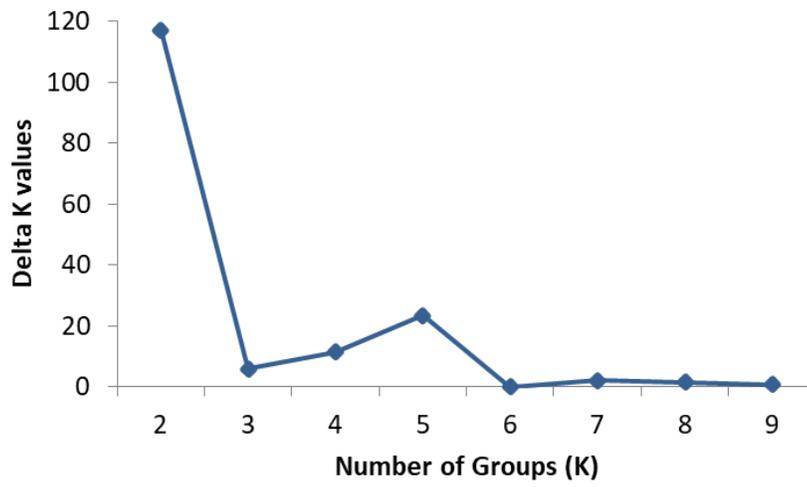
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536 Figure 1. Descriptive genetics of the microsatellite loci used for individuals of *E. benthamii* in
 537 municipality Colombo, state of Paraná, Brazil. The red horizon line corresponds to the mean value of the
 538 parameter across all loci.
 539



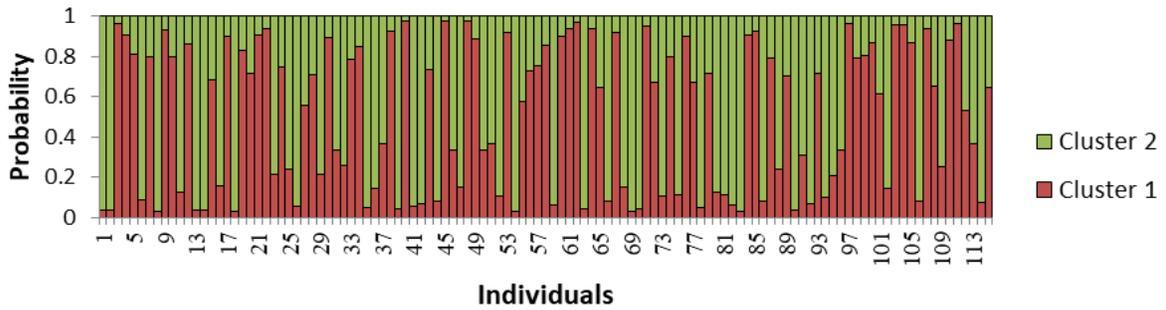
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541 Figure 2. Values of delta K corresponding to each number of groupings simulated in a population of
542 *E.benthamii* in municipality Colombo, state of Paraná, Brazil. $\Delta K = \text{mean} (|L''(K)|) / \text{sd} (L(K))$
543



544

545 Figure 3. Bayesian-bases analysis of population structure for $K = 2$, $L'(K)= 343,11$ and $\Delta K= 117,20$ in
546 municipality Colombo, state of Paraná, Brazil.
547



548

549

550

551 Table 2- Comparative of descriptive genetics parameters between two populations of *E. benthamii* in
552 municipality Colombo, state of Paraná, Brazil.

553

Parameters	Population 1	Population 2
Sample Size	54	61
N. of alleles	8.31	7.39
Expct. heterozygosity	0.67	0.61
Fix. Ind.	0.01	0.02
Mean DBH	56 cm	54 cm

554 Table 3: Parameters estimated by pairwise kinship and genomic wide selection in a *E. benthamii* population in
 555 Colombo, state of Paraná, Brazil.

556

Trait	r_{gy}	σ_a^2	σ_y^2	σ_e^2	h_2	θ_{xy}	h_2'
Dbh	-0.037	3.17	104.02	100.85	0.03		0.13
Height	-0.078	0.35	23.00	22.00	0.02		
Population						0.085	

557 Predictive ability (r_{gy}), additive variance (σ_a^2), phenotypic variance (σ_y^2), error variance (σ_e^2), heritability (h_2), pairwise
 558 kinship coefficient (θ_{xy}) and heritability estimated using pairwise kinship coefficient (h_2').

559

560

Figures

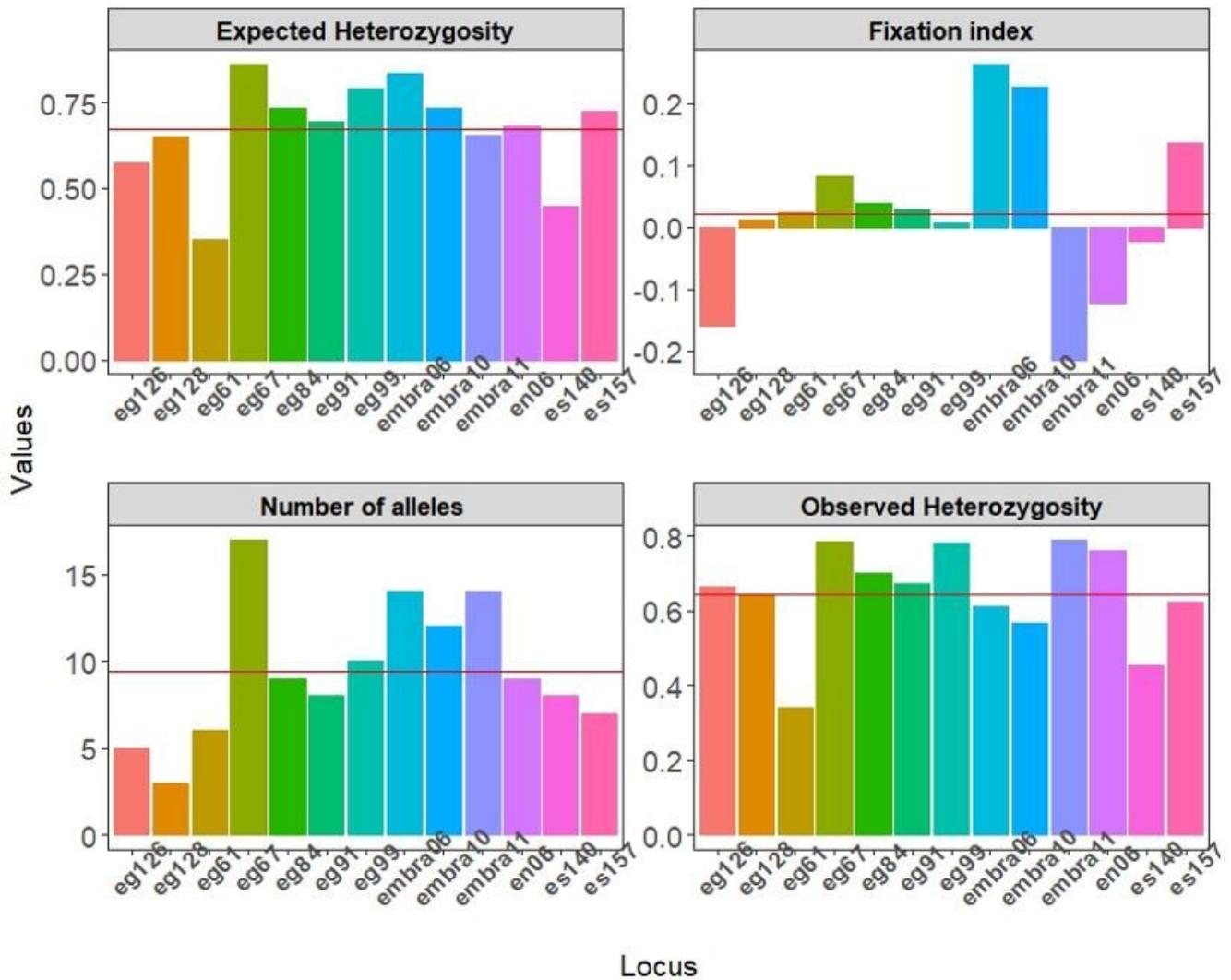


Figure 1

Descriptive genetics of the microsatellite loci used for individuals of *E. benthamii* in municipality Colombo, state of Paraná, Brazil. The red horizon line corresponds to the mean value of the parameter across all loci.

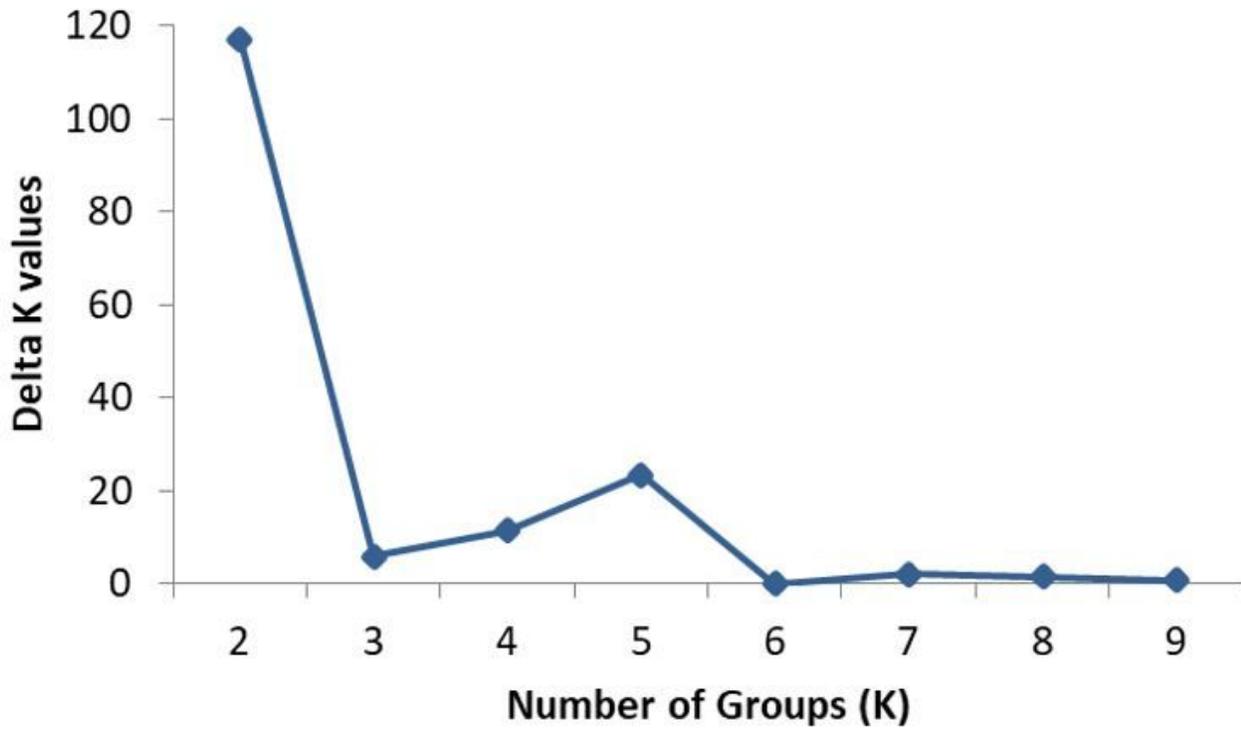


Figure 2

Values of delta K corresponding to each number of groupings simulated in a population of *E.benthamii* in municipality Colombo, state of Paraná, Brazil. $\Delta K = \frac{\text{mean}(|L'(K)|)}{\text{sd}(L(K))}$

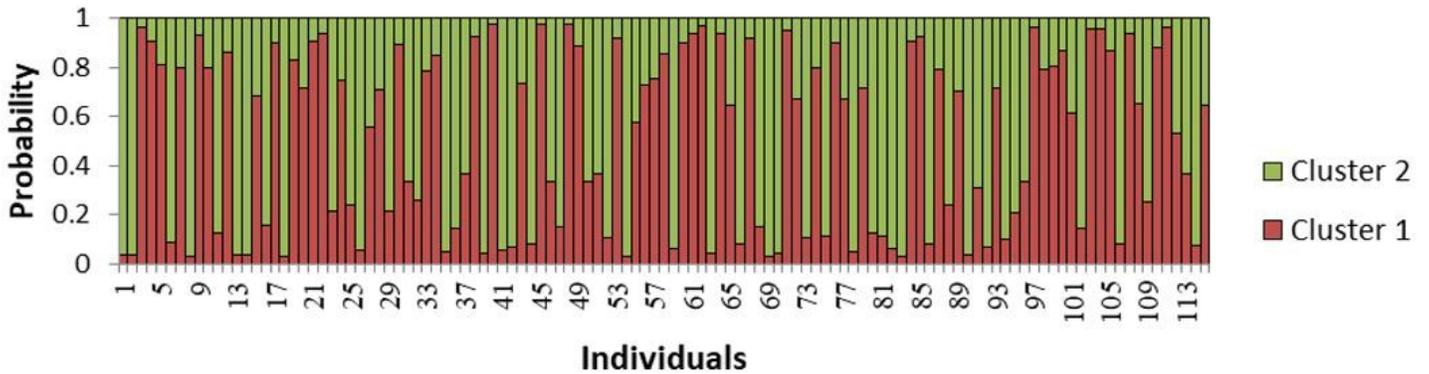


Figure 3

Bayesian-bases analysis of population structure for $K = 2$, $L'(K) = 343,11$ and $\Delta K = 117,20$ in municipality Colombo, state of Paraná, Brazil.