

Inheritance of foliar stable carbon isotope discrimination and third-year height in *Pinus taeda* clones on contrasting sites in Florida and Georgia

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Abstract Quantifying foliar stable carbon isotope discrimination (Δ) is a powerful approach for understanding genetic variation in gas exchange traits in large populations. The genetic architecture of Δ and third-year height is described for more than 1,000 clones of *Pinus taeda* tested on two contrasting sites. \hat{h}^2 for Δ was 0.14 (± 0.03), 0.20 (± 0.07), and 0.09 (± 0.04) at Florida, Georgia, and across sites, respectively. \hat{H}^2 for stable carbon isotope discrimination ranged from 0.25 (± 0.03) at the Florida site to 0.33 (± 0.03) at the Georgia site, while the across-site estimate of \hat{H}^2 was 0.19 (± 0.02). For third-year height, \hat{h}^2 ranged from 0.13 (± 0.05) at the Georgia site to 0.20 (± 0.06) at the Florida site with an across-site estimate of 0.09 (± 0.05). Broad-sense heritability estimates for third-year height were 0.23 (± 0.03), 0.28 (± 0.03), and 0.13 (± 0.02) at the Florida

site, Georgia site, and across sites, respectively. Type B total genetic correlation for Δ was 0.70 ± 0.06 , indicating that clonal rankings were relatively stable across sites, while for third-year height, rankings of clones were more unstable across the two trials ($\hat{r}_{BTG} = 0.55 \pm 0.08$). Third-year height and Δ were negatively correlated at the parental ($\hat{r}_{ADD} = -0.42 \pm 0.33$), full-sib family ($\hat{r}_{FS} = -0.54 \pm 0.25$), and clonal ($\hat{r}_{TG} = -0.30 \pm 0.11$) levels, suggesting that genetic variation for Δ in *P. taeda* may be a result of differences in photosynthetic capacity. We conclude that Δ may be a useful selection trait to improve water-use efficiency and for guiding deployment decisions in *P. taeda*.

Keywords Stable carbon isotope discrimination · Clones · *Pinus taeda*

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Introduction

Genetic variation in growth is ultimately dependent on genetic variation in some combination of underlying physiological and morphological traits. The possibility of shortening selection time in tree-breeding or clone-screening programs by using instantaneous measures of physiological processes and inferring long-term productivity potential from these measures has been considered for several decades (Cannell 1979; Burdon 1982; Dickmann 1991; Martin et al. 2005). Reducing the time required to evaluate progeny or clones would be of great benefit, especially for southern pine clonal programs where tissue maturation makes it difficult to vegetatively propagate any particular genotype for more than a few years.

Unfortunately, most physiological processes are difficult to measure quickly and so cannot be quantified in a timely fashion for large breeding or screening populations. Carbon

isotope discrimination measurements are an exception. This approach quantifies the ratio of ^{13}C to ^{12}C in foliage or other plant tissue relative to a geologic standard, expressed as $\delta^{13}\text{C}$ (carbon isotope composition) or as discrimination (Δ) which corrects for variations in source air $\delta^{13}\text{C}$ (Farquhar et al. 1989). The $\delta^{13}\text{C}$ of leaf tissue is a direct index of the ratio of leaf internal $[\text{CO}_2]$ to ambient $[\text{CO}_2]$, or c_i-c_a (Farquhar et al. 1989) during the time that leaf tissue is being formed. Physiologically, the c_i -to- c_a ratio, and in turn the $\delta^{13}\text{C}$ of foliage, is therefore determined by the time-integrated ratio of net photosynthesis rate to stomatal conductance. Further, if comparisons are made under identical evaporative demand, $\delta^{13}\text{C}$ can also be interpreted as an index of water-use efficiency, the ratio of carbon fixation to water loss (Farquhar et al. 1982). Foliar samples collected for $\delta^{13}\text{C}$ analysis must be standardized by crown position and age class, but otherwise sample collection and preparation is simple and rapid, enabling the analysis of many individuals over a short period of time, making this a potentially powerful approach for understanding genetic variation in gas exchange traits in large populations.

Several previous studies have reported genetic variation for stable carbon isotope discrimination in forest tree species, populations, families, and clones. For example, significant genetic variation for stable carbon isotope discrimination and composition has been reported in several conifers, including *Pseudotsuga menziesii* (Mirb.) Franco (Zhang et al. 1993; Aitken et al. 1995), *Picea* species and hybrids (Sun et al. 1996; Johnsen et al. 1999; Silim et al. 2001), *Larix occidentalis* Nutt. (Zhang et al. 1994), and *Pinus* species and hybrids (Olivas-Garcia et al. 2000; Brendel et al. 2002; Gebremedhin 2003; Prasolova et al. 2003; Emhart 2005). However, there have been relatively few studies designed to estimate heritability of stable carbon isotope discrimination in forest trees, and to our knowledge, this is the first reported study dealing with the

genetic architecture of stable carbon isotope discrimination using a complex pedigree in *Pinus taeda* L. (loblolly pine).

The objectives of this study were: (1) to quantify heritability of stable carbon isotope discrimination and the genetic correlation of discrimination with third-year growth, in a widely crossed *P. taeda* pedigree involving 32 parents; (2) to determine whether genetic parameters for growth and stable carbon isotope discrimination were stable across two sites in the lower coastal plain region of Florida and Georgia.

Materials and methods

Plant material and experimental design

The parental population consisted of 22 first-generation and ten second-generation selections from the larger Loblolly Pine Lower Gulf Elite Population which consists of selections (for growth) from all three southern pine tree improvement cooperatives: North Carolina State University–Industry Cooperative Tree Improvement Program, Western Gulf Forest Tree Improvement Program, and Cooperative Forest Genetics Research Program. These 32 loblolly pine parents were mated in a partial diallel design, and 1,200 clones from 61 full-sib families were established in field trials (Baltunis 2005; Baltunis et al. 2007). On average, each parent was involved in about four crosses.

The propagation of the clonal rooted cuttings has previously been described (Baltunis et al. 2005). In total, six field trials were established with rooted cuttings along with zygotic seedlings from the same 61 full-sib families (Baltunis et al. 2007). The current study used two of the trials containing 1,027 different clones; seedlings were not included in analyses. A total of 940 clones were established in a trial in Putnam County, FL, USA, while 871 clones were established in a trial in Randolph County, GA, USA (Table 1). Approximately 15 clones from each of 61 full-sib

Table 1 Details of two *P. taeda* clonal trials in Florida and Georgia

	Florida trial	Georgia trial
Location	Putnam County, FL, USA	Randolph County, GA, USA
Latitude	29° 38' 24" N	31° 47' 32" N
Longitude	81° 49' 27" W	84° 41' 32" W
Elevation	7 m	137 m
Soil series (taxonomic class)	Pomona series (sandy, siliceous, hyperthermic Ultic Alaquods)	Red bay series (fine-loamy, kaolinitic, thermic Rhodic Kandiodults)
Drainage	Poorly to very poorly drained	Well drained
Average annual rainfall	1,250 mm	1,340 mm
Average annual minimum–maximum temperatures	15.2°C min 27.8°C max	12.7°C min 25.2°C max
Mean stable carbon isotope discrimination	19.6‰	21.2‰
Mean third-year height	332 cm	518 cm

families were established as rooted cuttings at the two trials (Baltunis et al. 2007). Single-tree plots of a clone were established using a resolvable alpha incomplete block design (Williams et al. 2002) in eight replicates in each trial. At the Florida trial, third-year height and stable carbon isotope discrimination were measured on trees in four replicates. However, the four replicates in the Georgia trial that were measured for stable carbon isotope discrimination experienced herbicide damage midway through the growing season, and therefore, third-year height was measured on ramets of clones in the other four replicates. All eight replications at the Georgia trial had the same site prep and cultural treatments applied.

Measurements

Third-year height was measured at each of the trials after growth had ceased in winter 2005. For stable carbon isotope discrimination, approximately 300-g fresh weight foliage was sampled from three branches on the south side of each tree, upper 1/3 of the crown, from the most-recent, fully expanded flush. This was typically the second or third flush of foliage formed in the 2004 growing season. Foliage was collected over a 2-day period for each site in July 2004 at the Florida site and August 2004 at the Georgia site. Samples were placed in labeled paper bags and kept on ice until they could be placed in a drying oven at the end of each 2-day sampling period. Samples were kept in drying ovens at 60°C until grinding. Samples were finely ground in a coffee grinder and were placed in scintillation vials. The grinder was cleaned thoroughly between samples to avoid tissue contamination.

The relative abundance of ¹³C and ¹²C was determined in 3-mg subsamples with a Delta Plus isotope ratio mass spectrometer located at the Cornell University Stable Isotope Laboratory. Stable carbon isotope composition ($\delta^{13}\text{C}$) was expressed as ¹³C-to-¹²C ratio relative to international Pee Dee Belemnite standard (Craig 1954). Stable carbon isotope discrimination values (Δ) were calculated from $\delta^{13}\text{C}$ values using Eq. 1 (Farquhar et al. 1989), where δ_p is the stable carbon isotope composition of the plant material and δ_a is the stable carbon isotope composition of air (assumed to be -8‰):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \tag{1}$$

The accuracy and precision of the analysis for foliar $\delta^{13}\text{C}$ were ascertained by making random repeated measurements of $\delta^{13}\text{C}$ in each batch of the samples and using an internal foliar standard in each of the sample batches. We concluded that stable carbon isotope measurements are repeatable and accurate with a standard error of 0.14‰. In total, 6,383 foliar samples were analyzed for Δ .

Statistical analyses

Both single-site and pooled-site analyses were conducted for stable carbon isotope discrimination and third-year height using a parental model. All analyses were done using ASREML (Gilmour et al. 2005) and standard errors were calculated using the Taylor series expansion method for variance component and genetic parameter estimates in ASREML (Gilmour et al. 2005). For the pooled-site analysis, a bivariate parental linear mixed-effects model was used to obtain estimates of variance and covariance components for stable carbon isotope discrimination and third-year height:

$$y_i = X_i b_i + Z_{m_i} m_i + Z_{u_i} u_i + Z_{f_i} f_i + Z_{c_i} c_i + Z_{n_i} n_i + Z_{o_i} o_i + Z_{p_i} p_i + Z_{q_i} q_i + e_i, \tag{2}$$

where

- y_i is the vector of observations indexed (i) by Δ or third-year height (ht),
- b_i is the vector of fixed effects (i.e., mean, taxa, trials, and replicates within trials) and X_i is the known incidence matrix relating the observations in y_i to the fixed effects in b_i where $x_i b_i = \begin{bmatrix} X_{\Delta} & 0 \\ 0 & X_{ht} \end{bmatrix} \begin{bmatrix} b_{\Delta} \\ b_{ht} \end{bmatrix}$,
- m_i is the vector of random incomplete blocks nested within replicate and trial effects $\sim \text{MVN}(0, K \otimes I)$ where $K = \begin{bmatrix} \hat{\sigma}_{\text{INC}_{\Delta}}^2 & \hat{\sigma}_{\text{INC}_{\Delta ht}} \\ \hat{\sigma}_{\text{INC}_{\Delta ht}} & \hat{\sigma}_{\text{INC}_{ht}}^2 \end{bmatrix}$ and Δ and third-year height measurements at the Florida trial (Trial C) are correlated,
- u_i is the vector of random parent (female and male) general combining ability effects $\sim \text{MVN}(0, G \otimes A)$ where $G = \begin{bmatrix} \hat{\sigma}_{\text{GCA}_{\Delta}}^2 & \hat{\sigma}_{\text{GCA}_{\Delta ht}} \\ \hat{\sigma}_{\text{GCA}_{\Delta ht}} & \hat{\sigma}_{\text{GCA}_{ht}}^2 \end{bmatrix}$ and A =numerator relationship matrix,
- f_i is the vector of random specific combining ability effects $\sim \text{MVN}(0, S \otimes I_s)$ where $s = \begin{bmatrix} \hat{\sigma}_{\text{SCA}_{\Delta}}^2 & \hat{\sigma}_{\text{SCA}_{\Delta ht}} \\ \hat{\sigma}_{\text{SCA}_{\Delta ht}} & \hat{\sigma}_{\text{SCA}_{ht}}^2 \end{bmatrix}$ and I_s is an identity matrix of size equal to the number of full-sib families,
- c_i is the vector of random clones within full-sib family effects $\sim \text{MVN}(0, C \otimes I_c)$ where $C = \begin{bmatrix} \hat{\sigma}_{\text{CLONE}_{\Delta}}^2 & \hat{\sigma}_{\text{CLONE}_{\Delta ht}} \\ \hat{\sigma}_{\text{CLONE}_{\Delta ht}} & \hat{\sigma}_{\text{CLONE}_{ht}}^2 \end{bmatrix}$ and I_c is an identity matrix of size equal to the number of clones,
- n_i is the vector of random trial by parent (female and male) effects $\sim \text{MVN}(0, L \otimes I)$, where $L = \begin{bmatrix} \hat{\sigma}_{\text{TxGCA}_{\Delta}}^2 & \hat{\sigma}_{\text{TxGCA}_{\Delta ht}} \\ \hat{\sigma}_{\text{TxGCA}_{\Delta ht}} & \hat{\sigma}_{\text{TxGCA}_{ht}}^2 \end{bmatrix}$,
- o_i is the vector of random trial by full-sib family effects $\sim \text{MVN}(0, M \otimes I)$ where $M = \begin{bmatrix} \hat{\sigma}_{\text{TxFAM}_{\Delta}}^2 & \hat{\sigma}_{\text{TxFAM}_{\Delta ht}} \\ \hat{\sigma}_{\text{TxFAM}_{\Delta ht}} & \hat{\sigma}_{\text{TxFAM}_{ht}}^2 \end{bmatrix}$,
- p_i is the vector of random trial by clone within full-sib family effects $\sim \text{MVN}(0, N \otimes I)$ where $N = \begin{bmatrix} \hat{\sigma}_{\text{TxCLONE}_{\Delta}}^2 & \hat{\sigma}_{\text{TxCLONE}_{\Delta ht}} \\ \hat{\sigma}_{\text{TxCLONE}_{\Delta ht}} & \hat{\sigma}_{\text{TxCLONE}_{ht}}^2 \end{bmatrix}$,
- q_i is the vector of random replicate within trial by full-sib family effects $\sim \text{MVN}\left(0, \begin{bmatrix} I_{\Delta} \hat{\sigma}_{\text{REPFAM}_{\Delta}}^2 & 0 \\ 0 & I_{ht} \hat{\sigma}_{\text{REPFAM}_{ht}}^2 \end{bmatrix}\right)$,

\mathbf{e}_i is the random vector of residual terms \sim $MVN(\mathbf{0}, \mathbf{R} \otimes \mathbf{I})$ where

residuals were assumed to be heterogeneous across sites, $\mathbf{R} = \begin{bmatrix} \hat{\sigma}_{\text{ERROR}_{\Delta}}^2 & \hat{\sigma}_{\text{ERROR}_{\Delta\text{ht}}}^2 \\ \hat{\sigma}_{\text{ERROR}_{\Delta\text{ht}}}^2 & \hat{\sigma}_{\text{ERROR}_{\text{ht}}}^2 \end{bmatrix}$, $\mathbf{0}$ is the null matrix, \mathbf{I}_i is the identity matrix of dimension equal to the number of observations for Δ or third-year height, and in the case of measurements at the Florida trial (trial C) there is a covariance among residuals ($\hat{\sigma}_{\text{ERROR}_{\Delta\text{ht}}}^2$), \mathbf{Z}_{m_i} , \mathbf{Z}_{u_i} , \mathbf{Z}_{f_i} , \mathbf{Z}_{c_i} , \mathbf{Z}_{n_i} , \mathbf{Z}_{o_i} , \mathbf{Z}_{p_i} , and \mathbf{Z}_{q_i} are the known incidence matrices relating the observations in \mathbf{y}_i to effects in \mathbf{m}_i , \mathbf{u}_i , \mathbf{f}_i , \mathbf{c}_i , \mathbf{n}_i , \mathbf{o}_i , \mathbf{p}_i , and \mathbf{q}_i , respectively.

Causal components of variance, heritabilities, and genetic correlations

Additive (\hat{V}_{A_i}), dominance (\hat{V}_{D_i}), epistasis (\hat{V}_{I_i}), total genetic (\hat{V}_{G_i}), and phenotypic (\hat{V}_{P_i}) variances were estimated for Δ and third-year height according to Foster and Shaw (1988). Individual-tree narrow-sense and broad-sense heritabilities were estimated for stable carbon isotope discrimination and third-year height based on the genetic parameter estimates from single-site and pooled-site analyses:

$$\hat{h}_i^2 = \frac{\hat{V}_{A_i}}{\hat{V}_{P_i}} \approx \frac{4\hat{\sigma}_{\text{GCA}_i}^2}{2\hat{\sigma}_{\text{GCA}_i}^2 + \hat{\sigma}_{\text{SCA}_i}^2 + \hat{\sigma}_{\text{CLONE}_i}^2 + 2\hat{\sigma}_{\text{TxGCA}_i}^2 + \hat{\sigma}_{\text{TxFAM}_i}^2 + \hat{\sigma}_{\text{TxCLONE}_i}^2 + \hat{\sigma}_{\text{REPxFAM}_i}^2 + \hat{\sigma}_{\text{ERROR}_i}^2} \tag{3}$$

is the estimated individual-tree narrow-sense heritability from the pooled-site analysis.

$$\hat{H}_i^2 = \frac{\hat{V}_{G_i}}{\hat{V}_{P_i}} \approx \frac{2\hat{\sigma}_{\text{GCA}_i}^2 + \hat{\sigma}_{\text{SCA}_i}^2 + \hat{\sigma}_{\text{CLONE}_i}^2}{2\hat{\sigma}_{\text{GCA}_i}^2 + \hat{\sigma}_{\text{SCA}_i}^2 + \hat{\sigma}_{\text{CLONE}_i}^2 + 2\hat{\sigma}_{\text{TxGCA}_i}^2 + \hat{\sigma}_{\text{TxFAM}_i}^2 + \hat{\sigma}_{\text{TxCLONE}_i}^2 + \hat{\sigma}_{\text{REPxFAM}_i}^2 + \hat{\sigma}_{\text{ERROR}_i}^2} \tag{4}$$

is the estimated individual-tree broad-sense heritability from the pooled-site analysis. Heritability estimates from single-site analyses are the same except for dropping $2\hat{\sigma}_{\text{TxGCA}_i}^2$, $\hat{\sigma}_{\text{TxFAM}_i}^2$, and $\hat{\sigma}_{\text{TxCLONE}_i}^2$ from the phenotypic variance in the denominator.

The extent of genotype by environment interaction was investigated for stable carbon isotope discrimination and third-year height. Type B genetic correlations were calculated for additive effects, full-sib family effects, and the total genetic or clonal values across the trials (Yamada 1962; Burdon 1977);

$$\hat{r}_{B_{\text{ADD}_i}} = \frac{\hat{\sigma}_{\text{GCA}_i}^2}{\hat{\sigma}_{\text{GCA}_i}^2 + \hat{\sigma}_{\text{TxGCA}_i}^2} \tag{5}$$

is the type B genetic correlation for additive effects across trials.

$$\hat{r}_{B_{\text{FS}_i}} = \frac{2\hat{\sigma}_{\text{GCA}_i}^2 + \hat{\sigma}_{\text{SCA}_i}^2}{2\hat{\sigma}_{\text{GCA}_i}^2 + 2\hat{\sigma}_{\text{TxGCA}_i}^2 + \hat{\sigma}_{\text{SCA}_i}^2 + \hat{\sigma}_{\text{TxFAM}_i}^2} \tag{6}$$

is the type B genetic correlation for full-sib families across trials.

$$\hat{r}_{B_{\text{TG}_i}} = \frac{2\hat{\sigma}_{\text{GCA}_i}^2 + \hat{\sigma}_{\text{SCA}_i}^2 + \hat{\sigma}_{\text{CLONE}_i}^2}{2\hat{\sigma}_{\text{GCA}_i}^2 + 2\hat{\sigma}_{\text{TxGCA}_i}^2 + \hat{\sigma}_{\text{SCA}_i}^2 + \hat{\sigma}_{\text{TxFAM}_i}^2 + \hat{\sigma}_{\text{CLONE}_i}^2 + \hat{\sigma}_{\text{TxCLONE}_i}^2} \tag{7}$$

is the type B genetic correlation for total genetic or clonal values across trials.

Ranging from 0 to 1, a value of $\hat{r}_{B_{\text{ADD}}}$ near 1 indicates little genotype by environment interaction and that the parents ranked the same across the trials, while a low $\hat{r}_{B_{\text{ADD}}}$ (near 0) indicates that parental ranks were not stable across the sites and hence, genotype by environment interaction exists. A high $\hat{r}_{B_{\text{FS}}}$ indicates that full-sib families ranked similarly across the sites, while a high $\hat{r}_{B_{\text{TG}}}$ indicates that the ranking of total genetic values of the clones were stable across the trials.

Genetic correlations between stable carbon isotope discrimination and third-year height were estimated for additive effects, full-sib family effects, and the total genetic values of the clones using the estimated genetic variances and covariances from the bivariate, pooled-site analysis:

$$\hat{r}_{\text{ADD}} = \frac{\hat{\sigma}_{\text{GCA}_{\Delta\text{ht}}}}{\sqrt{\hat{\sigma}_{\text{GCA}_{\Delta}}^2 \hat{\sigma}_{\text{GCA}_{\text{ht}}}^2}} \tag{8}$$

is the genetic correlation between Δ and third-year height for additive effects, and $\hat{\sigma}_{\text{GCA}_{\Delta\text{ht}}}$ is the covariance between general combining ability effects for Δ and third-year height.

$$\hat{r}_{\text{FS}} = \frac{2\hat{\sigma}_{\text{GCA}_{\Delta\text{ht}}} + \hat{\sigma}_{\text{SCA}_{\Delta\text{ht}}}}{\sqrt{(2\hat{\sigma}_{\text{GCA}_{\Delta}}^2 + \hat{\sigma}_{\text{SCA}_{\Delta}}^2)(2\hat{\sigma}_{\text{GCA}_{\text{ht}}}^2 + \hat{\sigma}_{\text{SCA}_{\text{ht}}}^2)}} \tag{9}$$

is the genetic correlation between Δ and third-year height for full-sib families, and $2\hat{\sigma}_{GCA_{\Delta ht}} + \hat{\sigma}_{SCA_{\Delta ht}}$ is the covariance between the full-sib family effects for Δ and third-year height.

$$\hat{r}_{TG} = \frac{2\hat{\sigma}_{GCA_{\Delta ht}} + \hat{\sigma}_{SCA_{\Delta ht}} + \hat{\sigma}_{CLONE_{\Delta ht}}}{\sqrt{(2\hat{\sigma}_{GCA_{\Delta}}^2 + \hat{\sigma}_{SCA_{\Delta}}^2 + \hat{\sigma}_{CLONE_{\Delta}}^2)(2\hat{\sigma}_{GCA_{ht}}^2 + \hat{\sigma}_{SCA_{ht}}^2 + \hat{\sigma}_{CLONE_{ht}}^2)}} \quad (10)$$

is the genetic correlation between Δ and third-year height for the total genetic value of clones, and $2\hat{\sigma}_{GCA_{\Delta ht}} + \hat{\sigma}_{SCA_{\Delta ht}} + \hat{\sigma}_{CLONE_{\Delta ht}}$ is the covariance between the total clonal value effects for Δ and third-year height.

Results and discussion

Trial means for Δ and third-year height

Mean third-year height and stable carbon isotope discrimination from the two trials were 425.3 cm and 20.4‰, respectively. However, the Florida and Georgia sites were very different from each other in their soils, drainage, and productivity (Table 1). This is reflected in the greater growth observed at the Georgia site (Table 1), where mean third-year height was 520 cm *versus* 330 cm at the Florida site. Trees at the Florida site were showing micronutrient deficiency symptoms during the growing season, and consequently boron was applied midway through the growing season. Additionally, the Florida site was in the path of two hurricanes. The combination of saturated soils and high winds resulted in many leaning trees. Stable carbon isotope discrimination was also higher at the Georgia site. Mean discrimination at the Georgia and Florida sites were 21.2‰ and 19.6‰, respectively (Table 1). Although the Florida site receives less annual rainfall on average, it is the wetter of the two sites because of the drainage properties of the soil, and standing water was observed at the time of foliage collection. However, water probably was not limiting growth at either of the two sites.

Genetic components of variance

The estimates of total genetic variance for stable carbon isotope discrimination ($\hat{V}_{G_{\Delta}}$) were similar at both the Florida and Georgia trials: $\hat{V}_{G_{\Delta}} = 0.1001(\pm 0.01)$ at the Florida trial and $\hat{V}_{G_{\Delta}} = 0.1151(\pm 0.01)$ at the Georgia trial. Additionally, the majority of $\hat{V}_{G_{\Delta}}$ at these trials was a result of additive genetic variance (Fig. 1). $\hat{V}_{A_{\Delta}}$ was 0.0563 (± 0.02) and 0.0695 (± 0.02) at the Florida and Georgia trials, respectively. However, the estimate of nonadditive genetic variance partitioned differently at the two trials. For

example, all of the nonadditive genetic variance at the Florida trial was epistatic genetic variance ($\hat{V}_{I_{\Delta}} = 0.0438 \pm 0.01$), while at the Georgia trial, the majority of the nonadditive genetic variance was a result of dominance genetic variance ($\hat{V}_{D_{\Delta}} = 0.0318(\pm 0.02)$ versus $\hat{V}_{I_{\Delta}} = 0.0137(\pm 0.02)$). A slightly different partitioning of the total genetic variance was observed from the pooled-site analysis for stable carbon isotope discrimination with a slightly greater nonadditive to additive genetic variance ratio primarily due to epistatic genetic variance. $\hat{V}_{G_{\Delta}}$, $\hat{V}_{A_{\Delta}}$, $\hat{V}_{D_{\Delta}}$, and $\hat{V}_{I_{\Delta}}$ were 0.0719 (± 0.01), 0.0339 (± 0.02), 0.0019 (± 0.01), and 0.0362 (± 0.01), respectively.

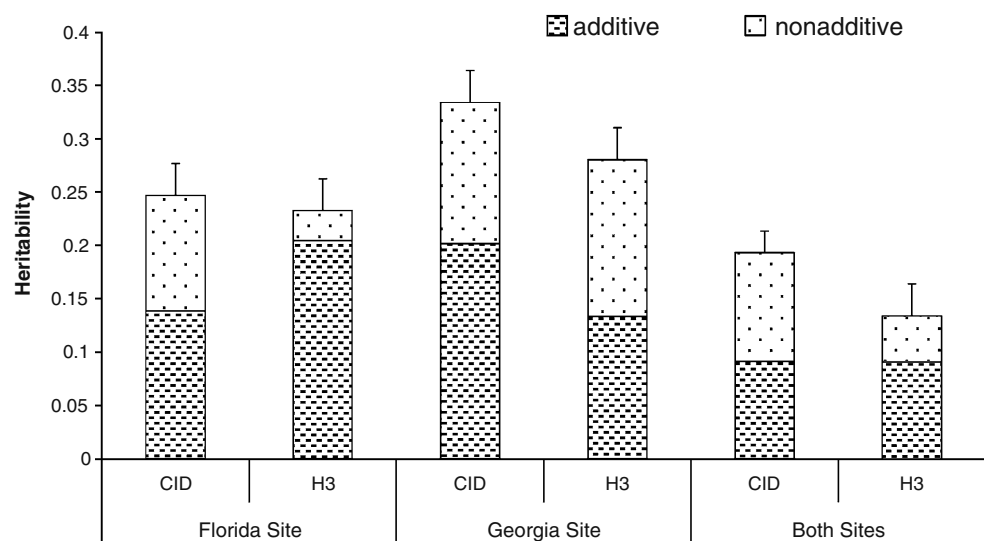
For third-year height, $\hat{V}_{G_{ht}}$ was 1,147.0 (± 186.0) and 984.4 (± 115.5) at the Florida and Georgia trials, respectively. The majority of the genetic variance for third-year height at the Florida trial was additive ($\hat{V}_{A_{ht}} = 1009.0 \pm 344.7$) and the estimate of epistatic genetic variance was negative, similar to that reported for earlier growth measurements (Baltunis et al. 2007). At the Georgia trial, there was a somewhat higher ratio of nonadditive to additive genetic variance for third-year height. The estimate of $\hat{V}_{A_{ht}}$ was 469.0 (± 199.5), while $\hat{V}_{D_{ht}}$ and $\hat{V}_{I_{ht}}$ were 290.9 (± 190.5) and 224.5 (± 167.3), respectively. The pooled-site estimates of genetic variance components followed a similar pattern to that of the Florida trial. The estimate of $\hat{V}_{G_{ht}}$ was 559.8 (± 114.9) with more than twice as much additive genetic variance compared with nonadditive genetic variance. Additionally, the estimate of $\hat{V}_{I_{ht}}$ for third-year height was negative in the pooled-site analysis.

Heritability estimates

Both stable carbon isotope discrimination and third-year height had low heritabilities in this population. Narrow-sense heritability (\pm standard error) for stable carbon isotope discrimination ranged from 0.14 (± 0.03) at the Florida site to 0.20 (± 0.07) at the Georgia site (Fig. 1). The across-site estimate of \hat{h}^2 was 0.09 (± 0.04), indicating the presence of genotype by environment interaction at the additive genetic level. Broad-sense heritability for stable carbon isotope discrimination ranged from 0.25 (± 0.03) at the Florida site to 0.33 (± 0.03) at the Georgia site, while the across-site estimate of \hat{H}^2 was 0.19 (± 0.02 ; Fig. 1). Narrow-sense heritability for third-year height ranged from 0.13 (± 0.05) at the Georgia site to 0.20 (± 0.06) at the Florida site with an across-site estimate of 0.09 (± 0.05 ; Fig. 1). Broad-sense heritability estimates for third-year height were 0.23 (± 0.03), 0.28 (± 0.03), and 0.13 (± 0.02) at the Florida site, Georgia site, and across sites, respectively (Fig. 1).

These heritability estimates for stable carbon isotope discrimination were at the low end of the range of heritability estimates reported in other tree species (Table 2) and much

Fig. 1 Broad-sense heritability estimates partitioned into additive ($=\hat{h}^2$) and nonadditive components for stable carbon isotope discrimination (Δ) and third-year height ($H3$) at two loblolly pine clonal trials growing on contrasting sites in Florida and Georgia. Standard error bars for broad-sense heritability estimates are shown



lower than those reported for annual crops (e.g., Condon and Richards 1992; Zacharisen et al. 1999; Rebetzke et al. 2006). For example, Johnsen et al. (1999) reported \hat{h}^2 for stable carbon isotope discrimination as 0.54 (± 0.26) for a 7×7 diallel of black spruce (*Picea mariana* (P. Mill.) B.S. B.). Estimates of narrow-sense heritability for stable carbon isotope composition in hoop pine (*Araucaria cunninghamii* Ait. ex D. Don) in Australia ranged from 0.4 to 0.72 (Prasolova et al. 2000, 2001). Additionally, in a clonal study of hybrid poplar clones (Monclus et al. 2005), broad-sense heritability for stable carbon isotope discrimination was 0.71.

Inheritance of stable carbon isotope discrimination in *Pinus* appears to be lower than for other genera (Table 2). Brendel et al. (2002) reported significant narrow-sense heritability for stable carbon isotope composition in *Pinus pinaster* Ait. with an estimate of 0.17 (± 0.06). Similarly, Prasolova et al. (2003) reported narrow-sense heritability estimates as 0.09 from winter foliage samples and 0.15 from summer foliage samples for stable carbon isotope composition in clones of F_1 hybrids between *Pinus elliotii* Engelm var. *elliotii* and *Pinus caribaea* var. *hondurensis* Barr. et Golf. In a clonal study of *P. elliotii*, within-family broad-sense heritability for stable carbon isotope discrim-

Table 2 Comparison of published heritability estimates for stable carbon isotope discrimination (or composition) in trees

Species	Population structure	Heritability	Reference
<i>Araucaria cunninghamii</i>	23 OP families	$h^2 > 0.4$ depending on canopy position	Prasolova et al. 2000
<i>Araucaria cunninghamii</i>	22 OP families (field trial)	$h^2 = 0.72$ (9-year-old field trial)	Prasolova et al. 2001
	26 OP families (greenhouse trial)	$h^2 = 0.66$ (10-month-old seedlings in greenhouse)	
<i>Castanea sativa</i>	48 OP families (eight families from six populations)	$h^2 = 0.31$	Lauteri et al. 2004
<i>Picea mariana</i>	7×7 diallel (seven parents)	$h^2 = 0.54$	Johnsen et al. 1999
<i>Pinus elliotii</i>	60 clones from four FS families	$H_{WF}^2 = 0.2$ (within-family broad-sense heritability pooled across all families)	Emhart 2005
<i>Pinus elliotii</i> x <i>Pinus caribaea</i> var. <i>hondurensis</i>	122 clones	$h^2 = 0.15$ (summer)	Prasolova et al. 2003
		$h^2 = 0.09$ (winter)	
<i>Pinus pinaster</i>	12 x 12 half diallel 12 parents 63 full-sib families	$h^2 = 0.17$	Brendel et al. 2002
<i>Pinus taeda</i>	30 clones	$H^2 = 0.07$	Gebremedhin 2003
<i>Pinus taeda</i>	60 clones from one FS family	$H_{WF}^2 = 0.23$ (in 2001) $H_{WF}^2 = 0.17$ (in 2003)	Emhart 2005
<i>Pinus taeda</i>	32 parents 61 full-sib families 1,027 clones	$h^2 = 0.09$ $H^2 = 0.19$	This study
<i>Populus deltoides</i> x <i>Populus nigra</i>	29 clones	$H^2 = 0.71$	Monclus et al. 2005

ination was 0.2 (pooled across four full-sib families) and 0.18 (averaged across four full-sib families) in two separate years of sampling (Emhart 2005). There have been two previous reports of heritability estimates for stable carbon isotope discrimination (or composition) in *P. taeda* based on estimates from a small number of genotypes. In a greenhouse study, Gebremedhin (2003) reported broad-sense heritability for stable carbon isotope composition from 30 clones as very low (0.07), while Emhart (2005) reported that within-family broad-sense heritability for one full-sib family of *P. taeda*, was 0.23 and 0.17 in 2001 and 2003, respectively.

Genotype by environment interaction

Estimates of genetic variation and heritability based on observations from a single site can be upwardly biased if genotype by environment interaction exists for a trait (Comstock and Moll 1963). The pooled-site estimates of heritability for both stable carbon isotope discrimination and third-year height were lower than the estimates based on single-site analyses, indicating the presence of genotype by environment interaction. Significant type B genetic correlations were observed across sites for additive, full-sib family, and total genetic effects for Δ and third-year height (Table 3). There appears to be some genotype by environment interaction for additive effects and full-sib family effects for stable carbon isotope discrimination, indicating rank changes of the 32 parents and 61 full-sib families based on genetic predictions, while relatively lower genotype by environment interaction was observed for full-sib family effects for third-year height as indicated by type B genetic correlations (Table 3). However, moderately high type B total genetic correlations were found, indicating that the ranking of clones were similar across the two sites for stable carbon isotope discrimination, while for third-year height, ranking of clones was more unstable across the two

trials compared to parental and full-sib family rankings (Table 3, Fig. 2).

Generally, little genotype by environment interaction for stable carbon isotope discrimination has been found in other trees and crops. For example, several previous studies have looked at the effects of varying levels of water stress on stable carbon isotope discrimination (or composition). In *Picea glauca* (Moench) Voss, ranking of genotypes were stable for stable carbon isotope composition in seedlings grown under irrigated and dry treatments with no significant genotype by environment interaction (Sun et al. 1996). Olivás-García et al. (2000) reported no seed source by watering treatment interaction for *Pinus ponderosa* Dougl. ex Laws seedlings subjected to either well-watered or water-stressed treatments. Johnsen et al. (1999) reported no evidence of genotype by site interaction for stable carbon isotope discrimination in a 7x7 diallel of *P. mariana* planted on three sites. However, significant genotype by environment interaction for stable carbon isotope discrimination was observed in mature *P. ponderosa* which was attributed to variation in growth phenology among the seed sources (Cregg et al. 2000).

There have been only two previous reports dealing with genotype by environment interaction for stable carbon isotope discrimination (or composition) in loblolly pine, and both of these studies have utilized a limited number of genotypes. Gebremedhin (2003) reported insignificant clone by watering treatment interaction for stable carbon isotope composition for 30 clones of loblolly pine in a greenhouse study. Whereas, Stover (2005) identified a significant clone by nitrogen treatment interaction for stable carbon isotope composition in nine loblolly pine clones. The level of inference may be limited due to the small number of genotypes in these two previous studies; nevertheless, perhaps the genotype by environment interaction observed in the current study was a result of nutrient limitations as opposed to water limitations at the Florida trial. On the other hand, nutrient availability had no significant effect on Δ in *Pinus radiata* D. Don (Korol et al. 1999). Even though significant genotype by environment interaction was identified for stable carbon isotope discrimination and third-year height in the current study, stable genotypes that can be identified perform well on these two contrasting sites (Fig. 2).

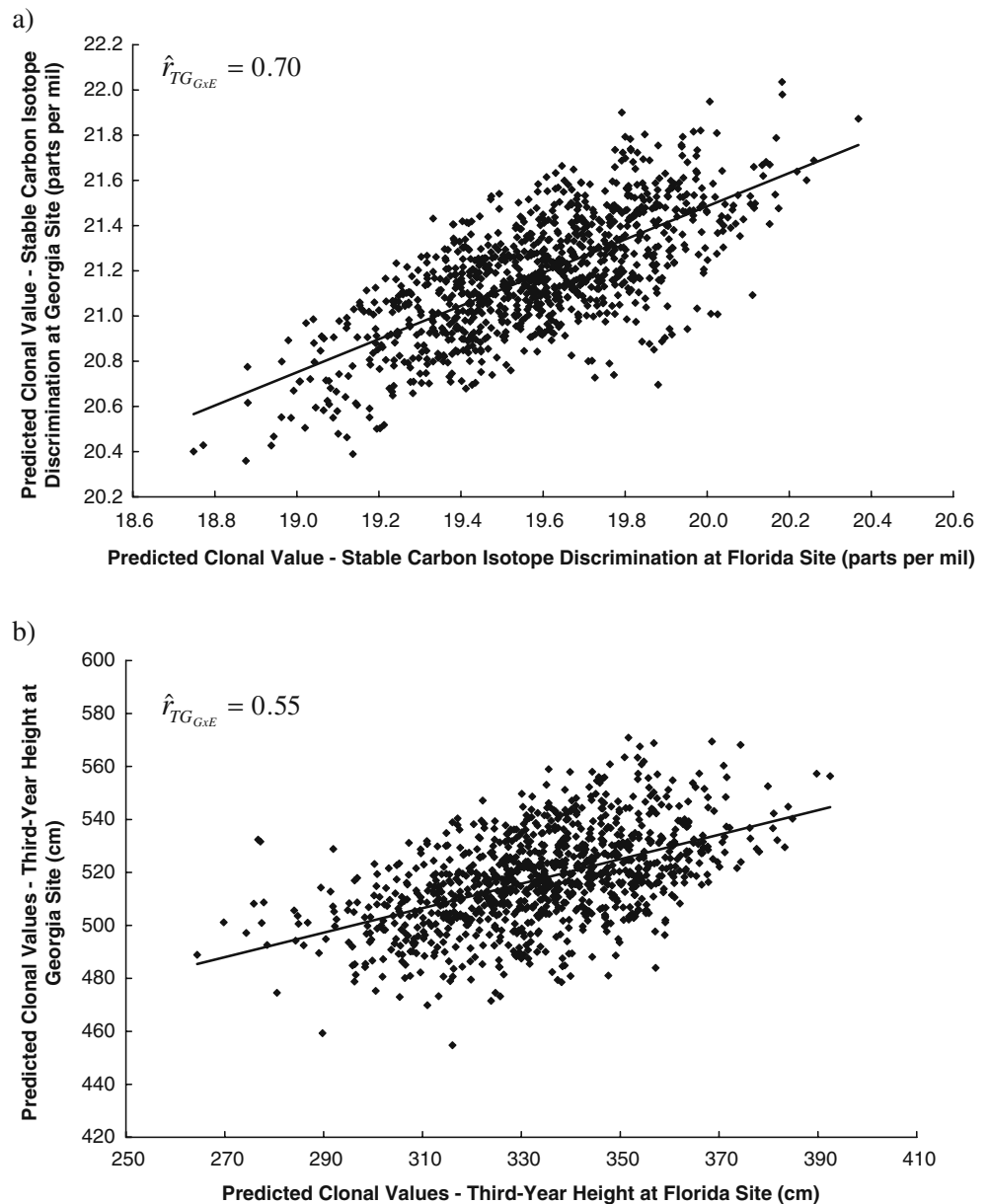
Genetic correlations between Δ and third-year height

Third-year height and stable carbon isotope discrimination were negatively correlated at the parental (-0.42 ± 0.33), full-sib family (-0.54 ± 0.25), and clonal (-0.30 ± 0.11) levels (Table 3, Fig. 3). Genetic variation in stable carbon isotope discrimination can be the result of either differences in stomatal conductance or photosynthetic capacity

Table 3 Type B genetic correlations as indicators for genotype by environment interaction for stable carbon isotope discrimination and third-year height and genetic correlations between stable carbon isotope discrimination and third-year height

	Stable carbon isotope discrimination	Third-year height
Genotype by environment interaction		
$\hat{r}_{B_{ADD}}$	0.64 (± 0.18)	0.62 (± 0.18)
$\hat{r}_{B_{FS}}$	0.56 (± 0.16)	0.71 (± 0.13)
$\hat{r}_{B_{TG}}$	0.70 (± 0.06)	0.55 (± 0.08)
Genetic correlation between traits		
\hat{r}_{ADD}	-0.42 (± 0.33)	
\hat{r}_{FS}	-0.54 (± 0.25)	
\hat{r}_{TG}	-0.30 (± 0.11)	

Fig. 2 Predicted total genetic values of clones at Florida site versus Georgia site depicting stability of clones for **a** stable carbon isotope discrimination **b** third-year height

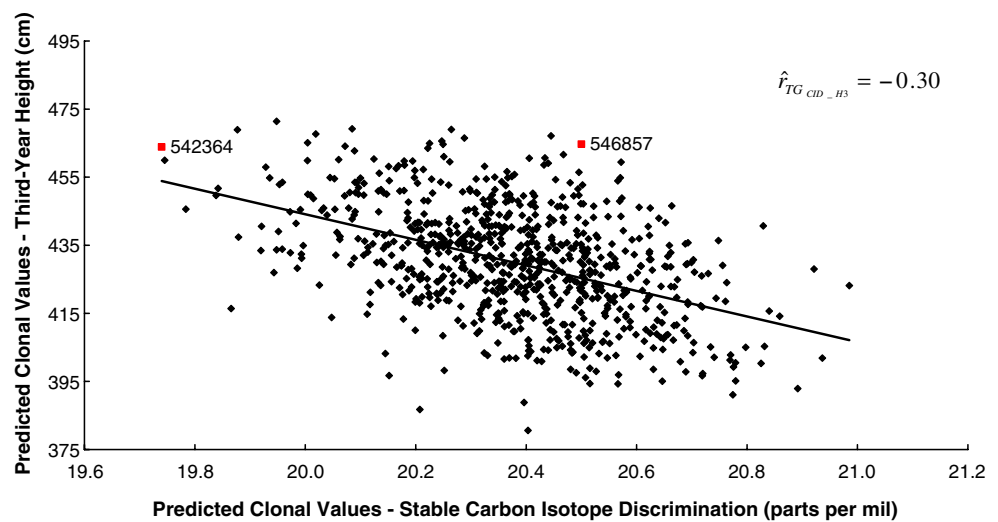


(Farquhar et al. 1989), and both have been identified as causes of variation in stable carbon isotope discrimination in forest trees. For example, differences in stomatal conductance have been implicated as the cause of genetic variation in stable carbon isotope discrimination (or composition) in *P. glauca* \times *Picea engelmannii* Parry ex Engelm. (Grossnickle and Fan 1998), *P. ponderosa* (Olivas-Garcia et al. 2000), and *P. menziesii* (Aitken et al. 1995). In contrast, differences in photosynthetic capacity have been identified as causes of genetic variation in stable carbon isotope discrimination (or composition) in 7-year-old clones of F₁ hybrids between *P. elliotii* var. *elliotii* and *P. caribaea* var. *hondurensis* (Xu et al. 2000), *P. glauca* (Sun et al. 1996), *P. mariana* (Johnsen et al. 1999),

Eucalyptus globulus Labill. (Pita et al. 2001), and in *Populus* \times *euramericana* Dode clones (Voltas et al. 2006).

The negative genetic correlation observed in the current study between stable carbon isotope discrimination and third-year height might suggest that genetic variation in stable carbon isotope discrimination in *P. taeda* may be largely the result of differences in photosynthetic capacity, as opposed to differences in stomatal conductance among genotypes that typically lead to positive correlations between discrimination and growth. Significant negative genetic correlations between growth and Δ have been reported in previous studies. Johnsen et al. (1999) reported that height and Δ in 22-year-old *P. mariana* had a genetic correlation of -0.97 . Similarly, Xu et al. (2000) reported a

Fig. 3 Predicted clonal values for stable carbon isotope discrimination *versus* third-year height from loblolly pine clonal trials growing on two sites in Florida and Georgia



genetic correlation of -0.96 in hybrid pine clones, and Voltas et al. (2006) reported a genetic correlation between stem volume and Δ of -0.60 for *Populus x euroamericana* hybrid clones. The genetic correlations between Δ and various above- and below-ground growth traits in *Castanea sativa* Mill. were generally strong and negative (Lauteri et al. 2004). However, insignificant genetic correlations between stable carbon isotope discrimination (or composition) and growth have been reported. For example, Brendel et al. (2002) reported a nonsignificant genetic correlation between ring width and stable carbon isotope composition in *P. pinaster*. Similarly, there was no significant genetic correlation between above-ground biomass and Δ in *Populus deltoides x Populus nigra* hybrid clones (Monclus et al. 2005). Additionally, Cregg et al. (2000) reported that negative associations between stable carbon isotope discrimination and growth in mature *P. ponderosa* have been found related to stomatal conductance rather than photosynthetic rate.

It is tempting to speculate that dramatic differences in photosynthetic capacity would explain clonal growth variation in *P. taeda*; however, based on smaller-scale gas exchange studies in southern pines, this may be incorrect. For years, physiological geneticists have attempted to detect genetic variation in photosynthesis rate or other gas exchange parameters for southern pine families, often with the intent of understanding the physiological mechanisms responsible for growth differences among families (or other taxa levels). In general, these studies have found little or no genetic variation among taxa in net photosynthesis capacity or rate but instead have found that genetic differences in productivity can be attributed primarily to variation in radiation interception potential as determined by leaf area and other crown structural traits (Boltz et al. 1986; Samuelson 2000; McGarvey et al. 2004; Stover 2005;

Emhart et al. 2007). The determinants of photosynthetic capacity are likely to be diverse and may occur at multiple scales (i.e., molecular, organ, whole-tree levels).

Genetic mechanisms that underlie complex physiological traits

One important research goal for this experimental plant population is to accumulate phenotypic information for use in genetic association studies (Neale and Savolainen 2004). In association studies, molecular genotypic data are associated with phenotypic variation in order to identify the genes (and alleles) that control traits of interest. Carbon isotope discrimination should be amenable to association analysis in loblolly pine given that it is heritable, with one-quarter to one-third of the phenotypic variation being due to genetic factors. While genetic correlations for carbon isotope discrimination were moderately high across the two sites, the contrasting architecture of the nonadditive genetic variance in Florida and Georgia (due to epistasis and dominance, respectively) suggests potential interaction of distinct gene systems with a common core of main effect loci that could function at both sites. Association analysis should generate new insights into the nature and diversity of genetic mechanisms that govern photosynthetic capacity and stomatal conductance in conifers and lead to new approaches to understand how the environment influences the expression of these traits to varying degrees in naturally selected tree populations.

Conclusions

Although we found that foliar stable carbon isotope discrimination in *P. taeda* had a low heritability, it still

may be useful as a selection trait in breeding programs to improve water-use efficiency, for guiding deployment decisions, to identify genotypes with contrasting growing strategies for further studies, and for elucidating the underlying mechanisms of complex physiological traits. Previous studies have reported on the usefulness of Δ as a trait in breeding programs in trees. For example, Zhang et al. (1996) suggested that stable carbon isotope discrimination could be used as a marker for breeding programs to improve growth of *P. menziesii* and *L. occidentalis*. Similarly, Sun et al. (1996) concluded that it would be possible to use stable carbon isotope composition as a selection trait for water-use efficiency and select *P. glauca* genotypes for high water-use efficiency without compromising yield. In addition, Δ has been recommended for genetic selection of drought-tolerant sugar beet varieties (*Beta vulgaris* sp.; Rytter 2005), Kentucky bluegrass (Ebdon and Kopp 2004), and wheat (*Triticum aestivum* L.; Rebetzke et al. 2002).

Stable carbon isotope discrimination may also be useful in making deployment decisions. Given a certain amount of growth, genotypes can be identified that have greater water-use efficiency by selecting clones that discriminate less. For example, Fig. 3 shows the genetic value of clones for Δ versus third-year height. Clones 542364 and 546857 are two of the highest-ranking clones in the population for third-year height with similar predicted third-year growth potentials. However, clone 542364 discriminates less than clone 546857 against the heavier carbon isotope and, therefore, should be more water-use efficient than clone 546857. This may be relevant for making deployment decisions because the more water-use efficient clones could be more productive on water-limited sites. Greater water-use efficiency is not equivalent to drought resistance, however, so further testing of these contrasting clones would be warranted. This will be possible as the stands develop because they are being maintained as living laboratories for accumulation of phenotypic information to be used in genetic association studies (Gonzalez-Martinez et al. 2006).

Stable carbon isotope discrimination data may serve as a useful screening method for choosing genotypes for detailed physiological studies. For example, clones with nearly identical growth potential, but a wide range of predicted Δ (Fig. 3), could be chosen for study to determine how variation in photosynthetic capacity and stomatal conductance influence Δ in this population, and whether variation in other traits (such as crown or root architecture, or biochemical efficiency of carbon fixation pathways) serve “compensatory” roles in producing similar growth rates among genotypes. Alternatively, selecting genotypes with a certain level of stable carbon isotope discrimination will produce a broad range in the growth potential of the selected clones. Fifty-nine clones had a

predicted genetic value approximately equivalent to the population mean stable carbon isotope discrimination (20.4‰; Fig. 3). Selection of clones at the tails of this distribution could provide genotypes with contrasting growing strategies for future physiological studies.

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