

# Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in *Picea mariana*: analyses from a half-diallel mating design using field-grown trees

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**Abstract:** We performed genetic analyses of growth, carbon isotope discrimination ( $\Delta^{13}\text{C}$ ), and foliar N concentration using a half-diallel subset of a  $7 \times 7$  complete diallel planted on three sites ranging in water availability. Trees were 22 years old. Heritabilities; general and specific combining abilities; as well as phenotypic, genetic, and environmental correlations were calculated using the best linear unbiased prediction and restricted maximum-likelihood methods. The four traits measured showed variable levels of genetic control. The calculated heritabilities for the traits were as follows: height,  $0.39 \pm 0.22$  (estimate  $\pm$  SD); diameter,  $0.14 \pm 0.10$ ;  $\Delta^{13}\text{C}$  value,  $0.54 \pm 0.26$ ; and foliar N, 0.00. Phenotypic correlations were moderate ( $r = -0.35$ ), genetic correlations were strong ( $r = -0.97$ ), and environmental correlations were weak ( $r = -0.18$ ) between height growth and  $\Delta^{13}\text{C}$ . The strong negative genetic correlation between  $\Delta^{13}\text{C}$  and growth supports earlier work with a subset of families indicating photosynthetic differences caused genetic variation in  $\Delta^{13}\text{C}$ . Inbreeding greatly decreased growth while not impacting  $\Delta^{13}\text{C}$ . High heritability, lack of inbreeding depression, and low environmental correlations indicate that a major proportion of  $\Delta^{13}\text{C}$  genetic control may be relatively simple. Because  $\Delta^{13}\text{C}$  is highly heritable, highly genetically correlated to growth, less environmentally sensitive than growth, and has the possibility of early selection, the trait is a good candidate trait for indirect selection for growth.

**Résumé :** Les auteurs ont réalisé des analyses de la **variabilité génétique** se rapportant à la croissance, à la variation en carbone isotopique ( $\Delta^{13}\text{C}$ ) et à la concentration foliaire en azote à l'aide d'un **sous-ensemble** semi-diallel d'un plan diallel complet établi sur trois sites variant quant à leur potentiel hydrique. Les arbres étaient âgés de 22 ans. Les **héritabilités**, les aptitudes **générales** et **spécifiques** à la combinaison de **même** que les corrélations phénotypiques, **génétiques** et environnementales ont été estimées à l'aide des méthodes de meilleure prédiction linéaire non biaisée et du maximum de vraisemblance restreinte. Les quatre **caractères mesurés** ont démontré des niveaux variables de **contrôle génétique**. Les **héritabilités calculées** étaient de  $0,39 \pm 0,22$  pour la hauteur (estimé  $\pm$  écart type), de  $0,14 \pm 0,10$  pour le diamètre, de  $0,54 \pm 0,26$  pour  $\Delta^{13}\text{C}$  et de 0,00 pour l'azote foliaire. Les corrélations phénotypiques entre la croissance en hauteur et  $\Delta^{13}\text{C}$  étaient **modérées** ( $r = -0,35$ ), alors que les corrélations **génétiques** étaient fortes ( $r = -0,97$ ) et les corrélations environnementales faibles ( $r = -0,18$ ) entre ces **mêmes** variables. La forte corrélation **génétique** négative notée entre  $\Delta^{13}\text{C}$  et la croissance s'accorde avec les **résultats** de travaux antérieurs découlant de l'étude d'un **sous-ensemble** de familles, et qui ont permis d'identifier l'effet de différences de rendement photosynthétique sur  $\Delta^{13}\text{C}$ . La **consanguinité** a résulté en une forte baisse de croissance, **mais** elle n'avait pas d'impact sur  $\Delta^{13}\text{C}$ . Une forte **héritabilité**, l'absence de dépression consanguine ainsi que de faibles corrélations environnementales indiquent que la majeure **partie** de la **mécanisme** de **contrôle** génétique **sous-jacente** à  $\Delta^{13}\text{C}$  serait relativement simple. **Parce** que  $\Delta^{13}\text{C}$  est **très** héritable, **fortement** **corrélé** **génétiquement** avec la croissance, moins sensible aux variations environnementales que la croissance, et qu'il **confère** la **possibilité** d'une sélection **hâtive**, il **apparaît** comme un bon **caractère candidat** pour la sélection **indirecte** quant à la croissance.

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## Introduction

A series of previous studies have examined genetic variation in growth characteristics of *Picea mariana* (Mill.) BSP (black spruce) and associated variation in physiological characteristics (Johnsen and Major 1995 and 1999, Flanagan and Johnsen 1995, Major and Johnsen 1996 and 1999). These studies made use of full-sib families from a complete diallel cross experiment replicated on three different sites at the Petawawa Research Forest (PRF) (Morgenstem 1974; Boyle 1987). Standard statistical analysis of growth characteristics on the trees from the diallel cross indicated important genotype by environment ( $G \times E$ ) interactions (Boyle 1987). The subsequent studies attempted to determine the physiological basis for the observed differences in growth, and the significant genotype by environment interactions using a subset of full-sib families that displayed a distinct  $G \times E$ . The physiological characteristics studied included leaf photosynthetic gas exchange, water relations characteristics, and leaf carbon isotope discrimination.

Measurement of the carbon isotope ratio of leaf tissue provides an assimilation-weighted average of the ratio of leaf intercellular  $\text{CO}_2$  partial pressure ( $p_i/p_a$ ) to atmospheric  $\text{CO}_2$  partial pressure (Farquhar et al. 1989). This ratio ( $p_i/p_a$ ) is important because it is a function of photosynthetic capacity and stomatal conductance. Changes in  $p_i/p_a$  are a function of changes in either, or both, photosynthetic capacity or stomatal conductance. Since leaf carbon isotope ratio provides information about processes integrated over the whole life of the leaf, it is particularly useful for examining subtle genetic differences in photosynthetic characteristics.

Significant differences in leaf carbon isotope discrimination were observed among four full-sib families of black spruce (Flanagan and Johnsen 1995). The source of variation in carbon isotope discrimination was differences in photosynthetic capacity, since genotypic variation in photosynthesis was not explained by genotypic variation in stomatal conductance (Johnsen and Major 1995; Major and Johnsen 1996). A strong negative correlation was also observed between average carbon isotope discrimination and average tree height for the four families on the driest, least productive site at PRF (Flanagan and Johnsen 1995). The growth of individual plants may be either positively or negatively correlated with leaf discrimination values depending on whether variation in discrimination is associated with changes in photosynthetic capacity or stomatal conductance (Farquhar et al. 1988, 1989). The negative correlation we observed between growth and carbon isotope discrimination was consistent with variation in photosynthetic capacity being the primary cause of variation in isotope discrimination (Flanagan and Johnsen 1995). However, there was no significant correlation between discrimination values and growth on the other two study sites, where water availability and productivity were higher.

Our previous study indicated that carbon isotope techniques greatly contributed to understanding physiological mechanisms that influence the growth of black spruce, and so the techniques show promise for incorporating physiological assessment into established tree breeding programs. Because of the significant progress made, we extended the study to examine a half-diallel subset of the complete diallel

experiment at PRF. Our analyses allowed calculation of heritabilities, genetic correlations, and general and specific combining abilities that are necessary for disentangling genetic and environmental effects.

## Materials and methods

### Plant material and experimental design

Full-sib families of *Picea mariana* were used from a complete  $7 \times 7$  diallel cross experiment that was started at the PRF in 1970 (Morgenstem 1974, Boyle 1987). The seven parental trees used for the diallel cross were from a plantation at PRF. The exact origin of the trees is unknown except that they were grown from seed collected in the Lake Simcoe - Rideau region in Ontario (Morgenstem 1974).

The seeds resulting from the diallel matings were germinated, and seedlings were grown for 2 years in a greenhouse before being planted at three field sites at PRF in 1973 (Morgenstem 1974). At each site, trees from a full-sib family were planted in either 9-tree (sites 1 and 3) or 16-tree (site 2) square plots. The arrangement of family plots was randomized within a block at each site. At sites 1 and 3 there were three replicate blocks, and at site 2 there were four replicate blocks. Site 1 was established with 45 families from the original 49 families produced by the diallel cross, site 2 was planted with 37 of the families, and site 3 was initiated with 32 of the families. We sampled 27 families on site 1, including 6 self-crosses; 22 families on site 2, including 3 self crosses; and 21 families on site 3, including 2 self crosses. We sampled the families resulting from crosses associated with the top diagonal of the complete diallel (no reciprocal crosses were used).

For our experiment, we attempted to sample 12 trees per family at each site. On sites 1 and 3, four trees were randomly chosen from each family plot in each of the three replicate blocks per site. On site 2, three trees were randomly chosen from each family plot in each of the four replicate blocks. In some cases, fewer than 12 trees were sampled per family because of missing trees, usually associated with death of the inbred trees. Of the total number of trees sampled, 18 families were represented on all three sites. A total of 820 analyses of leaf carbon isotope ratio were performed.

### Carbon isotope discrimination

Carbon isotope analysis was done on foliage collected during October 1993. Small branches, from the four cardinal compass directions, from the top one third of the canopy were cut from a tree. Only foliage produced during the previous summer was collected from the branches and combined for an individual tree. The tissue was dried at  $65^\circ\text{C}$  and ground to a fine powder with a mortar and pestle or with a tissue grinder.

A 2- to 3-mg subsample of the ground tissue was sealed in an evacuated Vycor tube with cupric oxide wire and silver foil. The tubes were heated to  $850^\circ\text{C}$  for 6 h followed by 8- to 9-h period of cooling to room temperature (Ehleringer and Osmond 1989). The carbon dioxide generated from the combustion was purified cryogenically within 2 days, and its  $^{13}\text{C}/^{12}\text{C}$  ratio measured on a gas isotope ratio mass spectrometer (Sira 12, VG Instruments Inc.) at the Ottawa-Carleton OCGC Stable Isotope Facility. Carbon isotope discrimination values (A) were calculated from carbon isotope composition values (a) as shown below (Farquhar et al. 1989):

$$\delta = \frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1$$

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

**Table 1.** Tree height, diameter at 1.65 m height, leaf  $\Delta^{13}\text{C}$  values, and leaf total nitrogen concentration for *Picea mariana* grown on the three different sites at the Petawawa Research Forest.

Characteristic	Site 1	Site 2	Site 3
Tree height (cm)	747.7 (6.9)	722.3 (6.3)	820.9 (6.6)
Tree diameter (cm)	9.30 (0.11)	9.31 (0.11)	10.35 (0.12)
Leaf $\Delta^{13}\text{C}$ value (‰)	19.81 (0.06)	19.23 (0.06)	19.83 (0.06)
Leaf nitrogen ( $\text{mg}\cdot\text{g}^{-1}$ )	0.96 (0.02)	0.91 (d.02)	1.06 (0.01)

Note: Values are means with SD given in parentheses. Leaf tissue was collected in October 1993. Height and diameter measurements were made in the autumn of 1992.

where  $R_{\text{Sample}}$  is the  $^{13}\text{C}/^{12}\text{C}$  content of a sample,  $R_{\text{Standard}}$  is the  $^{13}\text{C}/^{12}\text{C}$  content of the international PDB standard (Ehleringer and Osmond 1989),  $\delta_a$  is the  $\delta^{13}\text{C}$  value for source atmospheric  $\text{CO}_2$  (-0.0079 or -7.9‰), and  $\delta_p$  is the  $\delta^{13}\text{C}$  value for plant foliage

The accuracy and precision of our analyses were checked by making repeated measurements of the carbon isotopic composition of a U.S. National Bureau of Standards sample (NBS-21, Spectrographic Graphite), an internal laboratory standard (Fisher Graphite), and several plant samples. We conclude that our carbon isotope measurements are repeatable and accurate to at least  $\pm 0.1\%$ .

### Tree height and diameter and leaf nitrogen concentration

Total tree height was determined by placing a 10-m pole, which was marked off in centimetre increments, next to a tree. While the pole was held in place by one person, a second person read the tree height and recorded the value. Diameter was measured at breast height (1.65 m aboveground) with a tape measure. Tree height and diameter measurements were made for all trees in the three study plots in 1992. The concentration of nitrogen in leaf tissue was measured on the same ground tissue samples that were used for carbon isotope analysis. Total nitrogen content was measured using standard combustion with thermal conductivity detector methodology using a LECO CNS.

### Statistical analyses

The data were analyzed using the following linear models for the traits of tree height, diameter at breast height, carbon isotope discrimination, and foliar nitrogen concentration. For univariate analyses:

$$[1] \quad y_{ijklmn} = \mu + cF_{ijklmn} + B_{jk} + g_l + g_m + s_{lm} + Lg_{jl} + Lg_{jm} + Ls_{jlm} + Bf_{jklm} + e_{ijklmn}$$

where  $\mu$  is an overall mean;  $c$  is the slope of the regression of measurements on inbreeding coefficient;  $F_{ijklmn}$  is the covariate, inbreeding coefficient;  $L_j$  is the fixed effect of the  $j$ th location;  $B_{jk}$  is the fixed effect of the  $k$ th block in the  $j$ th location;  $g_l$  is one half of the random additive genetic effect of the  $l$ th female parent,  $\sim N(0, \sigma_g^2)$ ;  $g_m$  is one half of the random additive genetic effect of the  $m$ th male parent,  $\sim N(0, \sigma_g^2)$ ;  $s_{lm}$  is the random nonadditive genetic interaction between the  $l$ th and  $m$ th parents,  $\sim N(0, \sigma_s^2)$ ;  $Lg_{jl}$  and  $Lg_{jm}$  are the random interactions between one half of the additive genetic effects of parents  $l$  and  $m$  and the  $j$ th location,  $\sim N(0, \sigma_{lg}^2)$ ;  $Ls_{jlm}$  is the random interaction between the nonadditive genetic interaction of parents  $l$  and  $m$  and the  $j$ th location,  $\sim N(0, \sigma_{ls}^2)$ ;  $Bf_{jklm}$  is the random interaction between full-sib family  $lm$  and  $k$ th block of the  $j$ th location,  $\sim N(0, \sigma_{bf}^2)$ ;  $e_{ijklmn}$  is random error,  $\sim N(0, \sigma_e^2)$ ; and  $y_{ijklmn}$  is the  $n$ th observation in full-sib family  $lm$  in the  $k$ th block of the  $j$ th location. For multivariate analyses:

$$[2] \quad y_{ijklmn} = \mu_i + c_i F_{ijklmn} + L_{ij} + B_{ijk} + a_{ijklmn} + s_{ilm} + Lf_{ijlm} + Bf_{jklm} + e_{ijklmn}$$

where  $y_{ijklmn}$ ,  $\mu_i$ ,  $c_i$ ,  $F_{ijklmn}$ ,  $L_{ij}$ , and  $B_{ijk}$  are as defined above for univariate analyses except now they pertain to trait  $i$ ;  $a_{ijklmn}$  is the random additive genetic effect of the  $ijklmn$ th individual for trait  $i$ , -MN(O,AG), where  $\mathbf{A}$  describes the additive genetic relationships among individuals and  $\mathbf{G}$  is a matrix whose values are the additive variances and covariance among traits;  $s_{ilm}$  is the random non-additive genetic interaction between parents  $l$  and  $m$  for the  $i$ th trait, -MN(O,IS), where  $\mathbf{I}$  is a matrix of block identity matrices and  $\mathbf{S}$  is a matrix whose elements are the variances and covariances for nonadditive full-sib family effects among traits;  $Lf_{ijlm}$  is the random interaction between the  $j$ th location and the  $lm$ th full-sib family for trait  $i$ , -MN(O,IM), where  $\mathbf{I}$  is a matrix of block identity matrices and  $\mathbf{M}$  is a matrix whose elements are the covariances for location by family interaction among traits;  $Bf_{jklm}$  is the random interaction between the  $k$ th block in the  $j$ th location with the  $lm$ th full-sib family for the  $i$ th trait, -MN(O,IN), where  $\mathbf{I}$  is a matrix of block identity matrices and  $\mathbf{N}$  is a matrix whose elements are the covariance for block by family interaction among traits; and  $e_{ijklmn}$  is random error, -MN(O,IE), where  $\mathbf{I}$  is an identity matrix and  $\mathbf{E}$  is a matrix whose elements are the error variances and covariances among traits.

Univariate analyses (eq. 1) were conducted using GAREML (Huber 1993) with a reduced animal model (Quaas and Pollak 1980) to account for unequal additive genetic variances within full-sib families due to inbreeding. Variance components were estimated using the restricted maximum likelihood method (REML) and then used as priors (starting values) for multivariate analyses.

The program MTDFREML (Boldman et al. 1995) was used for multivariate analyses to calculate REML estimates for all variances and covariances in eq. 2. The convergence criterion was  $1 \times 10^{-9}$  for the simplex variance and after convergence the algorithm was restarted twice more using the estimates to assure that the algorithm consistently converged on the same answers. The use of MTDFREML provides putatively unbiased estimates of the variance components since the  $\mathbf{A}$  matrix describes the additive relationships among individuals and accounts for inbreeding effects on the additive variance, and the use of the inbreeding coefficient as a covariate adjusts the observations for the average effect of inbreeding depression. After convergence, best linear unbiased estimates (BLUE) of fixed effects and best linear unbiased predictions (BLUP) of random variables were produced. Significance of variance components was tested by calculating  $\mathbf{Z}$  statistics as the variance component over the square root of the variance of the estimate. Standard deviations of the heritability estimates were calculated as per Huber (1992). Phenotypic Pearson correlations were calculated using primary data. Phenotypic correlations, are composed of genetic and environmental correlations and can be very useful in understanding genetic and environmental controls of a trait; the magnitude and even sign of the different correlation

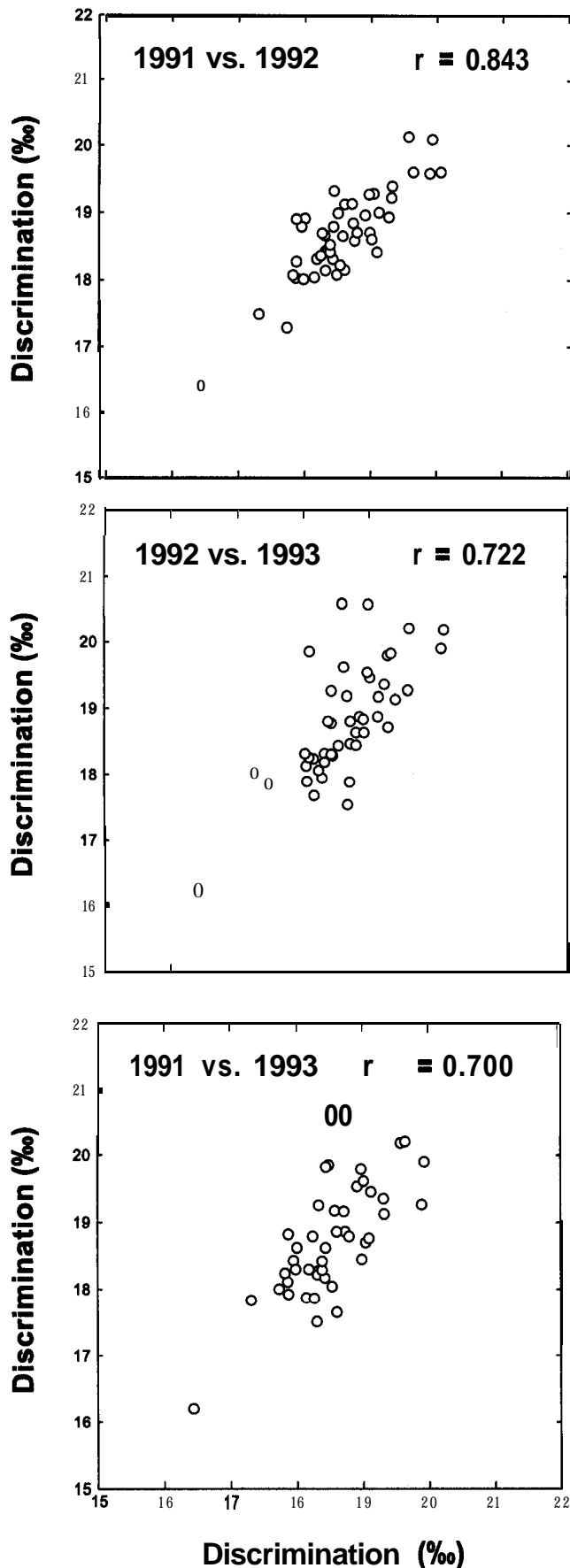


Fig 1. Relationships between measured leaf carbon isotope discrimination values from individual *Picea mariana* trees in 1991, 1992, and 1993 on site 2 at the Petawawa Research Forest.

estimates can be different (Falconer 1981). MTDFREML produced genetic and environmental correlations among the traits.

## Results

The average values for tree height and diameter, carbon isotope discrimination, and leaf total nitrogen content for black spruce in the three field sites are shown in Table 1 (no self crosses were included in these averages). The  $\Delta^{13}\text{C}$  values showed the same relative rankings and magnitude of differences among sites as a subset of four full-sib families analyzed in 1991 and 1992 (Flanagan and Johnsen 1995). A strong correlation occurred between  $\Delta^{13}\text{C}$  values measured on the same trees in successive years (Fig. 1), indicating that the ranking of individual trees remained almost constant among years.

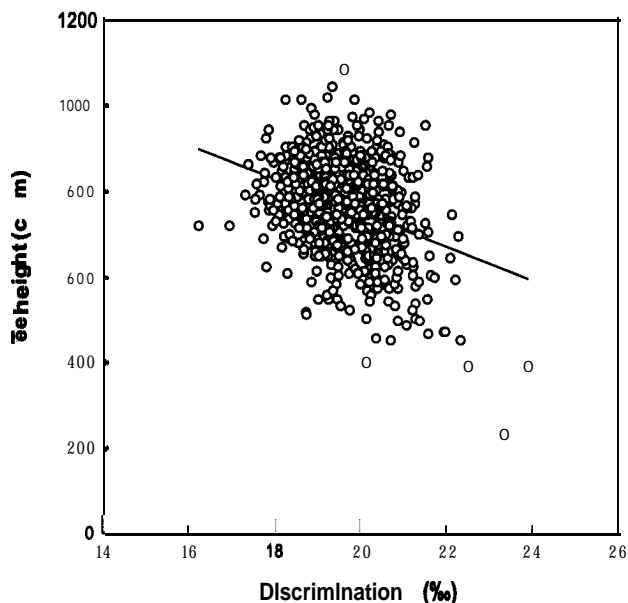
There was a strong phenotypic correlation between tree height and diameter (Table 2). However, there was only a moderate to low phenotypic correlation between height and  $\Delta^{13}\text{C}$  values (Fig. 2, Table 2). Leaf total nitrogen concentration was uncorrelated with any of the other traits that were measured (Table 2). Correlations among traits were similar on all three sites (not shown).

The four traits we measured showed variable levels of genetic control. The calculated heritabilities ( $h^2$ ) for the traits were as follows: height,  $0.39 \pm 0.22$  (estimate  $\pm$  SD); diameter,  $0.14 \pm 0.10$ ;  $\Delta^{13}\text{C}$  value,  $0.54 \pm 0.26$ ; and leaf nitrogen, 0.00. The general combining ability (GCA) variance component was high for tree height and discrimination values, while the specific combining ability (SCA) was moderate to low for these traits (Table 3). In contrast the GCA and SCA values were moderate for tree diameter (Table 3). There was no evidence for a genotype by site interaction for any trait. The rather low Z statistics for variance components and high standard deviations for  $h^2$  estimates are due to the experiment including a relatively low number of parents and offspring per parent for quantitative genetic analysis.

There was a strong genetic correlation between tree height and  $\Delta^{13}\text{C}$  values (Fig. 3, Table 4). The negative correlation indicated that faster growth was genetically associated with lower  $\Delta^{13}\text{C}$  values. The four full-sib families used in our preliminary studies of photosynthetic gas exchange and carbon isotope discrimination fit closely on a regression line between tree height and  $\Delta^{13}\text{C}$  values for the whole population of outcrossed families used in this study (Fig. 4). The subset of four families used in previous studies included approximately 40% of the variation present in the entire diallel population for both height and  $\Delta^{13}\text{C}$  (Fig. 4).

Families resulting from selfs did not fit on the same regression line between tree height and  $\Delta^{13}\text{C}$  values (Fig. 4). Selfing had large deleterious effects on height (-32.0%) and diameter (-36.4%) growth, but it had little impact on carbon isotope discrimination (2.6%). Thus, the full-sib family breeding values for  $\Delta^{13}\text{C}$  were basically two times the parent values and occurred on trees that were very small due to inbreeding (Fig. 4). This indicates that genetic expression of

**Fig 2.** Relationship between measured tree height and leaf  $\Delta^{13}\text{C}$  value (phenotypic correlation), for *Picea mariana* trees grown on the three different sites at the Petawawa Research Forest.



carbon isotope discrimination is stable despite the size of the trees in which the genes are present.

## Discussion

Our data show significant genetic variation in leaf carbon isotope discrimination, consistent with our previous studies with a subset of four full-sib families of black spruce (Flanagan and Johnsen 1995). Genetic variation in carbon isotope discrimination has also been demonstrated in a number of other plant species (Hall et al. 1993; Richards and Condon 1993) including *Psuedotsuga menziesii* (Mirb.) Franco (Zhang et al. 1993), *Larix occidentalis* Nutt. (Zhang et al. 1994), *Piceu glauca* (Moench) Voss (Sun et al. 1996) and the *Piceu sitchensis* (Bong.) Carr. - interior spruce complex (Fan et al. 1999). In four of the families studied here, variation in photosynthetic capacity is the primary cause of genetic differences in discrimination (Johnsen and Major 1995; Major and Johnsen 1996) consistent with observations in peanut (Hubick et al. 1988). No (Johnsen and Major 1995) or very minor (Major and Johnsen 1996) differences were observed for stomatal conductance rate and for turgor loss point (Johnsen and Major 1999; Major and Johnsen 1999) in the four intensively studied families of black spruce.

In our previous study of four full-sib families (Flanagan and Johnsen 1995), a negative correlation between discrimination and growth was only observed on the driest of the three sites. However, in this current study we observed a weak negative phenotypic correlation on each site individually and across all sites combined. This apparent inconsistency is attributable to the fact that, in this current study, both the family and total sample sizes are much larger, which increased the precision of correlation estimates. The diallel mating system used here also allowed us to partition the phenotypic correlations into genetic and environmental components. The additive genetic correlation between growth

**Table 2.** Phenotypic correlations among tree height, tree diameter, leaf  $\Delta^{13}\text{C}$  value, and leaf total nitrogen concentration for *Picea mariana* grown on the three different sites at the Petawawa Research Forest.

	Tree height	Tree diameter	$\Delta^{13}\text{C}$ value
Tree diameter	0.767	—	—
Leaf $\Delta^{13}\text{C}$ value	-0.250	-0.352	—
Leaf nitrogen	0.119	0.150	0.086

and  $\Delta^{13}\text{C}$  was negative and extremely strong. The sign of this genetic correlation provides further insight into the physiological basis of genetic variation in  $\Delta^{13}\text{C}$  observed (discussed below). The high genetic correlation coefficient indicates that photosynthesis is a very important physiological component of growth performance among the families in this population.

Mechanistic models of carbon isotope discrimination during photosynthetic gas exchange predict that discrimination will be reduced when photosynthetic capacity is increased and stomatal conductance remains constant (Farquhar et al. 1982, 1989). This suggests that carbon isotope discrimination values should be negatively correlated with plant growth when variation in discrimination is the result of changes in photosynthetic capacity (Farquhar et al. 1989). The higher photosynthetic capacity should translate into higher growth if all other things remained constant. In contrast, if variation in discrimination is caused primarily by a change in stomatal conductance, then carbon isotope discrimination values should be positively correlated with growth. This is because an increase in stomatal conductance should result in higher carbon assimilation rates, thereby increasing growth, and also increase discrimination against  $^{13}\text{C}$  during photosynthetic gas exchange (Farquhar et al. 1989). Our data show strong negative genetic correlations between discrimination values and estimates of tree growth, a result consistent with genetic variation in photosynthesis driving the genetic variation in carbon isotope discrimination. This interpretation assumes that the genetic variation in  $^{13}\text{C}$  discrimination observed in leaf tissue is representative of  $^{13}\text{C}$  discrimination integrated across the entire growing season. Our observed negative correlation between  $^{13}\text{C}$  discrimination and growth is also consistent with our previous gas-exchange studies that indicated variation in discrimination was primarily controlled by differences in photosynthetic capacity rather than stomatal conductance (Flanagan and Johnsen 1995; Johnsen and Major 1995).

Given that genetic variation in photosynthetic capacity was the major cause of differences in  $\Delta^{13}\text{C}$  values, we expected that leaf nitrogen content would also be strongly correlated with discrimination values. Several studies have demonstrated strong correlations between leaf nitrogen content and photosynthetic capacity in comparisons among different, wide-ranging species (Field and Mooney 1986; Reich et al. 1997). In contrast, we observed very poor phenotypic, genetic, and environmental correlations between total leaf nitrogen content (on a leaf dry mass basis) and leaf  $\Delta^{13}\text{C}$  values and tree height or tree diameter (Table 2). Sullivan et al. (1997) have also observed that maximum photosynthetic

**Table 3.** Variance components matrix from the multivariate analyses of tree height, tree diameter, leaf  $\Delta^{13}\text{C}$  value, and leaf total nitrogen content for three different sites at the Petawawa Research Forest.

Source	Tree height	Tree diameter	$\Delta^{13}\text{C}$ value	Leaf N
General combining ability (GCA)	0.13	0.10	0.17	0.00
Specific combining ability (SCA)	0.06	0.16	0.04	0.00
Site $\times$ GCA	0.00	0.00	0.00	0.00
Site $\times$ SCA	0.00	0.00	0.00	0.00
Block $\times$ family	0.14	0.07	0.11	0.03
Error	0.74	2.74	0.60	0.04

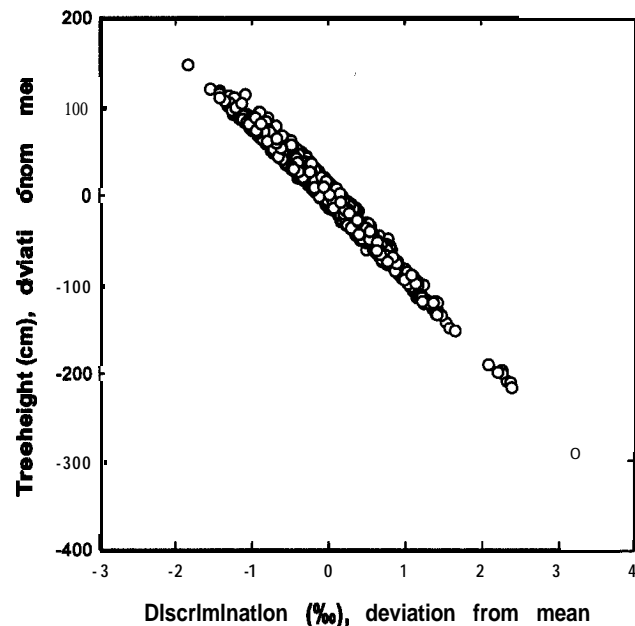
**Table 4.** Additive genetic correlations among tree height, tree diameter, leaf  $\Delta^{13}\text{C}$  value, leaf total nitrogen concentration for *Picea mariana* grown on the three different sites at the Petawawa Research Forest.

	Tree height	Tree diameter	$\Delta^{13}\text{C}$ value
Tree diameter	0.95	—	—
Leaf $\Delta^{13}\text{C}$ value	-0.97	-0.64	—
Leaf nitrogen	0.00	0.00	0.00

capacity was not well correlated with leaf tissue nitrogen concentration in natural populations of black spruce in Saskatchewan. The absence of a strong correlation between leaf nitrogen and photosynthesis may result because a significant fraction of leaf nitrogen is used for other functions such as herbivore defense. Field and Mooney (1986) noted that relatively poor correlations occurred between leaf nitrogen and photosynthesis in sclerophyllous species with long-lived leaves because of the need to use nitrogenous based compounds in herbivore defense chemicals. Expressing leaf nitrogen content on a dry mass basis may also contribute to a poor correlation with photosynthetic capacity. Changes in the amount of starch in a leaf could alter leaf nitrogen content expressed on a leaf area basis, depending on variation in leaf mass-to-area ratio (Sullivan et al. 1997). Our data suggest that the scale-of-interest (among genera, among species, within species, etc.) is important when considering the generality of relationships between foliar nitrogen and photosynthetic capacity.

In this study, we have examined variation in physiological process components among families and individuals that vary greatly in size. We interpret that the genetic variation in carbon isotope discrimination has greatly contributed to the genetic variation in tree growth. However, the interpretation of cause versus effect always needs to be carefully considered. In seedling studies it has been shown that physiological traits can be allometrically related to seedling size and so growth variation can confound the direct interpretation of mean genotypic differences (Johnsen and Bongarten 1991). In our current study using large trees, variation in environmental factors within a forest canopy stemming from differential tree size might also influence leaf carbon isotope discrimination values and potentially confound interpretation

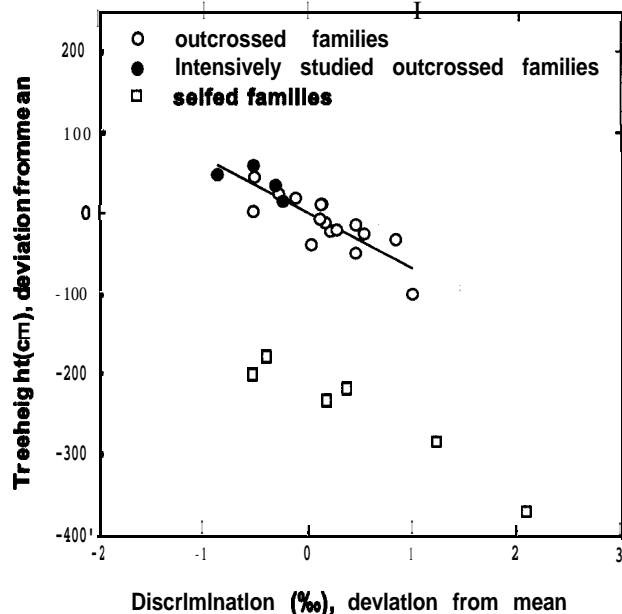
**Fig. 3.** Relationship between the calculated individual tree breeding values for tree height and leaf  $\Delta^{13}\text{C}$  values (additive genetic correlation), for *Picea mariana* trees grown on the three different sites at the Petawawa Research Forest.



of the genetic differences in  $\Delta^{13}\text{C}$  values. In the next three paragraphs, we explore some potentially important confounding factors and evaluate their impacts.

Foliage within a canopy may assimilate some  $\text{CO}_2$  released from respiration, which is relatively depleted in  $^{13}\text{C}$ , in addition to  $\text{CO}_2$  from the well-mixed convective boundary layer. This can cause leaves lower in the canopy to have lower  $^{13}\text{C}$  contents than upper canopy leaves, even if all other environmental and physiological factors were constant (Broadmeadow and Griffiths 1993). We sampled leaves from the top one third of the canopy, and the contribution of respired  $\text{CO}_2$  would be negligible based on recent studies in natural black spruce canopies (Brooks et al. 1997) and a red pine (*Pinus resinosa* Ait.) plantation with higher tree density than the PRF black spruce plantations (Berry et al. 1997). There would be too little variation in the carbon isotope ratio of source  $\text{CO}_2$  in the small height range for the large majority of leaf samples collected for this study. Some of the inbred families were very stunted and so would have had the opportunity to assimilate a greater fraction of soil-respired

Fig. 4. Relationship between the mean full-sib family genetic values of tree height and leaf  $\Delta^{13}\text{C}$  values, for trees grown on the three different sites at the Petawawa Research Forest. Full-sib family genetic values were calculated as the sum of male GCA effects, female GCA effects, SCA effects, and, for selfed families, inbreeding effects. Note that intensively studied families were used in Flanagan and Johnsen (1995), Johnsen and Major (1995, 1999), and Major and Johnsen (1996, 1999).



$\text{CO}_2$  that was depleted in  $^{13}\text{C}$ , but the data in Fig. 4 were not consistent with significantly higher apparent discrimination because of exposure to  $\text{CO}_2$  depleted in  $^{13}\text{C}$  in the inbred trees.

Variation in light intensity within a canopy can also cause variation in  $p_i/p_a$  and, therefore, cause variation in leaf  $\Delta^{13}\text{C}$  values (Farquhar et al. 1989; Berry et al. 1997; Brooks et al. 1997). With the exception of the very stunted inbred trees that were exposed to significantly lower average light intensities, the leaves collected from the majority of the trees were exposed to full sunlight near the top of the forest canopy. In addition, leaves were collected from all four cardinal compass directions to avoid any directional shading effects. We also dismiss the shading effect as being a dominant factor for the stunted inbred trees because in most cases the leaf  $\Delta^{13}\text{C}$  values of the inbred trees were not different from the tallest trees in the plantation (Fig. 4).

Variation in hydraulic architecture within a tree can have a significant influence on stomatal conductance,  $p_i/p_a$ , and leaf  $\Delta^{13}\text{C}$  values (Waring and Silvester 1994; Walcroft et al. 1996). This has been noted particularly in trees with very long branches (e.g., *Pinus radiata* D. Don). It is possible, therefore, that the relationship we observed between tree height and discrimination could be associated with hydraulic architecture effects on leaf  $\Delta^{13}\text{C}$  values, so that taller trees had reduced water supply to the upper branches and, therefore, had lower stomatal conductance in relation to photosynthetic capacity and consequently lower  $\Delta^{13}\text{C}$  values. If this were the case, we would expect large variation (approximately 5%, as has been observed in *P. radiata*) in leaf carbon isotope composition among branches at the top and

**Table 5.** Environmental correlations among tree height, tree diameter, leaf  $\Delta^{13}\text{C}$  value, and leaf total nitrogen concentration for *Picea mariana* grown on the three different sites at the Petawawa Research Forest.

	Tree height	Tree diameter	$\Delta^{13}\text{C}$ value
Tree diameter	0.79	—	—
Leaf $\Delta^{13}\text{C}$ value	-0.18	-0.28	—
Leaf nitrogen	0.00	0.00	0.00

bottom of black spruce canopies, a pattern that has not been observed (Brooks et al. 1997). In addition, carbon isotope discrimination has a low environmental correlation with height growth (Table 5). Inbreeding had large deleterious effects on height (-32.0%) and diameter (-36.4%) growth, but it had virtually no impact on carbon isotope discrimination (2.6%). Finally, our previous gas exchange work has shown  $\Delta^{13}\text{C}$  differences were due to variation in photosynthesis and not stomatal conductance (Johnsen and Major 1995; Major and Johnsen 1996). Based on all the points raised in the previous three paragraphs, we feel confident in the robustness of the calculations of strong genetic differences in leaf carbon isotope discrimination.

The fact that inbreeding greatly depressed growth while not impacting  $\Delta^{13}\text{C}$  again highlights the obvious point that net photosynthesis ( $P_n$ ) alone does not control tree growth. It appears that, in this population, that high  $P_n$  might be a prerequisite for high growth rate but other physiological mechanisms must be in place to achieve a fast growth rate. Selfing does not appear to disrupt the photosynthetic potential of the trees, but it does appear to disrupt subsequent physiological processes contributing to growth. However, we have not yet measured actual gas exchange of the selfed families. High heritability, lack of inbreeding depression, and low environmental correlations indicate that a major proportion of  $\Delta^{13}\text{C}$  genetic control may be relatively simple. Reciprocals should be investigated to determine the role of extranuclear DNA in  $\Delta^{13}\text{C}$  as inheritance of chlorophyll DNA has been shown to be paternal in *Picea* (Stine et al. 1989; Sutton et al. 1991).

Quantitative genetic theory predicts that traits important to fitness will have low heritability because variation should have been reduced by natural selection (Falconer 1981). We calculated high heritabilities for  $\Delta^{13}\text{C}$  in the PRF families and  $\Delta^{13}\text{C}$  was highly genetically correlated to growth, indicating photosynthetic characteristics were important contributors to tree growth. Tree growth rate is potentially an important component of fitness. It is possible that discrimination is negatively correlated to other aspects of fitness that are unimportant in a plantation environment (e.g., reproductive success traits). In addition, the plantation trees utilized were the result of artificial regeneration, planted at a constant spacing, and under smaller-than-natural environmental variation. Thus, the seedling culture and testing environment were vastly different than the environment naturally regenerated trees would be exposed to. This may have influenced our estimates as heritabilities are environment specific (Falconer 1981).

Our previous related studies of carbon isotope discrimination, gas exchange, and water relations utilized a subset of families that displayed a distinct  $G \times E$  in growth. However, this larger scaled and more in-depth genetic analysis indicates  $G \times E$  in study traits is unimportant. These results are not necessarily inharmonious. The four families in the subset may well display a physiologically based and repeatable interaction with site, a premise supported by the fact that many of the component drought-tolerance traits measured have displayed  $G \times E$  effects and trends (Major and Johnsen 1996, 1999; Johnsen and Major 1999). However, relative to the diallel population as a whole, genetic main effects in discrimination and growth contribute far greater to total variance and thus statistical importance. This subtle  $G \times E$  variation nested within this population may well represent "preadapted" genetic variation that could potentially become important if environments change. It is this type of variation that is important to capture in genetic conservation programs (Ledig 1986; Ledig et al. 1998).

Differences in photosynthetic and other physiological characteristics have not regularly been shown to be an important contributor to genetic variation in growth characteristics (Greenwood and Volkaert 1992). However, the black spruce populations discussed here have been intensively studied for a variety of detailed physiological characteristics and important and repeatable variation has been demonstrated. In addition, carbon isotope discrimination provides information about physiological processes integrated over the whole life of the leaf, so it is particularly useful for examining subtle genetic differences in photosynthetic characteristics that might not be discernable with more traditional techniques. This last fact also allows sampling at a scale relevant to tree breeding and genetic conservation programs, which is difficult to impossible with gas exchange and most other physiological assessments. We found carbon isotope discrimination to be highly heritable and highly genetically correlated with growth, while displaying far less environmental variation than growth. Thus, our data strongly suggest carbon isotope discrimination may be a good selection criterion to incorporate into black spruce, and perhaps other tree species, tree improvement programs. If the trait is genetically expressed at an early age, then it presents a powerful early selection tool; experiments are being performed currently to examine for this potential. In addition, the importance of photosynthetic characteristics contributing to growth differences may be population specific or even plastid specific and must be assessed in other control-crossed populations as well as reciprocal crosses within the current population.

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