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11 **Genetic parameters and QTL analysis of $\delta^{13}\text{C}$ and ring width**
12 **in maritime pine**

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1Abstract

2Classical quantitative genetics and quantitative trait dissection analysis (QTL)
3approaches were used in order to investigate the genetic determinism of wood
4cellulose carbon isotope composition ($\delta^{13}\text{C}$, a time integrated estimate of water use
5efficiency) and of diameter growth and their relationship on adult trees (15 years) of
6a forest tree species (maritime pine). We used a half diallel experimental set-up to (1)
7estimate heritabilities for $\delta^{13}\text{C}$ and ring width and (2) to decompose the phenotypic
8 $\delta^{13}\text{C}$ / growth correlation into its genetic and environmental components. We found
9considerable variation for $\delta^{13}\text{C}$ (range of over 3‰) and for ring width (range of over
105 mm) and significant heritabilities (narrow sense 0.17 / 0.19 for $\delta^{13}\text{C}$ and ring width,
11respectively, 100% additivity). The significant phenotypic correlation between $\delta^{13}\text{C}$
12and ring width was not determined by the genetic component, but was attributable to
13environmental components. Using a genetic linkage map of a full-sib family, four
14significant and four suggestive QTLs were detected for $\delta^{13}\text{C}$, the first for $\delta^{13}\text{C}$ in a
15forest tree species, as far as known to the authors. Two significant and four
16suggestive QTLs were found for ring width. No co-location of QTLs was found
17between $\delta^{13}\text{C}$ and growth.

18Keyword Index :

19stable isotope, ^{13}C , growth, water use efficiency, heritability, quantitative trait, Pinus
20pinaster, tree rings

1 Introduction

2 In given environmental conditions, trees with high water-use efficiency at the leaf
3 level (intrinsic WUE, defined as the ratio of net CO₂ assimilation rate A to stomatal
4 conductance for water vapour g) can maintain higher growth rates under water
5 limited conditions than trees with lower WUE (Sun *et al.* 1996; Nguyen-Queyrens *et*
6 *al.* 1998). Measurements of plant carbon isotope composition ($\delta^{13}\text{C}$) provide time-
7 integrated estimates of WUE (Farquhar, O'Leary & Berry 1982; Farquhar &
8 Richards 1984) that can be applied to adult trees (Zhang & Marshall 1994; Zhang &
9 Marshall 1995; Guehl *et al.* 1995; Sun *et al.* 1996). Assessments of differences in
10 WUE among- and within-tree species are facilitated by the crown- and time-
11 integrative nature of tree ring $\delta^{13}\text{C}$.

12 Forest tree species are known to be among the most polymorphic species of the flora
13 (Hamrick, Godt & Sherman-Broyles 1992). Genotypic differences in leaf $\delta^{13}\text{C}$ of
14 conifer species were found among provenances in common garden studies (Zhang,
15 Marshall & Jaquish 1993; Zhang *et al.* 1994; Zhang *et al.* 1995; Guehl *et al.* 1995;
16 Nguyen-Queyrens *et al.* 1998). It has been suggested that provenance differences of
17 $\delta^{13}\text{C}$ might be determined by differences in stomatal sensitivity to changes in vapour
18 pressure deficit (Zhang *et al.* 1995) and/or differences in plant hydraulic
19 characteristics (Guehl *et al.* 1995). However, differences of $\delta^{13}\text{C}$ among genetic
20 families within provenances of *Picea mariana* (Mill.) were found to be mainly
21 determined by differences in photosynthetic capacity (Johnsen & Major 1995; Major
22 & Johnsen 1996). Similar indications were obtained for maritime pine (*Pinus*
23 *pinaster* Ait.) by Guehl *et al.* (1995).

24 Tree growth is an important goal for forest tree breeding programs. To avoid
25 inadvertent negative selection for growth when selecting for high WUE, it is
26 important to know if $\delta^{13}\text{C}$ and growth are genetically linked. Positive but weak
27 phenotypic relationships between $\delta^{13}\text{C}$ and height or diameter growth (Flanagan &
28 Johnsen 1995; Johnsen *et al.* 1999; Nguyen-Queyrens *et al.* 1998) have been found
29 among trees within different forest tree species. Genetic parameters calculated for
30 physiological or morphological traits can disentangle phenotypic relationships into
31 genotypic and environmental components. Johnsen *et al.* (1999) found strong genetic
32 correlations between $\delta^{13}\text{C}$ and tree height or tree diameter. They concluded that A
33 was determining $\delta^{13}\text{C}$ and growth performance and thus constituted probably the link
34 between the two traits. However, since $\delta^{13}\text{C}$ as an indicator of WUE could be either
35 controlled by A and / or by g , there is not necessarily a strong relationship between A
36 and $\delta^{13}\text{C}$. This suggests that the existence of a genetic correlation between $\delta^{13}\text{C}$ and
37 growth is depending on the factor by which WUE is controlled.

38 Adaptive traits, like $\delta^{13}\text{C}$, are characterised by high phenotypic variation among and
39 within populations of forest tree species (Meinzer *et al.* 1992; Zhang *et al.* 1993;
40 Zhang *et al.* 1995; Flanagan *et al.* 1995; Nguyen-Queyrens *et al.* 1998). Moreover,
41 high heritabilities for $\delta^{13}\text{C}$ have been found for non-woody (Matus, Slinkard & Van
42 Kessel 1995; Asay, Johnson & Palazzo 1998) and woody species (Johnsen *et al.*
43 1999). The development of genetic mapping (Tanksley 1993) has made it possible to
44 localize genetic factors controlling quantitative traits (QTLs, Quantitative Trait
45 Loci). High heritability of a trait is a favourable factor for quantitative trait dissection
46 analysis.

1In crop plant breeding, improvement of WUE has been an important aim and
2therefore the first QTLs for $\delta^{13}\text{C}$ were detected in tomato (Martin *et al.* 1989).
3Mansur *et al.* (1993) found in a preliminary investigation of $\delta^{13}\text{C}$ on soybean one
4large genomic region that could be responsible for as much as 53% of the observed
5variation. In a study of three weeks old barley plants (Pakniyat *et al.* 1997), twelve
6AFLP markers were detected for $\delta^{13}\text{C}$, two of these markers alone accounted for
753.2% of the variation. QTLs for water use efficiency, as measured by the ratio of
8dry weight to water used, were found in soybean (Mian *et al.* 1996; Mian, Ashley &
9Boerma 1998).

10Detection of QTL on woody species, however, is still in development, due to long
11generation time and therefore the lack of controlled crosses. Genetic maps have often
12to be constructed from F1 full-sib progenies. Carlson *et al.* (1991) were the first to
13show that RAPD primers could be screened for informative markers segregating in a
141:1 ratio in diploid tissue of full-sib progenies. Grattapaglia and Sederoff (1994)
15extended this idea in constructing parental maps of an interspecific eucalyptus hybrid
16family in a mapping strategy named “two-way pseudo-testcross”. It was further used
17in conifers (Kubisiak *et al.* 1996, Arcade *et al.* 2000) with RAPDs and AFLPs. For
18maritime pine the genome coverage required for linkage map construction and QTL
19analysis was achieved by using RAPD markers (Plomion *et al.* 1995a; Plomion,
20O'Malley & Durel 1995b) and AFLP analysis (Costa *et al.* 2000).

21Our objectives were: 1) To estimate the variability and heritability of $\delta^{13}\text{C}$ and ring
22width in a forest tree species (*Pinus pinaster* Ait.) using a half-diallel experimental
23design. 2) To investigate the phenotypic correlation between $\delta^{13}\text{C}$ and growth. 3) To
24separate the phenotypic correlation between $\delta^{13}\text{C}$ and growth into a genetic and an
25environmental component. 4) To dissect $\delta^{13}\text{C}$ and ring width into mendelian inherited
26components (quantitative trait dissection analysis) using a *Pinus pinaster* Ait. full-sib
27family. 5) To compare QTLs for $\delta^{13}\text{C}$ with QTLs for growth.

1 **Material and Methods**

2 **Half Diallel**

3 A twelve by twelve half-diallel of maritime pine (*Pinus pinaster* Ait.) was used to
 4 estimate the variability, heritability and genetic correlations among the studied traits.
 5 Parental trees were crossed in 1980 and seeds from the controlled crosses sown in a
 6 nursery in spring 1982 and planted in autumn 1982. The 12 parents were trees
 7 phenotypically selected for stem growth and straightness in the local provenance of
 8 the Landes de Gascogne. The half-diallel was located in Cestas (Gironde, France,
 9 44°N44' 0°W44') on a semi-humid podzolic soil. Spacing was 4 m between rows and
 10 1.1 m between individual trees, i.e. 2272 trees/ha. No selfed crosses were analysed,
 11 therefore the half-diallel consisted of 66 families (12 female and 11 male parents) of
 12 125 to 15 individuals each. Three families were not available, therefore only 63
 13 families were analysed. A parentage test was performed using 3 microsatellites
 14 (Gerber *et al.* 2000) confirming the authenticity of the progenies used in the half
 15 diallel. The experimental design consisted of 74 incomplete randomised blocks (the
 16 large number of blocs is due to the fact that the presented half-diallel is part of a
 17 much larger complete diallel). For the present study, 564 trees were cut in march
 18 1997 (trees were fifteen years old). Disks were sampled, dried in a greenhouse and
 19 analysed for carbon isotopic composition ($\delta^{13}\text{C}$) and ring width as described below.

20 **Full-sib family**

21 A three-generation outbred pedigree comprising 202 fifteen-year-old trees was used
 22 to study the genetic architecture of the studied traits, i.e. the number, genome
 23 location and effect of Quantitative Trait Loci. The four grand parents were trees
 24 phenotypically selected for stem growth and straightness in the local provenance of
 25 the Landes de Gascogne and grafted in clonal archives. These grand parents were
 26 tested in a polycross progeny test and classified according to their breeding value as
 27 "Vigor +" (for vigorous trees) and "Vigor -" (for less vigorous trees). Each of the
 28 parental trees is the result of the cross of one "Vigor +" and one "Vigor -"
 29 grandparent. The two parental trees were crossed in 1980 and seeds from the
 30 controlled cross-sown in spring 1982. They produced progeny seedlings that were
 31 planted in autumn 1982. The family was located in Malente (Gironde, France, 44° N
 32 30' 0° W 47') on a semi-humid podzolic soil. Spacing was 4 m between rows and 1.1
 33 m between individual trees, i.e. 2272 trees / ha. The trees were felled in March 1997
 34 and stem discs were cut, dried in a greenhouse and analysed for $\delta^{13}\text{C}$ and ring width.
 35 From all the analysed families, 16 trees with no visible growth in the last four years
 36 before harvest were removed from the analysis.

37 **Ring width measurements**

38 Wood subsamples were taken from four positions on the circumference of the stem
 39 discs for the last four years of growth (1993 to 1996). As the trees were cut in march
 40 1997, this includes any growth during winter 96/97 utilising reserve material from
 41 the summer 1996 growth period. Two different methods were used for ring width
 42 measurements. For the half diallel experiment, ring width was measured using the
 43 indirect X ray-method first described by Polge (1966). For the full-sib family, the
 44 ring widths were measured at the four sampling points on the circumference using a

1semi-automatic system consisting of a digitising tablet linked to a computer
 2(precision 0.1 mm standard deviation). The width was averaged for each ring. To
 3make the ring width data comparable to the $\delta^{13}\text{C}$ measurements, for each tree the
 4mean growth was calculated for the years 1993 to 1996 (mean ring width: MRW),
 5using an arithmetic mean.

6

7Isotope measurements

8The $\delta^{13}\text{C}$ was measured of a bloc of four rings, which represents a ring-width
 9weighted mean of the $\delta^{13}\text{C}$ of each ring. The sampled blocks of wood were cut by
 10hand into small pieces, pre-ground in a centrifugal mill (Tecator, Cyclotech 1093
 11Sample Mill, Höganäs, Sweden) and milled to a fine powder in a ball mill (Retsch,
 12MM2000, Haan, Germany). Cellulose was extracted after an acidic acid /nitric acid
 13procedure described in Brendel, Iannetta & Stewart (2000). In brief, the method uses
 14a concentrated nitric acid / 80% acetic acid on-to-ten dilution (0.2 cm^3 in 2 cm^3) to
 15digest lignin, proteins and hemicelluloses in 50 mg of powdered wood sample. The
 16digested molecule fragments are then washed out using ethanol, remainders of acid
 17are removed during a water wash. The samples are dried chemically with a pure
 18ethanol / acetone progression and physically in a vacuum centrifugal evaporator
 19(speed vac) at 100 hPa for 2 h. The original protocol (Brendel *et al.* 2000) was
 20modified to include two extraction cycles, a 0.5 molar NaOH wash replacing the
 21water wash to remove acids more thoroughly and prolonging the ethanol washes to 5
 22min at $60\text{ }^\circ\text{C}$. For $\delta^{13}\text{C}$ analysis, 1 mg cellulose subsamples were combusted and
 23analysed for ^{13}C composition using a continuous flow isotope ratio mass
 24spectrometer (Delta S, Finnigan MAT, Bremen, Germany). Carbon isotope
 25composition was calculated relative to the Pee Dee Belemnite standard as (Craig
 261957):

$$27 \delta^{13}\text{C} = \frac{R_{sa} - R_{sd}}{R_{sd}} \times 1000 [\text{‰}] \quad (1),$$

28where R_{sa} and R_{sd} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the standard, respectively.
 29The discrimination between the $\delta^{13}\text{C}$ of atmospheric CO_2 ($\delta_{air} \approx -8\text{‰}$) and the $\delta^{13}\text{C}$ of
 30plant material (δ_{plant}) was calculated as (Farquhar *et al.* 1984) :

$$31 \Delta = \frac{\delta_{air} - \delta_{plant}}{1 + \frac{\delta_{plant}}{1000}} \quad (2).$$

32Intrinsic WUE was estimated from discrimination using a modified equation from
 33(Farquhar *et al.* 1982) :

$$34 WUE = \frac{A}{g} = \frac{c_a}{1.6} \left(\frac{b - \Delta}{b - a} \right) \left[\frac{\text{mol CO}_2}{\text{mol H}_2\text{O}} \right] \quad (3),$$

1 where c_a is the atmospheric CO₂ concentration (estimated as $360 \cdot 10^{-6}$ mol mol⁻¹), b is
 2 the net fractionation caused by carboxylation (27‰) and Δ is the discrimination
 3 between the $\delta^{13}\text{C}$ of atmospheric CO₂ and the $\delta^{13}\text{C}$ of cellulose (Equation 2).

4

5 Estimation of genetic parameters

6 The normality of the distribution of the traits for both experimental set-ups was
 7 tested using Smirnov-Kolmogorov test. While $\delta^{13}\text{C}$ was normally distributed, a small
 8 distortion from the normality was observed for ring width (p-value = 0.01).
 9 However, this distortion was considered to be too small to necessitate an adjustment.

10 Analyses of variance for block and family effects in the half diallel were carried out
 11 with the OPEP software (Baradat 1989; Baradat & Labbé 1995) according to the
 12 following model derived from the ‘‘Henderson III’’ model (Searle 1971):

$$13 Y_{ijk} = \mu + B_i + F_j + \varepsilon_{ijk} \quad (4)$$

14 where Y_{ijk} is the value of the trait for the individual k belonging to the family j ,
 15 located in the block i , B_i is the fixed effect of the i th block, F_j is the random effect of
 16 the j th family and ε_{ijk} is the random residual comprising : individual deviation from
 17 family mean and family x block interactions. When block and family effects were
 18 significant, data were adjusted to the block effect, prior to the decomposition of
 19 family effect. The half diallel analysis was carried out with OPEP using the model
 20 presented below, it is derived from the simplification of the random diallel model
 21 described by Garretsen & Keuls (1977) (Baradat & Desprez-Lousteau 1997) which is
 22 adapted to non orthogonal trials with reciprocal crosses :

$$23 Y_{ijk} = \mu + a_i + a_j + s_{ij} + \varepsilon_{ijk} \quad (5),$$

24 where Y_{ijk} is the value of the trait for the individual k corresponding to the cross
 25 between the male i and the female j , a_i (a_j) is the general combining ability (GCA) of
 26 the i^{th} (j^{th}) parent, s_{ij} is the specific combining ability (SCA) of the cross between the
 27 i th and the j th parent and ε_{ijk} is the residual term. The additive and dominance
 28 variances are: $\sigma^2_A = 4\sigma^2_a$ and $\sigma^2_D = 4\sigma^2_s$, whereas the phenotypic variance is :
 29 $\sigma^2_P = \sigma^2(Y_{ijk}) = 2\sigma^2_a + \sigma^2_s + \sigma^2_\varepsilon$. The narrow and broad sense heritabilities were
 30 calculated as $h^2_{ns} = \sigma^2_A / \sigma^2_P$ and $h^2_{bs} = (\sigma^2_A + \sigma^2_D) / \sigma^2_P$, respectively. The percentage
 31 of additivity is calculated as the additive variance divided by the sum of additive plus
 32 dominance variances: $\sigma^2_A / (\sigma^2_A + \sigma^2_D)$. Genetic and environmental correlations were
 33 computed with OPEP using a multi-trait analysis of variance and covariance: (1)
 34 ‘‘estimated’’ genetic and environmental correlations were calculated according to the
 35 additive and dominance effects assessed in the random model (Equation 5; parental
 36 level), and (2) ‘‘predicted’’ correlations were assessed from the individual breeding
 37 values of each tree (individual level; figures 2 a-c). However, as the estimated and
 38 the predicted correlations gave similar results, only the correlation coefficients based
 39 on the prevalent ‘‘estimated’’ results were used in the discussion. Standard errors of
 40 estimates of heritabilities were computed using the robust Jackknife method (Lebart
 41 *et al.* 1979).

1QTL detection

2Two genetic maps corresponding to the female and male parents of the full-sib
3family were established using AFLP markers, genotyped on a subset of 90 F1. The
4whole mapping population was further genotyped with evenly spaced markers to
5increase the statistical power of QTL detection (Chagné *et al.* submitted). In order to
6reduce the intra-trial environmental background noise, the data were adjusted for the
7block effect. We used the two-way pseudo-test cross mapping strategy to construct
8the linkage map (Grattapaglia & Sederoff 1994). Twelve linkage groups were found
9for the female map, equalling the number of chromosomes for *Pinus pinaster*. For
10the male map 15 linkage groups were detected, however the combination of the male
11and the female maps into a consensus map using $3/4/1/4$ -segregating markers yields 12
12linkage groups for each parent. For QTL analysis however, only the $1/2/1/2$ -segregating
13markers could be used.

14For QTL analysis, MultiQTL software (A. Korol, <http://www.multiqtl.com>) was used.
15In a first step, QTLs were detected by interval mapping using a LOD threshold of 1.5
16and a one-QTL-model (one QTL per linkage group). In a second step, these QTLs
17were taken as co-factors (composite interval mapping, CIM; introduced by Jansen &
18Stam 1994 and Zeng 1994), allowing individual QTL to be detected independently to
19the background noise. In a third step, a two QTL model (Korol *et al.* 1998) using
20CIM was applied, first testing if two QTLs are significant and then testing if two
21QTLs are more significant than one QTL. Standard deviations for the positions of the
22QTLs were calculated using a bootstrap method.

23As there are difficulties involved when using asymptotic approximations of LOD
24statistics (fixed LOD level) for QTL detection (Doerge & Churchill 1996), a
25permutation approach was used to determine appropriate significance thresholds.
26Two theoretical critical thresholds were considered, the first corresponding to a per
27linkage group type I error of 5% allowing the detection of “suggestive” QTL and the
28second corresponding to a genome wise type I error of 5% allowing the detection of
29“significant” QTL. Theoretical critical threshold corresponding to a genome wise
30type I error of 5% were calculated for each chromosome taking into account the
31number of markers in each chromosome. If α_m is the critical threshold at the marker
32level corresponding to a 5% genome wise type I error, the α_c (critical threshold at the
33chromosome level) for a chromosome comprising n markers would be:
34 $\alpha_c = 1 - (1 - \alpha_m)^n$. These theoretical thresholds were compared to the thresholds
35associated with the LOD obtained by CIM at the chromosome level after 1000
36permutations of the data. The proportion of phenotypic variance explained by each
37QTL was estimated using the coefficient of determination (R^2 , estimated by CIM,
381000 permutations), which is based on the partial correlation of a putative QTL with
39the trait adjusted for cofactors in the multi-locus model.

1 Results

2 Trait distributions

3 Means, ranges and variabilities for $\delta^{13}\text{C}$ were very similar between the half diallel
4 and the full-sib experimental designs (Table 1, Figure 1). For MRW (mean ring
5 width) growth was higher in the half diallel by 0.66 mm and also the range of
6 observed values was larger (Table 1). However, the coefficient of variation was
7 slightly higher for the full-sib experimental design than for the half diallel. This was
8 also true when the coefficients of variation for MRW were calculated using the block
9 effect adjusted data (data not shown).

10 Half diallel

11 Analysis of variance including family and block effect (Equation 4) indicated
12 variation among families for both $\delta^{13}\text{C}$ and MRW, which justified the decomposition
13 of the family effect according to Equation 5. Taking into account the significant
14 block effect for $\delta^{13}\text{C}$, data were adjusted prior to the genetic decomposition. The
15 narrow sense heritabilities were highly significant ($p < 0.005$) for $\delta^{13}\text{C}$ and for MRW
16 and close in their values (Table 2). No dominance effects were detected for these two
17 traits, thus narrow sense and broad sense heritabilities are equal and additivities are
18 100%.

19 The phenotypic correlation between mean ring width and $\delta^{13}\text{C}$ associated faster
20 growth with less negative $\delta^{13}\text{C}$ values (higher WUE) and was significant with a
21 coefficient of correlation of $r = 0.45$ (Table 3 and Figure 2a). The correlation was not
22 significant (Table 3) for the genetic component (additive effect), whereas the
23 environmental component was highly significant with a strong correlation coefficient
24 ($r = 0.52$).

25 Full-sib family

26 A significant positive phenotypic correlation between MRW and $\delta^{13}\text{C}$ of the full-sib
27 family ($r = 0.39$; $p < 0.005$; Figure 3) was observed. For $\delta^{13}\text{C}$, eight QTLs were
28 found on seven linkage groups (chromosomes) and for MRW six QTLs on four
29 linkage groups (Table 4). Using the one-QTL model, six QTLs were detected for
30 $\delta^{13}\text{C}$ and two for MRW. With the two-QTL model, one pair of QTLs was found for
31 $\delta^{13}\text{C}$ and two pairs for MRW. For $\delta^{13}\text{C}$, two of the QTLs detected with the one-QTL
32 model and the QTL-pair detected with the two-QTL model and for MRW one QTL-
33 pair are "significant QTLs" at a probability corresponding to a 5% genome wise type
34 I error. All other detected QTLs are "suggestive QTLs" at a probability
35 corresponding to a 5% chromosome type I error. For $\delta^{13}\text{C}$, QTLs were detected on
36 the male and the female maps, however not on the same chromosomes. A multi-locus
37 model, including the male and female maps, explained 51.4% of the phenotypic
38 variation of $\delta^{13}\text{C}$, the major QTL at chromosome 6 alone explaining 12.4%. For
39 MRW, no QTLs were found on the female map and a multi-locus model for the male
40 map explained 42.9% of the observed phenotypic variation. No co-localisation for a
41 QTL of $\delta^{13}\text{C}$ and of MRW was found.

1 Discussion

2 Trait distributions

3 Standard deviations and ranges were similar for half diallel as well as for the full-sib
4 (Table 1). For $\delta^{13}\text{C}$ the coefficients of variation were nearly the same, whereas the
5 coefficient of variation for mean ring width was higher for the full-sib than for the
6 half diallel (Table 1). Variability of traits might be expected to be higher in a half
7 diallel with twelve parental trees, than in one full-sib family. However, it has to be
8 taken into account that parental trees for both experiments were selected from the
9 same provenance (Landes, Gascogne) and therefore might be genetically close.
10 Further, with a polygenic complex trait such as $\delta^{13}\text{C}$, due to transgression, even
11 parents with only a small difference in a measured trait can produce offspring with
12 extreme values (Prioul *et al.* 1997). For $\delta^{13}\text{C}$, the average of family variation of the
13 half diallel (0.55‰ standard deviation within a range of 0.0‰ to 1.0‰ standard
14 deviations) was similar to the variation found for the 186 trees of the full-sib family
15 (0.63‰ standard deviation). Similarly, for MRW, the average of standard deviations
16 within half-diallel families was 0.94 mm, close to the 0.99 mm standard deviation
17 found for the full-sib family. Using Equations 2 and 3, the measured $\delta^{13}\text{C}$ values
18 transformed into a range of WUE of 67 $\mu\text{mol CO}_2 / \text{mol H}_2\text{O}$ to 100 $\mu\text{mol} / \text{mol}$ for
19 the half-diallel and a range of 65 $\mu\text{mol} / \text{mol}$ to 95 $\mu\text{mol} / \text{mol}$ for the full-sib. This
20 represents for the half diallel and the full-sib a variation from one to one-and-a half
21 times the WUE.

22

23 Heritabilities and quantitative trait dissection analysis

24 The heritabilities for mean ring width and $\delta^{13}\text{C}$ were found to be significant, similar
25 between the two traits and of rather moderate value. Therefore selective crossings
26 can improve growth and WUE. The heritabilities for ring width are comparable to
27 values found in the literature for maritime pine or other conifers. Danjon (1994)
28 found for maritime pine trees from the same provenance as used in the present study
29 narrow sense heritabilities for diameter growth ranging from 0 to 0.45 for different
30 experimental set-ups (40 to 100 half or full-sib families). Blada (1999) found for a
31 *Pinus cembra* L. 10x10 full-diallel narrow sense heritabilities for diameter from 0.23
32 to 0.32 and broad sense heritabilities from 0.50 to 0.59.

33 For $\delta^{13}\text{C}$ there are not yet any publications known to the authors that estimated
34 heritability for maritime pine, and there are only a few publications of estimates of
35 heritability for $\delta^{13}\text{C}$ for other species. Narrow sense heritability estimates by Johnsen
36 *et al.* 1999 for *Picea mariana* are lower for diameter growth (0.14) than for $\delta^{13}\text{C}$
37 (0.54). For non-woody species, heritabilities for $\delta^{13}\text{C}$ can be high (broad sense
38 heritabilities for *Lens culinaris* Medikus 0.73, Matus *et al.* 1995 and for *Agropyron*
39 *desertorum* (Fischer ex Link) Schultes 0.90, Asay *et al.* 1998), however it was shown
40 that water stress could reduce the heritability of $\delta^{13}\text{C}$ (Ehdaie & Waines 1994,
41 Johnson *et al.* 1990). An explanation for the moderate heritabilities found in the
42 present study could therefore be the integrative properties of $\delta^{13}\text{C}$ measured on
43 cellulose of several rings, together with the possibility of frequent water stress. The
44 present study was located in the south-west of France, where summer drought is

2

1common (Nguyen-Queyrens *et al.* 1998). The half diallel was created from the
2descendants of trees selected for growth vigour. This might have restricted the
3genetic base compared to natural populations and hence lowered the detectable
4heritability of growth.

5Existing QTLs for maritime pine were localized for traits related to growth (Plomion,
6Durel & O'Malley 1996, Gerber, Lascoux & Kremer 1997). We were able to provide
7here the first example of QTL observations for $\delta^{13}\text{C}$ in a forest tree species. The four
8significant QTLs found for $\delta^{13}\text{C}$ explained nearly one-third of the phenotypic
9variation observed for this trait. Several experiments (Prioul *et al.* 1997) have shown
10that even for complex traits, such as growth or carbon isotope discrimination, the
11expected number of major loci is quite small, a small number of genetic factors is
12predominantly determining a quantitative trait. No co-localisations of QTLs for $\delta^{13}\text{C}$
13and QTLs for MRW were observed, suggesting no common genetic control for these
14two traits. However, underestimation of number of QTL is inherent to the
15methodology of QTL detection.

16

17Relationships between $\delta^{13}\text{C}$ and MRW

18The phenotypic correlations between $\delta^{13}\text{C}$ and growth (ring width) found for the half
19diallel and full-sib experimental designs are significant with moderate coefficients of
20correlation (Figure 2a, Figure 3, Table 3) and the estimated regressions are similar in
21slope and intercept. Among trees in the same environmental conditions, this suggests
22that an increased growth relates to a higher WUE. Depending on a plants'
23physiology, a difference in WUE could be predominantly determined by stomatal
24conductance and/or by assimilation rate. The Farquhar model of carbon isotope
25discrimination (Farquhar, Ehleringer & Hubick 1989) predicts that an increasing
26photosynthetic capacity will decrease Δ . Positive as well as negative correlations
27have been found between photosynthesis and growth (Johnsen *et al.* 1995), however
28when assuming a positive correlation between photosynthetic capacity and growth, a
29positive correlation between $\delta^{13}\text{C}$ and growth could suggest a predominantly
30assimilation rate based control of $\delta^{13}\text{C}$. This is in agreement with results for black
31spruce: differences among families were found to be mainly determined by
32differences in photosynthesis (Johnsen *et al.* 1995), whereby differences in
33photosynthesis were rather the result of non-stomatal limitations than of stomatal
34limitation (Major *et al.* 1996). If $\delta^{13}\text{C}$ would be controlled by stomatal conductance,
35the Farquhar model predicts a negative correlation between $\delta^{13}\text{C}$ and growth.
36Therefore, the positive correlation between $\delta^{13}\text{C}$ and growth suggests that the
37variation of WUE among the measured trees is rather controlled by assimilation than
38by stomatal conductance. This was the case for the half diallel as well as for the full-
39sib family.

40The calculated genetic and environmental correlations between $\delta^{13}\text{C}$ and MRW
41indicate that the phenotypic correlation is mainly based on environmental influence.
42This result is in agreement with the lack of colocalisations between QTLs for $\delta^{13}\text{C}$
43and for MRW. The bloc effect was included in the estimation of the genetic
44parameters, hence the environmental correlation is probably due to micro-
45environmental influences on each individual tree. As the model also accounts for any

1 type of genetic effect, including intra-family genetic variation, the observed large
2 environmental variation therefore suggests for the two measured traits a high
3 sensitivity to micro-environmental conditions. This also suggests for growth and
4 water use efficiency a high non-genetic plasticity to adjust to environmental
5 conditions. The strong environmental correlation that was found for the two traits is
6 therefore probably due to a substantial environmental influence of less negative $\delta^{13}\text{C}$
7 with increased growth and *vice versa*.

8 The non-significant genetic correlation in the presented study is in opposition to the
9 strong genetic correlation between $\delta^{13}\text{C}$ and tree growth (height and diameter) found
10 for black spruce (Johnsen *et al.* 1999). Several factors might have contributed to this
11 discrepancy. 1) Johnsen *et al.* (1999) suggested assimilation rate as common control
12 for $\delta^{13}\text{C}$ and growth. However, correlations between assimilation rate and growth
13 found in the literature range from negative over non significant to positive
14 relationships (Johnsen *et al.* 1995). Therefore, even if $\delta^{13}\text{C}$ is determined by
15 assimilation rate, if growth is not determined by assimilation rate, then there might
16 be no correlation between $\delta^{13}\text{C}$ and growth. 2) Furthermore, genetic control was
17 rather moderate for both traits, which might have lowered the significance of a
18 genetic correlation. 3) It has also to be taken into account that in the present study
19 carbon isotope discrimination measured on cellulose of main stem wood was
20 compared to the average diameter growth of four years, whereas Johnsen *et al.*
21 (1999) compared $\delta^{13}\text{C}$ of needle material with height or trunk diameter. These
22 complex traits might include the action of a number of slightly different genes and a
23 common genetic control might exist for the combination of needle $\delta^{13}\text{C}$ to height or
24 diameter and not for the combination of trunk cellulose to mean annual growth of the
25 same growth period.

26 The lack of a genetic correlation between $\delta^{13}\text{C}$ and MRW found in the half-diallel is
27 in agreement with the lack of co-localizations between QTLs for $\delta^{13}\text{C}$ and for MRW
28 found in the full-sib experimental design. Albeit the lack of a genetic correlation,
29 both traits were found to be heritable and significant QTLs were detected. This opens
30 new perspectives for the investigation of the genetic determinism of water use
31 efficiency and the identification of groups of genes involved in drought responses.

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7

1 Tables

2 Table 1 Means, standard deviations (SD), coefficients of variation (SD / mean),
 3 ranges (maximum – minimum) and probability (p-value in %; * significant at 5%
 4 level; ns not significant) of block and family effects (Equation 4) of the two
 5 experimental designs for $\delta^{13}\text{C}$ (in [‰]) and mean ring width (MRW; in [mm])

		N	Mean	SD	Coef. of Var.	Range	Block effect	Family effect
$\delta^{13}\text{C}$	Half diallel	564	-26.21	0.61	0.02	3.64	2.8*	2.3*
	Full-sib	186	-26.48	0.63	0.02	3.23	0.0*	---
MRW	Half diallel	564	2.88	1.10	0.38	6.77	ns	0.2*
	Full-sib	186	2.11	0.99	0.47	5.15	0.9*	---

6

7 Table 2 Half diallel: Heritabilities for $\delta^{13}\text{C}$ and mean ring width (MRW) with
 8 standard deviations and 95% confidence interval in parentheses. As there were no
 9 dominance effects detected and therefore additivity is 100%, the narrow sense (ns)
 10 and broad sense (bs) heritabilities are equal.

	$h^2_{ns} = h^2_{bs}$	%additivity
$\delta^{13}\text{C}$ [‰]	0.17 ± 0.06 (0.06 – 0.29)*	100%
MRW [mm]	0.19 ± 0.06 (0.06 – 0.31)*	100%

11* significant at the 5% level

12

13 Table 3 Half diallel: Correlations between mean ring width and $\delta^{13}\text{C}$; r is the
 14 correlation coefficient on the family level, estimated by OPEP software with
 15 standard deviation and 95% confidence interval in parentheses; r' is the correlation
 16 coefficient estimated by linear regression analysis using the calculated individual tree
 17 breeding values which are shown in Figure 2.

	r	r'
Phenotypic correlation	0.45 ± 0.057 (0.33 – 0.56)*	0.46*
Genetic correlation	0.27 ± 0.21 (0.07 – 0.15) NS	0.02 NS
Environmental correlation	0.52 ± 0.16 (0.20 – 0.83)*	0.79*

18* significant at the 5% level; NS : not significant

Table 4 Full sib: Results of the composite interval mapping analysis for $\delta^{13}\text{C}$ and 2mean ring width (MRW) using MultiQTL software. The p-value associated with the 3LODs were calculated using 1000 permutations of the data, standard deviation of 4position (SD) was calculated using the bootstrap method (1000 permutations); in 5case of a significant QTL-pair for a chromosome, Lod and p-value are given for the 6tests of (I) two QTLs *versus* no QTLs and (II) two QTLs *versus* one QTL (difference 7of Lod for two and for one QTL).

	map	Chr ^a	N ^b	Position \pm SD ^c	Lod	P-value ^d	Dir. ^e	R ²	R ² _{total}
$\delta^{13}\text{C}$	male	3a	149	4.3 \pm 13.2	1.78	0.021*	+	0.047	0.268
	male	6	164	102.7 \pm 21.6	4.40	0.001***	+	0.124	
	male	8	85	0.0 \pm 15.7	1.85	0.021*	-	0.050	
	male	9	183	104.6 \pm 28.5	1.90	0.033*	-	0.047	
	female	2	84	209.1 \pm 56.4	2.30	0.019*	+	0.065	0.246
	female	5	164	99.9 \pm 22.0	1.98	0.0033***	-	0.062	
	female	12	153	1: 0.0 \pm 36.7 2: 135.4 \pm 27.1	I: 4.24 II: 1.88	I: 0.002*** II: 0.036*	- +	0.119	
MRW	male	2b	180 153	1: 47.2 \pm 15.9 2: 51.8 \pm 11.1	I: 2.49 II: 1.57	I: 0.048* II: 0.027*	- +	0.181	0.429
	male	5	145	56.8 \pm 33.4	2.41	0.022*	-	0.065	
	male	6	174 168	1: 7.5 \pm 31.0 2: 133.7 \pm 22.0	I: 4.24 II: 2.68	I: 0.001*** II: 0.002*	- +	0.124	
	male	11	165	11.6 \pm 30.9	1.73	0.040*	+	0.059	

8^a chromosome ID

9^b number of full-sibs with available data for QTL detection

10^c Lod score peak position (from the top) of the chromosome (cM)

11^d Probability for the null hypothesis of no QTL at the chromosome level

12 *** : probability corresponding to a 5% genome wise type I error (significant QTL)

13 * : probability corresponding to a 5% chromosome type I error (suggestive QTL)

14^e direction of influence of presence of the allele for each QTL

1Figure legends

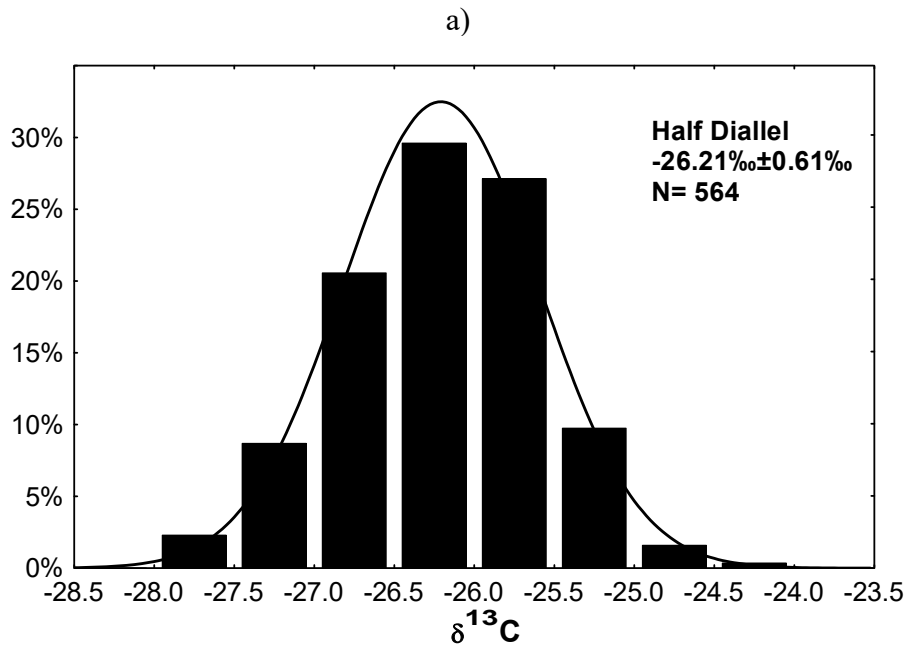
2**Figure 1** Distribution of cellulose $\delta^{13}\text{C}$ (raw data; not corrected for block effect) for
3a) the half diallel experiment and b) the full-sib experiment; parameters for the
4normal distributions as in Table 1

5**Figure 2** Linear correlations between mean ring width (MRW) and $\delta^{13}\text{C}$ for the half
6diallel experiment using data corrected with the individual tree breeding values
7(OPEP software): a) phenotypic, b) genetic (additive effects) and c) environmental
8correlations; all data are adjusted for block effect, data for genetic and environmental
9correlations are centred and standardized by the mean.

10**Figure 3** Phenotypic linear correlation between mean ring width (MRW) and $\delta^{13}\text{C}$
11for the full-sib experiment; data adjusted for block effect

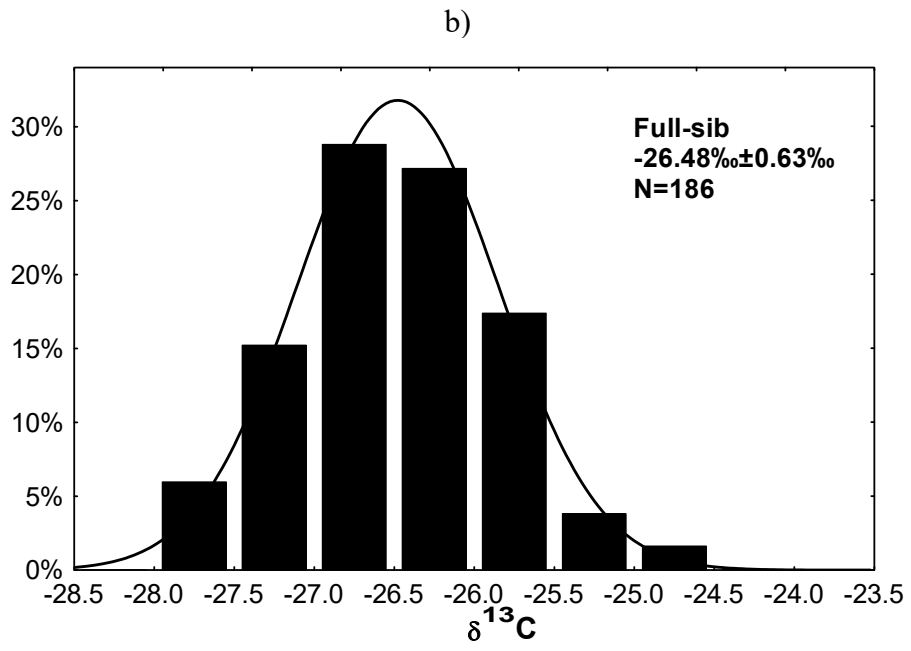
1 **Figure 1 a b**

2



3

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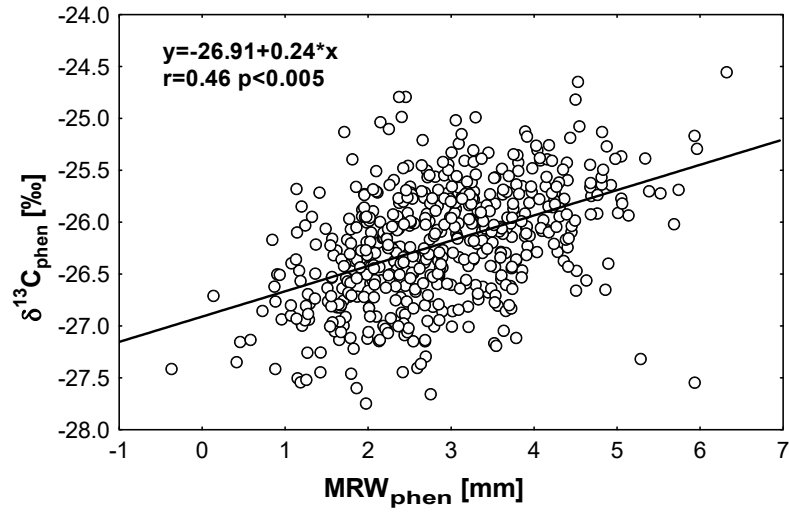


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1 **Figure 2 a b c**

2

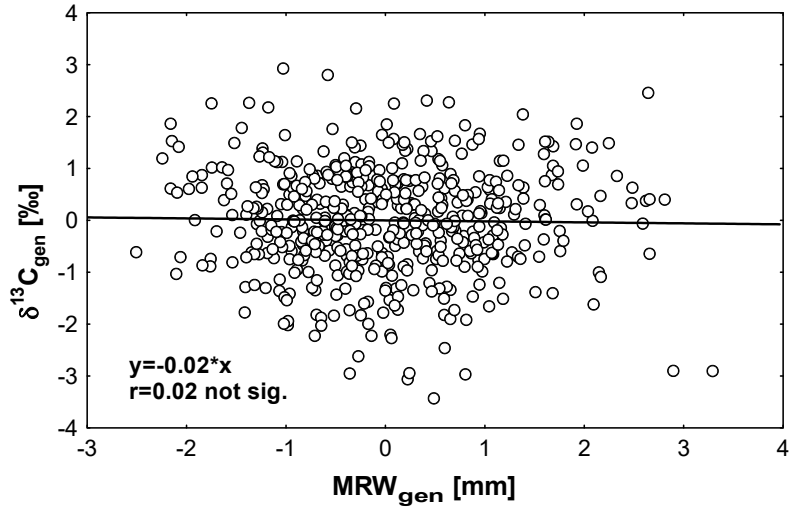
a) phenotypic correlation



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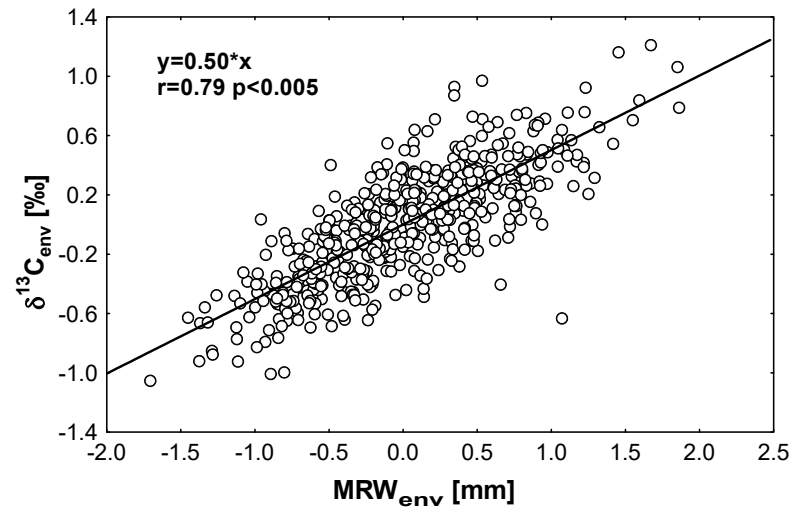
b) genetic correlation



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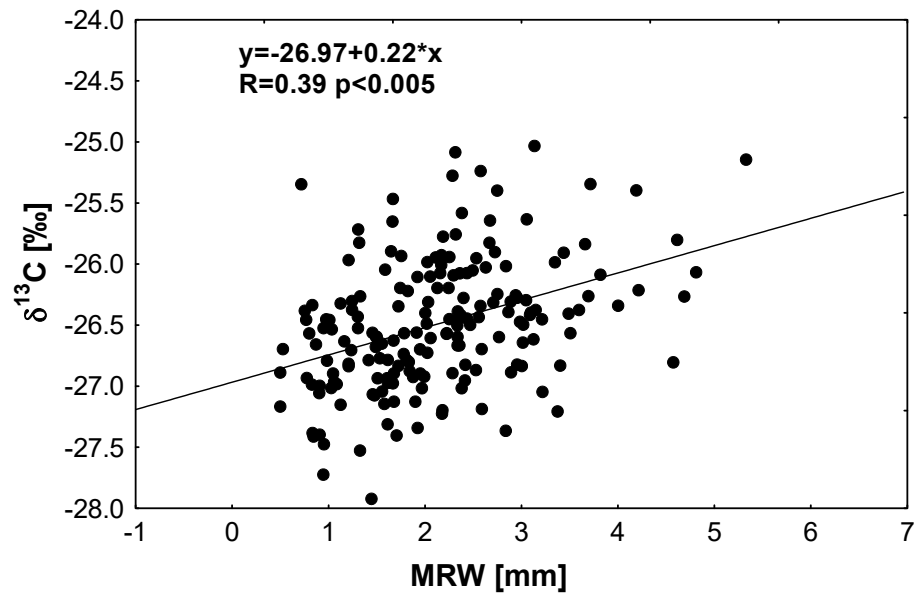
6

c) environmental correlation



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2

1 **Figure 3**2
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4