

Genetic Parameter Estimates for Diameter Growth, Pilodyn Penetration and Spiral Grain in *Picea abies* (L.) KARST.

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Abstract

Univariate and multivariate Restricted Maximum Likelihood procedures were applied to estimate (co)variance components and related genetic parameters for diameter growth, pilodyn penetration and spiral grain in *Picea abies*. The data are from 15 to 18-year-old progeny tests, comprising cross- and open-pollinated families.

Narrow-sense heritabilities ranged between 0.09 and 0.27 for diameter, 0.18 and 0.36 for raw pilodyn readings, and 0.29 and 0.47 for spiral grain. Adjusting raw pilodyn readings to a common diameter, by means of covariance analysis, improved the heritability (ranging from 0.30 to 0.62) of the indirect measure of wood density.

The additive genetic correlations between growth and the wood properties were adverse, being less marked between diameter and spiral grain. Pilodyn showed low and favourable additive genetic correlations with spiral grain. Reasonably strong genotype by environment interactions were found for diameter but not for the wood properties, giving some support for an environmental zonation of the breeding programme.

Key words: *Picea abies*, open-pollination, controlled crosses, REML, heritability, genetic correlation, genotype by environment interaction.

Introduction

Norway spruce's (*Picea abies* (L.) KARST.) breeding programme in Denmark has been running since the early 1970's (WELLENDOFF, 1988; WELLENDOFF *et al.*, 1994), aiming to improve growth rate, wood quality and health. As a result of this programme, and over a number of years, several trials have been established using open- and cross-pollinated progenies from plus trees selected in mature Danish stands of German origin. The trials reported here constitute, therefore, a great opportunity to determine the degree and type of genetic control (including additive and dominance effects), and the importance of genotype by environment interaction for a number of traits relevant to selection criteria used in the programme. So far, for spiral grain in *Picea* spp., estimates of genetic parameters have been reported for Sitka spruce only (HANSEN and ROULUND, 1997, 1998; HANSEN, 1999). In Norway spruce, there are no genetic parameter estimates available for spiral grain, despite its significant influence on the quality of the sawn timber (DANBORG, 1996).

Unfortunately, pooling data from several trials brings complications to the analysis. In this case, the representation of base parents across trials and the crossing design were unbalanced, and differences in age and growing conditions have resulted in heterogeneous variances amongst sites for a given trait.

The aim of this work was to estimate genetic parameters for diameter growth, pilodyn penetration (an indirect measure of wood density) and spiral grain, using the information available from a large set of open- and cross-pollinated progeny trials, established across a range of sites in Denmark. The analyses used a Restricted Maximum Likelihood (REML, after PATTERSON and THOMPSON, 1971) approach to estimate (co)variance

components, under univariate and multivariate linear mixed models. The genetic model used here accounted for both additive and dominance effects, using pedigree information to link observations. A multivariate REML procedure allowed the estimation of full variance-covariance matrices, through the use of correlated information across different sites or traits. Heterogeneous variances across sites were also modelled.

Material and Methods

Table 1 provides summary information about the analysed groups of replicated progeny tests, namely the identification of field trials, the description of genetic units, the number of common parents across groups, the studied characters and the respective measurement ages. The location of the field trials is illustrated in figure 1.

Table 1. – General information about the analysed series of Norway spruce progeny trials.

Groups	Trials	Locations (planting zone)	Traits measured (ages from field planting in years)	Genetic units
I	F245	Giesegård (east)	diameter (16)	cross-pollinated progenies (nested design) 15 males and 50 females (11 males in common with mother trees in group II)
	F246	Farum (east)	diameter (16) pilodyn (16) spiral grain (18)	
II	F226	Birkebak (west)	diameter (16)	open-pollinated progenies 50 mother trees (11 mother trees in common with males in group I)
	F228	Langesø (east)	diameter (18) pilodyn (18) spiral grain (18)	
	F229	Esrum (east)	diameter (16)	
III	F241	Bræthrolleborg (east)	diameter (16) pilodyn (18) spiral grain (18)	open-pollinated progenies 37 mother trees
	F242	Løvenholm (west)	diameter (15)	
	F243	C.E. Flb. pltg. (west)	diameter (18) pilodyn (18) spiral grain (18)	
	F244	Bjerslev pltg. (west)	diameter (15)	

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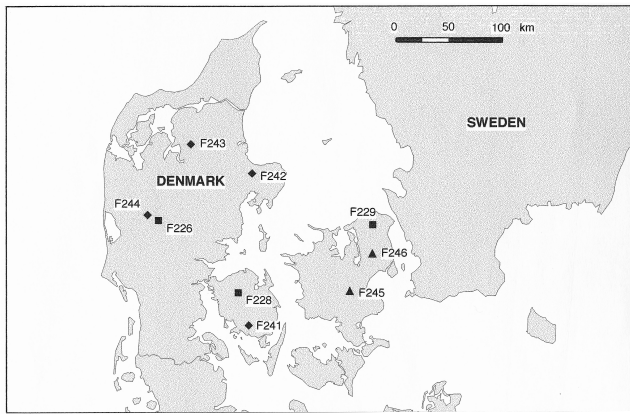


Figure 1. – Location of Norway spruce progeny trials across Denmark. ▲ cross-pollinated families, Group I. ■ open-pollinated families, Group II. ◆ open-pollinated families, Group III.

Plant material

The trials include material which was obtained from three groups of progeny. In group I, each of a set of 15 male parents was crossed with a subset of female parents, following a nested mating scheme. The crossing design was however unbalanced, with the majority of the subsets having 4 females mated to each male. Groups II and III are open-pollinated progenies from 50 and 37 female parents, respectively. All base parents are plus trees selected in mature Danish stands from west-continental seed sources (German origin).

The male parents in group I and the female parents in groups II and III were selected in two stages. Initially, 800 trees were selected for growth and health, with some consideration given to finer branches and straighter stems. One hundred genotypes were finally chosen for higher *density level* (following the terminology of OLESEN, 1976, to refer to basic density adjusted for the effect of differences in ring width), and were then grafted and allocated to two replicated clonal seed orchards (known locally as FP240 and FP241). The female parents used in the crossings of group I were selected on the basis of visual criteria only.

The parents forming the three series are only partially connected. Eleven of the 15 males in group I are also female parents (out of a total of 50) in group II. Group III has 37 females which are not present in groups I and II. There is a common reference bulked seedlot from Rye Nørskov (F300) also of west-continental origin.

The series of trials reported here include 90 out of 100 parents represented in the seed orchards, plus a male parent in group I that belongs to the general breeding population. Material from group I was tested at two sites, from group II at three sites and from group III at four sites.

Field layout

The experimental layout of all field trials is a split-plot design.

In group I, the families with a male parent in common were randomly allocated within a given main plot. The main plots were randomized within a large block. There were 6 blocks. The reference population (F300, Rye Nørskov) was represented in all main plots. Within a main plot, each full-sib family was represented by 4 trees in a row subplot. The trees were planted at a spacing of 1.5 m x 1.5 m. The trials were established in 1982.

In groups II and III, the open-pollinated families from parents selected in the same stand were randomly assigned

within a given main plot. The main plots were randomly allocated in each of 4 and 6 blocks in groups II and III, respectively. In each main plot, a bulked seedlot collected from the same stand location and the common reference population (F300, Rye Nørskov) were also present. Within a main plot, the families were represented by 2 x 4 tree subplots in II, and by 1 x 4 tree subplots in III. The spacing was 1.6 m x 1.5 m in II, and 1.5 m x 1.5 m in III. The trials in II and III were established in 1980 and 1981, respectively.

A systematic thinning was carried out in F228, F229, F241 and F243 at ages 14 or 15 years from planting. The trial F241 was further selectively thinned at age 17, by removing trees with poor growth.

Characters assessed

The variables measured were *diameter* at breast-height (DM), *spiral grain* (SG) and *pilodyn* penetration (PL).

SG was measured in standing trees, following a methodology similar to that described by HARRIS (1984) and HANSEN (1999). The grain direction on the tangential plane was marked with a freely pivoted needle penetrating through the bark, and a protractor with a spirit level was then used to measure the grain angle with respect to the vertical. As in other spiral grain studies with spruce (DANBORG, 1994a; HANSEN, 1999), positive and negative values were assigned to readings corresponding to left and right-handed directions, respectively. Two readings based on opposite radii were made in each tree, in order to cancel the measurement error associated with a stem axis leaning from the vertical: the average of the two observations was taken to obtain the true grain angle relative to the stem axis (BRAZIER, 1965; HARRIS, 1984; HANSEN, 1999). Depending on tree growth, the sampling position was either between the 10th and 11th or between the 11th and 12th branch whorls counted from the top. Therefore, the measurements refer to the ring numbers 11 or 12 from the pith.

PL was taken as the average of two readings made in opposite directions at breast-height, after removing the bark. PL had a spring strength of 6 J, and a 2.0 mm pin diameter was used.

Data analysis

Single-site analyses of variance components were carried out for PL and SG, in trials F246 and F228. For the cross-pollinated progenies (F246), the fitted linear model was:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{s} + \mathbf{W}_1\mathbf{m} + \mathbf{W}_2\mathbf{p} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is the vector of individual tree observations; $\mathbf{b}$ is the vector of fixed block effects; $\mathbf{a}$ is the vector of random additive genetic effects of individual trees; $\mathbf{s}$ is the vector of random non-additive genetic effects due to the specific combinations of males with females; $\mathbf{m}$ is the vector of random main plot effects; $\mathbf{p}$ is the vector of random subplot effects; $\mathbf{e}$ is the vector of random residual deviations of individual trees; $\mathbf{X}$, $\mathbf{Z}_1$, $\mathbf{Z}_2$, $\mathbf{W}_1$ and $\mathbf{W}_2$ are incidence matrices relating the observations to the model effects. It is assumed that the random terms are jointly normal with moments:

$$E(\mathbf{a}) = E(\mathbf{s}) = E(\mathbf{m}) = E(\mathbf{p}) = E(\mathbf{e}) = \mathbf{0}$$

$$\text{VAR} \begin{bmatrix} \mathbf{a} \\ \mathbf{s} \\ \mathbf{m} \\ \mathbf{p} \\ \mathbf{e} \end{bmatrix} = \mathbf{A}\sigma_a^2 \oplus \mathbf{I}\sigma_s^2 \oplus \mathbf{I}\sigma_m^2 \oplus \mathbf{I}\sigma_p^2 \oplus \mathbf{I}\sigma_e^2$$

where $\oplus$ is the direct sum of matrices related to the random terms in the model; $\mathbf{A}$ is the additive genetic relationship matrix between trees and $\mathbf{I}$ is an identity matrix; σ_a^2 is the additive genetic variance; σ_s^2 is the variance due to the specific combinations of males with females; σ_m^2 and σ_p^2 are, respectively, the main plot and subplot variances; σ_e^2 is the residual variance. The components σ_s^2 and σ_e^2 are expected to equal $1/4 \sigma_d^2$ and $3/4\sigma_d^2 + \sigma_e^2$ (respectively), where σ_d^2 is the variance due to dominance and σ_e^2 is the variance due to environmental effects within subplots. In the case of the open-pollinated progenies (F228), the $\mathbf{s}$ term was dropped from model (1) and σ_e^2 is expected to equal $\sigma_d^2 + \sigma_e^2$. In both cases, it is assumed that epistatic and maternal effects are negligible.

Multi-site analyses of (co)variance components were accomplished for DM measured in all trials within a given group, and for PL and SG measured in group III (i.e. trials F241 and F243). The approach considers the performance of the same trait in two separate environments as two different traits, and takes into account the genetic covariances across sites. For the cross-pollinated progenies (group I), the linear model fitted for DM was defined as:

$$\begin{bmatrix} \mathbf{y}_i \\ \mathbf{y}_j \end{bmatrix} = \begin{bmatrix} \mathbf{X}_i & 0 \\ 0 & \mathbf{X}_j \end{bmatrix} \begin{bmatrix} \mathbf{b}_i \\ \mathbf{b}_j \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{1i} & 0 \\ 0 & \mathbf{Z}_{1j} \end{bmatrix} \begin{bmatrix} \mathbf{a}_i \\ \mathbf{a}_j \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{2i} & 0 \\ 0 & \mathbf{Z}_{2j} \end{bmatrix} \begin{bmatrix} \mathbf{s}_i \\ \mathbf{s}_j \end{bmatrix} + \begin{bmatrix} \mathbf{W}_{1i} & 0 \\ 0 & \mathbf{W}_{1j} \end{bmatrix} \begin{bmatrix} \mathbf{m}_i \\ \mathbf{m}_j \end{bmatrix} + \begin{bmatrix} \mathbf{W}_{2i} & 0 \\ 0 & \mathbf{W}_{2j} \end{bmatrix} \begin{bmatrix} \mathbf{p}_i \\ \mathbf{p}_j \end{bmatrix} + \begin{bmatrix} \mathbf{e}_i \\ \mathbf{e}_j \end{bmatrix} \quad (2)$$

where $\mathbf{y}_i$ and $\mathbf{y}_j$ are, respectively, the vectors of individual tree observations in site i (F245) and j (F246); the $\mathbf{b}$ vectors refer to fixed effects (sites and blocks within sites); $\mathbf{a}$, $\mathbf{s}$, $\mathbf{m}$, $\mathbf{p}$, $\mathbf{e}$ correspond to random effects, and $\mathbf{X}$, $\mathbf{Z}_1$, $\mathbf{Z}_2$, $\mathbf{W}_1$, $\mathbf{W}_2$ are incidence matrices, all defined as before. On the assumption of multivariate normality, the random terms in model (2) have expectations

$$E \begin{bmatrix} \mathbf{a}_i \\ \mathbf{a}_j \end{bmatrix} = E \begin{bmatrix} \mathbf{s}_i \\ \mathbf{s}_j \end{bmatrix} = E \begin{bmatrix} \mathbf{m}_i \\ \mathbf{m}_j \end{bmatrix} = E \begin{bmatrix} \mathbf{p}_i \\ \mathbf{p}_j \end{bmatrix} = E \begin{bmatrix} \mathbf{e}_i \\ \mathbf{e}_j \end{bmatrix} = 0$$

and a variance-covariance matrix defined as

$$\begin{bmatrix} \mathbf{A}\sigma_{a_{ii}}^2 & \mathbf{A}\sigma_{a_{ij}}^2 \\ \mathbf{A}\sigma_{a_{ij}}^2 & \mathbf{A}\sigma_{a_{jj}}^2 \end{bmatrix} \oplus \begin{bmatrix} \mathbf{I}\sigma_{s_{ii}}^2 & \mathbf{I}\sigma_{s_{ij}}^2 \\ \mathbf{I}\sigma_{s_{ij}}^2 & \mathbf{I}\sigma_{s_{jj}}^2 \end{bmatrix} \oplus \begin{bmatrix} \mathbf{I}\sigma_{m_{ii}}^2 & 0 \\ 0 & \mathbf{I}\sigma_{m_{jj}}^2 \end{bmatrix} \oplus \begin{bmatrix} \mathbf{I}\sigma_{p_{ii}}^2 & 0 \\ 0 & \mathbf{I}\sigma_{p_{jj}}^2 \end{bmatrix} \oplus \begin{bmatrix} \mathbf{I}\sigma_{e_{ii}}^2 & 0 \\ 0 & \mathbf{I}\sigma_{e_{jj}}^2 \end{bmatrix}$$

where the off-diagonal elements of the two first square matrices in the direct sum are covariances between the trait measured in the two sites, namely due to additive genetic effects ($\sigma_{a_{ij}}$) and due to effects related to the specific combinations of males with females ($\sigma_{s_{ij}}$ expected to equal $1/4$ of the dominance covariance). The residual, main plot and subplot effects were assumed to be uncorrelated across sites, thus with zero covariances in diagonal matrices. Again, for open-pollination data (groups II and III), the $\mathbf{s}$ term was not fitted. For DM measured in groups II and III, the homogeneity of the additive genetic correlations across sites was judged by comparing the log likelihood of the model defined as above (i.e. using an unstructured variance-covariance matrix to fit the additive genetic effects) with that of an equal correlation model. In this model, a uniform correlation matrix with one parameter is pre and post multiplied by a diagonal matrix of order n (n = number of sites), where the elements are the additive genetic standard deviations at each site.

Covariance analysis was also applied for raw PL measures by using DM growth as a covariate, so that genetic effects in whole-ring density could be evaluated at an overall mean ring width for the site and sampling height considered.

Multiple trait analyses were carried out for each site, to estimate covariances between two different traits i and j measured in the same individual. Here, non-zero covariances between traits are expected for residual, main plot and subplot terms. Thus, the parameters are defined as in model (2), but with the following modifications in the variance-covariance matrix:

$$\text{VAR} \begin{bmatrix} \mathbf{m}_i \\ \mathbf{m}_j \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_{m_{ii}}^2 & \mathbf{I}\sigma_{m_{ij}}^2 \\ \mathbf{I}\sigma_{m_{ij}}^2 & \mathbf{I}\sigma_{m_{jj}}^2 \end{bmatrix}$$

$$\text{VAR} \begin{bmatrix} \mathbf{p}_i \\ \mathbf{p}_j \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_{p_{ii}}^2 & \mathbf{I}\sigma_{p_{ij}}^2 \\ \mathbf{I}\sigma_{p_{ij}}^2 & \mathbf{I}\sigma_{p_{jj}}^2 \end{bmatrix}$$

$$\text{VAR} \begin{bmatrix} \mathbf{e}_i \\ \mathbf{e}_j \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_{e_{ii}}^2 & \mathbf{I}\sigma_{e_{ij}}^2 \\ \mathbf{I}\sigma_{e_{ij}}^2 & \mathbf{I}\sigma_{e_{jj}}^2 \end{bmatrix}$$

where the off-diagonal elements are covariances for main plot, subplot and residual effects.

In all analyses, the main plot and/or subplot terms were dropped from the model when corresponding (co)variances were not significantly different from zero. This was determined on the basis of a *component/standard error* less than 1, or by the likelihood-ratio test (KENDALL and STUART, 1979) when the ratio *component/standard error* was between 1 and 2. The bulked stand seedlots in groups II and III and the common reference seedlot were not included in the analysis. In groups II and III, previous analyses showed that the variance between the base populations was small and not significant for the studied traits. Thus, a stand effect was not fitted in the models for analysing the data from groups II and III, on the assumption that the parents are from a single base population with an average breeding value of zero and a common genetic variance.

REML estimates of (co)variance components and their standard errors were obtained with the ASREML programme (GILMOUR *et al.*, 1999), which uses the Average Information REML algorithm described by GILMOUR *et al.* (1995). Narrow-sense heritabilities and residual, additive genetic and phenotypic correlations were estimated according to standard formulae (FALCONER and MACKAY, 1996). The standard errors of the estimates were calculated from variances of ratios, using an approximation based on a Taylor series expansion. For each trait and trial, the coefficient of genetic variation was calculated as the ratio between the *additive genetic standard deviation* and the *mean*. The sampling coefficient of variation of the heritability was calculated as the ratio between the heritability *standard error* and the *estimate*, and is used to compare the accuracy of the estimates.

Results

Variance components and heritability estimates

Table 2 lists the estimated trial means and variance components for DM, PL and SG. The narrow-sense heritabilities and their sampling coefficients of variation are given in table 3.

The average grain angle varied from 1.4° in trial F243 to 2.5° and 2.7° in the other examined trials (Table 2). The spirality was predominantly left-handed in the grain data, with a few sampled trees exhibiting a weak level of right-handed spirality.

Table 2. – Estimated trait means and variance components (with the ratio component/standard error in parenthesis). For group I, the residual variances refer to environmental effects within subplots only (i.e. 3/4 the dominance variance were extracted from the total variance due to residual deviations of individual trees). For groups II and III, the residual variances contain both environmental and dominance effects. In both cases, epistasis was assumed to be absent. For pilodyn, the results at the top correspond to raw measures and those at the bottom to adjusted values using diameter as a covariate. The main plot and subplot terms were dropped from the model when the variances were not significantly different from zero, as judged by a) a ratio component/standard error less than 1 or by b) the likelihood-ratio test when the ratio component/standard error was between 1 and 2.

Trait	Group	Trial	Mean	Variance components						Coefficient of genetic variation (%)
				Additive	Dominance	Main plot	Subplot	Residual	Phenotypic	
Diameter (mm)	I	F245	102.9	54.8 (1.3)	250.9 (2.7)	a)	b)	171.1	476.8	7.2
		F246	104.4	70.4 (1.3)	206.5 (2.0)	a)	a)	375.4	652.3	8.0
	II	F226	53.3	36.1 (2.5)	—	15.2 (2.9)	a)	192.3	243.6	11.3
		F228	112.6	176.7 (2.7)	—	a)	a)	475.1	651.8	11.8
		F229	100.4	140.1 (2.1)	—	a)	b)	549.5	689.6	11.8
	III	F241	97.4	81.1 (1.8)	—	28.2 (1.8)	a)	210.2	319.5	9.2
		F242	76.9	33.7 (1.3)	—	17.5 (1.8)	a)	317.2	368.4	7.6
		F243	108.1	75.9 (2.0)	—	a)	a)	318.5	394.4	8.1
		F244	66.7	31.9 (2.2)	—	12.0 (2.8)	a)	102.4	146.3	8.5
Pilodyn (mm)	I	F246	21.7	1.16 (1.2)	3.09 (1.7)	a)	0.54 (3.1)	1.72	6.51	5.0
				1.31 (1.5)	1.12 (0.8)	a)	0.57 (4.4)	1.39	4.39	5.3
	II	F228	19.3	1.80 (2.3)	—	0.56 (2.4)	0.44 (2.0)	3.31	6.11	7.0
				1.30 (2.4)	—	0.25 (1.9)	0.31 (2.1)	2.19	4.05	6.0
	III	F241	20.3	2.10 (1.7)	—	b)	a)	3.68	5.78	7.1
				2.77 (2.4)	—	a)	a)	1.68	4.45	8.2
	III	F243	18.6	1.12 (2.0)	—	a)	0.47 (2.2)	2.94	4.53	5.6
				1.17 (2.3)	—	a)	0.41 (2.6)	1.81	3.39	5.9
Spiral grain (degrees)	I	F246	2.7	1.21 (1.9)	0.24 (0.3)	b)	0.14 (2.2)	1.00	2.59	40.7
	II	F228	2.6	1.04 (3.0)	—	0.16 (2.1)	b)	1.58	2.78	39.2
	III	F241	2.5	0.99 (1.8)	—	a)	a)	1.80	2.79	39.8
	III	F243	1.4	0.38 (2.4)	—	a)	b)	0.92	1.30	44.0

This reflects the general pattern of spirality in Norway spruce, where the grain angle is typically left-handed in the juvenile wood, and gradually becomes straight or even right-handed with distance from the pith (DANBORG, 1994a).

For DM, the coefficient of genetic variation (CGV) varied across sites between 7.2% and 11.8%. However, the trials in group I and F242 of group III had lower CGV, as well as a lower variance component/standard error ratio (Table 2) for the additive genetic effects. For raw PL measures, the CGV ranged between 5.0% and 7.1% and, for PL adjusted for differences in DM, it varied between 5.3% and 8.2%. In both cases, the CGVs and also the variance component/standard error ratios for the additive genetic effects were again lower in group I (Table 2). For SG, the CGV was much higher, ranging between 39.2% and 44.0%. A lack of statistical significance for the additive genetic effects, as given by the likelihood-ratio test, was only found for DM in trials F242 and F246.

Dominance effects were substantial in group I, being larger than the additive genetic effects for DM and raw PL measures, but negligible for SG (Table 2). The dominance component accounted for 53% and 32% of the phenotypic variance in DM, and for 48% of the phenotypic variance in raw PL measures.

Wood density was adjusted to a common mean ring width at breast-height based on a covariance analysis of the raw PL measures, and using DM as the covariate. In F228, the significance of the additive genetic variance was larger (i.e. with ratios component/standard error of 2.3 and 2.7, Table 2) for both raw PL measures and DM, and the adjustment by covariance analysis reduced somewhat the additive genetic variance for the dependent variable. As noted by STEEL and TORRIE (1980), covariance analysis may remove part of the treatment effects when they also have an influence on the regressor variable. However, covariance analysis in F228 removed from the residual variance a substantial portion (i.e. 34%) of the variation in

Table 3. – Narrow-sense heritabilities for diameter, pilodyn and spiral grain in different series of Norway spruce progeny trials. The sampling coefficient of variation (in %) of the estimates is given in parenthesis. For pilodyn, the estimates refer to raw measures and to adjusted values, using diameter as a covariate.

Groups	Trials	Diameter	Pilodyn		Spiral grain
			Raw	Adjusted	
I	F245	0.12 (75.0)			
	F246	0.11 (72.7)	0.18 (83.3)	0.30 (63.3)	0.47 (46.8)
II	F226	0.15 (40.0)			
	F228	0.27 (33.3)	0.30 (40.0)	0.32 (40.6)	0.38 (31.6)
	F229	0.20 (45.0)			
III	F241	0.25 (56.0)	0.36 (55.6)	0.62 (38.7)	0.36 (52.8)
	F242	0.09 (77.8)			
	F243	0.19 (52.6)	0.25 (48.0)	0.35 (40.0)	0.29 (41.4)
	F244	0.22 (45.5)			

PL associated with DM, and increased slightly the additive genetic variance/standard error ratio. A similar tendency was observed in F241, where the decrease of the residual variance was larger (i.e. 54%), probably reflecting the low and non-significant genetic variation of the covariate (i.e. diameter at age 18, see *Discussion*). In trial F246, the reduction was even larger (i.e. 64%) for the dominance variance component of the adjusted PL, but was marginal (i.e. 19%) for the environmental variance within subplots. Covariance analysis increased the heritabilities for PL and usually improved the accuracy of the estimates, as indicated by their smaller sampling coefficients of variation (Table 3).

Narrow-sense heritabilities ranged between 0.09 to 0.27 for DM, 0.18 and 0.36 for raw PL measures, 0.30 and 0.62 for the adjusted PL and 0.29 and 0.47 for SG (Table 3).

Correlations between traits

Table 4 shows the correlation estimates between traits measured at the same age in a given trial. Due to a better sample representation (i.e. a larger number of half-sib families), the additive genetic correlations were estimated using the open-pollinated progenies only. The residual and phenotypic correlations were estimated using data from all trials.

As expected, the phenotypic correlations had lower standard errors than their additive genetic and residual counterparts. The phenotypic correlations between DM and raw PL measures were always positive and fairly stable in magnitude, ranging from 0.50 to 0.60. The phenotypic correlations between SG and DM were 0.13 and 0.20, indicating that left-handed grain inclinations tend to be kept in fast growing trees. For PL measures (both raw and adjusted) and SG, the phenotypic correlations were close to zero.

The residual correlations between SG and PL (both raw and adjusted) were low and generally negative (ranging from 0.0 to –0.26), in contrast to the positive genetic estimates (ranging from 0.0 to 0.33). For SG and DM, the signs of the residual and genetic correlations pointed out the same tendency as for the phenotypic estimates, but with stronger (i.e. 0.32 and 0.47) and weaker (i.e. 0.14 and 0.03) values for additive genetic and residual components (respectively).

The residual correlations between raw PL and DM were all high and positive, ranging from 0.54 to 0.67. However, the additive genetic correlation between these traits in F243 was substantially lower (i.e. 0.19) than that in F228 (i.e. 0.80) at the same age.

Correlations across sites

Table 5 presents the additive genetic correlations estimated for DM across sites within each group. As the heritability estimates were rather low for both of the sites in group I, the correlation due to dominance effects is also shown for comparison.

Strong correlations were found for F245 and F246 in group I (i.e. 1.13 and 0.99 for additive and dominance terms, respectively) and, in group II, between F228 and F229 (i.e. 0.84) and between F226 and F228 (i.e. 0.87). The correlations dropped between sites of greater geographical contrasts (i.e. F226 and F229, Figure 1) with an estimate of 0.38 (Table 5). However, applying an equal correlation structure across all sites in group II (data not shown), with a common parameter estimate of 0.74, did not reduce substantially (i.e. –1.5) the log likelihood when compared with the model using the full variance-covariance structure to fit the additive genetic terms.

In group III, F241 tended to be poorly correlated with all trials, with additive genetic correlations ranging between 0.0 and 0.42 (Table 5). Combining all sites in group III, an equal correlation model resulted in an estimate of 0.27 for the parameter and changed the log likelihood by –3.0 (data not shown) when compared with the model using the full matrix of additive genetic (co)variances. Following the same approach but excluding F241, resulted in a change in log likelihood of only –0.7 and a common correlation of 0.55 (data not shown), thus indicating more homogeneous correlations in the set with 3 trials (i.e. F242, F243 and F244).

Table 4. – Estimates for correlations (with standard errors in parenthesis) between diameter (DM), pilodyn (PL) and spiral grain (SG) measured at the same age in a given field trial. The residual correlations contain both dominance and environmental effects (assuming no epistasis).

Correlation	Trials	DM and raw PL	DM and SG	Raw PL and SG	Adjusted PL and SG
Additive genetic	F228	0.80 (0.11)	0.32 (0.23)	0.33 (0.25)	0.24 (0.25)
	F241	—	—	0.19 (0.42)	0.0 (0.36)
	F243	0.19 (0.33)	0.47 (0.28)	0.33 (0.30)	0.13 (0.29)
Residual	F246	0.67 (0.05)	—	—	—
	F228	0.54 (0.08)	0.14 (0.11)	–0.08 (0.14)	–0.26 (0.15)
	F241	—	—	–0.12 (0.21)	–0.06 (0.29)
	F243	0.63 (0.08)	0.03 (0.10)	0.0 (0.12)	–0.02 (0.14)
Phenotypic	F246	0.58 (0.03)	—	—	—
	F228	0.60 (0.02)	0.20 (0.03)	0.07 (0.04)	–0.06 (0.04)
	F241	—	—	–0.01 (0.06)	–0.03 (0.06)
	F243	0.50 (0.03)	0.13 (0.04)	0.09 (0.04)	0.03 (0.04)

Table 5. – Estimated genetic correlations and covariances across sites for diameter. Dominance correlations are given for group I only, otherwise being additive genetic estimates. The values above the diagonal are correlations, and those below the diagonal are covariances. In parenthesis, the component/standard error ratio is given for the covariances and the standard error is given for the correlations.

Group I			Group II			Group III			
<i>Additive genetic:</i>			<i>Additive genetic:</i>			<i>Additive genetic:</i>			
F245	F246		F226	F228	F229	F241	F242	F243	F244
F245	–	1.13 (0.14)	F226	–	0.87 (0.21)	F241	–	0.42 (0.46)	0.0 (0.38)
F246	69.9 (1.6)	–	F228	69.3 (2.9)	–	F242	21.7 (0.9)	–	0.78 (0.42)
<i>Dominance:</i>			F229	27.1 (1.2)	131.6 (2.7)	F243	0.0 (0.0)	39.4 (1.7)	–
F245	F246					F244	0.0 (0.0)	26.6 (1.8)	15.7 (0.9)
F245	–	0.99 (0.09)							0.32 (0.33)
F246	225.3 (2.6)	–							–

For the wood properties, the estimates of additive genetic correlations across sites were obtained with data from F241 and F243 only, and they were 0.88 for raw PL and 1.28 for SG (Table 6).

Discussion

Variance components and heritability estimates

The lower CGVs found for DM and PL in group I (Table 2), and consequently small heritabilities (Table 3), may reflect

Table 6. – Estimated additive genetic correlations and covariances across sites for raw pilodyn readings and spiral grain. The values above the diagonal are correlations, and those below the diagonal are covariances. In parenthesis, the component/standard error ratio is given for the covariances and the standard error is given for the correlations.

Pilodyn			Spiral grain		
F241	F243		F241	F243	
F241	–	0.88 (0.32)	F241	–	1.28 (0.25)
F243	1.35 (2.2)	–	F243	0.78 (3.2)	–

sampling effects associated with the reduced set of male parents used in the nested mating scheme. The survival was lower in trial F242, when compared with the other trials of the same group and age (i.e. 85% in F242 versus 98% in F244, not shown), contributing to larger environmental variation and reduced accuracy for the genetic variance estimation of DM growth (Table 2). Nevertheless, the ranges of the CGV were relatively small for the studied traits (Table 2), suggesting that scale effects may have the major influence on the heterogeneity of the additive genetic variances. Scale effects reflect differ-

ences between trials in the growth environment and measurement ages.

Large dominance effects were found for DM and raw PL measures (Table 2). Using a factorial mating design (NC Design II) in 12-year-old Douglas-fir, KING *et al.* (1988) reported also a significant dominance effect for pilodyn, but only accounting for 19% of the additive genetic variance. In addition, KING *et al.* (1988) found non-significant dominance variance for direct wood density determinations in annual rings 8–12 counted from the bark. As also reviewed by ZOBEL and JETT (1995), the additive genetic factors are likely to control the inheritance of wood density. Nevertheless, the dominance variance estimates obtained here must be interpreted with caution. Their reliability is limited by sample size, and thus a larger number of genetic entries (both males and females within males) could improve this estimate and lead to more accurate heritabilities. Particularly for DM and raw PL measures, the heritability estimates have low accuracies as evident from their high sampling coefficients of variation (i.e. 73% to 83%, Table 3).

It is not possible to determine whether the thinning (see *Material and methods*) had a significant effect on the variance estimates, because the comparison between thinned and unthinned trials is confounded with different growth environments and/or measurement ages. As noted by MATHESON and RAYMOND (1984), systematic thinning may be of minor importance, but selective thinning can affect the variance components thus influencing the heritability estimates. Selective thinning was carried out in F241, by removing trees with poor vigour of growth, at age 17 years. Data analysis of DM at age 18 (not shown), revealed indeed a strong decrease in the additive genetic variance when compared with the results (shown in Table 2) obtained at age 16. For the wood properties, however, the effects of selective thinning in F241 may have not been so pronounced. The additive and phenotypic variances of raw PL measures and SG at age 18 were comparable to those in F228 (Table 2) at the same age and similar growth conditions. The same applies to the CGV. Moreover, as shown in table 3, the heritabilities were similar in both trials (i.e. 0.30 and 0.36 for raw PL measures; 0.38 and 0.36 for SG).

In Norway spruce, a hyperbolic function has been defined by OLESEN (1976) to describe the negative relationship between basic density and ring width, considering the latter an independent variable that reflects factors having a direct effect on the basic density. Recently, DANBORG (1994b) has shown that the decrease in average density with increasing ring width is particularly due to a reduction in latewood percentage and, to a lesser extent, to a decrease in earlywood and latewood densities. Thus, for the species in question, it may be worth to adjust for differences in ring width when comparing the levels of a class variable (which can be genotypes, stands, silvicultural treatments, periods of tree growth, etc) in terms of inherent wood density, because the observed variation in this wood property reflects partly variations in ring width. In the analysis of PL, adjusted to a common mean ring width at breast-height by means of covariance analysis, the effect of the covariate was found significant ($P < 0.001$) in all cases, with the regression coefficient being fairly stable (each unit increase in DM contributed to an increase in PL varying from 0.05 mm to 0.06 mm, data not shown). Covariance analysis usually resulted in higher and more precise heritability estimates for PL, as a large portion of the within-subplot variance (which included dominance and environmental effects) of raw PL tended to be associated with variation in average ring width (as expressed by DM).

PL being more heritable than DM (Table 3) is in agreement with previous work in young spruce wood (LEE, 1993; YANCHUK and KISS, 1993; HANSEN and ROULUND, 1997; COSTA E SILVA *et al.*, 1998), where individual heritabilities were reported to range from 0.22 to 0.41 for raw PL measures. Higher heritability estimates have been found in other conifers (i.e. 0.8, KING *et al.*, 1988) or hardwoods (i.e. 0.6, GREAVES *et al.*, 1996). For SG measured at ring numbers 6, 8 or 10 from the pith in Sitka spruce, HANSEN and ROULUND (1997, 1998) reported individual heritabilities varying from 0.36 to 0.78, being higher than those found for DM and raw PL measures.

Correlations between traits

Although unfavourable, the phenotypic relationship between SG and DM was not pronounced as suggested by the low correlation coefficients (Table 4). For Norway spruce growing on fertile sites, DANBORG (1994a) reported that faster growing trees tended to have larger grain angles, although the relationship between SG and ring width was consistent and statistically significant only in one of the examined stands. On the other hand, on moderate sites, DANBORG (1994a) did not find any consistent differences between fast and slow growing trees for SG. The phenotypic correlations between PL and SG were also low (Table 4). Working with young Sitka spruce, HANSEN and ROULUND (1997, 1998) reported low phenotypic correlations between SG and DM or raw PL.

The phenotypic correlations between DM and raw PL measures (Table 4) are in agreement with a reported tendency of a strong adverse relationship between growth rate and whole-ring density in Norway spruce (OLESEN, 1976, 1977; DANBORG, 1994b; ROZENBERG and CAHALAN, 1997). On the other hand, the rather low value of 0.19, obtained for the additive genetic estimate in F243 at age 18 (Table 4), disagrees with the reported strong correlation. Apart from variations induced by sampling error, genetic correlation estimates between two characters may be influenced by genotype by time interactions in one or both of the traits. In F243, earlier measurements at age 14 (not shown) provided estimates for additive genetic, residual and phenotypic correlations of 0.54 (s.e. = 0.19), 0.67 (s.e. = 0.06) and 0.61 (s.e. = 0.02), respectively, between raw PL and DM. These estimates at age 14 approximate those obtain-

ed in F228 (Table 4) at age 18. However, in F243, the additive genetic correlation between ages 14 and 18 (not shown) was stronger for DM (i.e. 0.97) than for raw PL (i.e. 0.81). Thus, albeit high, the genetic correlation between density measures in typical juvenile rings (i.e. breast-height PL readings at age 14) and those in the juvenile-mature transition area (i.e. at age 18) was not perfect in F243. To some extent, this result may indicate differences between genotypes in their response to growth conditions during the 4-year-period, thus affecting the genetic covariance between raw PL and DM in F243 at age 18. As noted by OLESEN (1977) and DANBORG (1994b), random variation in climatic conditions has a large effect on whole-ring density and, for annual rings further from the juvenile wood boundary (i.e. about ring number 10 from the pith), may influence the radial pattern of density variation in Norway spruce.

The negative genetic relationship between wood density and diameter growth is usually stronger for Norway spruce than for Sitka spruce (ROZENBERG and CAHALAN, 1997). The additive genetic correlations between raw PL and DM obtained in F243 at age 14, and in F228 at age 18, agree with this tendency, considering the range (i.e. from 0.46 to 0.72) of estimates reported in the literature for Sitka spruce (LEE, 1993; HANSEN and ROULUND, 1997, 1998; COSTA E SILVA *et al.*, 1998). Besides, the residual correlations between raw PL and DM were also strong and positive (Table 4). These trends suggest that additive genetic and residual factors may determine the expression of the traits through similar internal physiological processes, probably involved in the regulation of cell division and expansion in xylem growth (thus affecting both latewood percentage and ring width). The substantial magnitudes of the residual correlations also support the results obtained in the covariance analysis of the raw PL measures, where variation in DM accounted for a reasonable portion of the within-subplot variance associated with the indirect measure of wood density (Table 2).

Correlations across sites

The allocation of sites to the eastern and western planting regions (Table 1) refers to an adjusted version of a previous proposal of an environmental zonation for Norway spruce in Denmark, based on growth (WELLENDORF *et al.*, 1999). Moderate to low site indices prevail in the western region, in contrast to more productive sites in the eastern region. The western zone is also characterized by more wind-exposed sites and harsher climatic conditions than the eastern region.

The strong genetic correlation estimates between the two sites in group I, and between F228 and F229 in group II (Table 5), agree with the zonation given in Table 1. However, in group II, the correlation between F226 and F228 was high (Table 5), which is not consistent with what could be expected from the outlined zonation. Besides, following the use of an equal correlation model across all sites in group II, the rather small change in log likelihood suggested that the individual correlations do not differ substantially from a common estimate of 0.74. Yet the most extreme sites in terms of geographic location (i.e. F226 and F229, Figure 1) had the lowest correlation (Table 5). For the sites in group III, the results pointed out a contrast between F241 and the other trials, which is consistent with the zonation indicated in Table 1.

The results suggest some level of genotype by environment interaction for DM particularly if contrasting planting regions are involved, but they are inconclusive with respect to potential risks within these zones. Furthermore, albeit not large, the difference in measurement ages may confound true genotype by

environment effects with variation induced by annual changes in climate.

For SG, the estimated additive genetic correlation across sites was outside the parameter space (Table 6), suggesting a very high value. The probability of obtaining multivariate sample estimates that exceed their defined range increases as the number of traits increase, their heritabilities decrease and sample sizes are smaller (HILL and THOMPSON, 1978). The heritabilities estimated for SG by multi-site analysis of F241 and F243 were moderate (Table 3), and were similar to those obtained in single-site analysis of these trials. In Denmark, HANSEN and ROULUND (1997, 1998) reported very high genetic correlations across sites for SG measured in young Sitka spruce, suggesting the absence of true genotype by environment interaction for this trait. This can explain partly the non-positive definite result, because estimates outside their defined bounds are likely to be obtained when true genetic correlations are close to their limits (HILL and THOMPSON, 1978; FOULLEY and OLLIVIER, 1986).

The results indicate that the wood properties are likely to be stable across environments, as suggested by ZOBEL and JETT (1995), and several other works with young spruce (YANCHUK and KISS, 1993; ROZENBERG and CAHALAN, 1997, cit. CHANTRE and GOUMA, 1994).

Conclusion

Genetic parameters were estimated for three key traits in Norway spruce. The estimates were based on a range of sites, and cross- and open-pollinated material from various sources. Heritabilities were lower for diameter growth than for pilodyn and spiral grain. Adjusting raw pilodyn readings to a common diameter, by means of covariance analysis, improved significantly the heritability of the indirect measure of wood density. The relationship between growth rate and wood properties was adverse, being usually stronger between diameter and raw pilodyn readings. Pilodyn was favourably but poorly correlated with spiral grain.

Estimates of dominance variance could also be obtained from two of the trials. The results indicated very large dominance effects (3 to 4 times the additive effects) in diameter and raw pilodyn readings, but not in spiral grain. Particularly for raw pilodyn measures, the result is not consistent with previous findings and needs confirmation. The specific mating scheme used (too few crosses per parent) does not allow for an accurate estimate of dominance variance, so results should be interpreted with caution.

Although the analysed sets of trials were not particularly suited to test the importance of genotype by environment interactions in this population, the results showed that the wood properties had high additive genetic correlations between the same trait measured in different trials. However, for diameter, low correlations were found between some sites, generally of contrasting growing conditions, suggesting that some environmental zonation may be warranted for improvement in growth rate.

Finally, the REML analysis used here proved to be a flexible and convenient framework for the estimation of genetic parameters from a heterogeneous dataset, involving both open- and cross-pollinated families of an unbalanced mating design, and several trials with different variances and degrees of genetic links between them. Although some of the limitations present in the dataset could not be overcome, the REML mixed model analysis was nevertheless able to combine the information available across trials, hence improving the accuracy of the

variance component estimates. Such flexibility and power are great advantages in most advanced generation breeding programmes.

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