Quantitative Genetic Parameters for Seven Characters in a Clonal Test of Salix eriocephala

I. Clonal Variation, Clone x Environment Interactions, Heritabilities, and Genetic Gains

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Summary

Quantitative genetic parameters were calculated for 7 characters important to biomass production using 20 clones of *Salix eriocephala*, planted on 2 sites in eastern Ontario, Canada. Significant clonal variation was detected for stem basal diameter, number of stems, number of branches, moisture content and specific gravity, but not for stem height or biomass. The clonal component accounted for 0.8% to 42.2% of the total variation in respective characters. No significant clone x site interaction was detected for any of the characters examined.

The broad sense heritabilities estimated for stem height, stem basal diameter and biomass were low (estimates based on individual ramets H^2_i=0.01 to 0.09, estimates based on clonal means H^2_c=0.05 to 0.31), those for number of stems and number of branches were moderate (H^2_i=0.13 to 0.15, H^2_c=0.37 to 0.51), while those for moisture content and specific gravity were high (H^2_i= 0.26 to 0.42, H^2_c=0.62 to 0.65).

Large genetic gains are achievable from selection for number of stems and branches, ranging from 11.7% to 16.2% and 6.4% to 9.6% in selection based on clonal means and individual ramets, respectively, and a 20% selection intensity. However, only limited gains are possible from clonal selection for increased height, diameter, or biomass growth, or for higher moisture content and specific gravity. These are in a range of 2.6% to 7.3% and 1.1% to 4.3% in selection based on clonal means and individual ramets, respectively.

Key words: Salix eriocephala, quantitative genetic parameters, variance components, clonal variation, clone x environment interaction, clonal selection, heritability, genetic gain.

Introduction

Production of woody biomass, as an alternative source of energy, from short rotation forest plantations, has received much attention in many developed countries since the energy crisis of the early 1970's (Abelson, 1982; Stott, 1984). Short rotation intensive culture (SRIC) plantations are designed to use fast growing hardwood trees which can be harvested repeatedly (after resprouting) on cycles of 10 years or less, and are genetically improved, often closely spaced and under intensive cultural practice (Anderson *et al.*, 1983; Mitchell, 1990).

The use of willows (*Salix L.*) in SRIC plantations for energy and chemicals has stimulated particular interest in many European countries (Siren, 1981, 1983) and in North America (Zsusfa, 1982 and 1990; Zsuffa *et al.*, 1984). Most of the recent genetic research interest in *Salix* has focused on interspecific hybridization and reproductive barriers (Argus, 1974; Mosseler, 1987), phylogenetic relationships between species (Chong, 1992), and allozyme variation and linkage analysis (Aravanopoulos, 1992). However, quantitative genetic parameters have seldom been assessed for this genus, especially for morphological characters (Mosseler et al., 1988; Kenney, 1990). In the present paper, 20 clones of *Salix eriocephala* M. were used to investigate the clonal variation, clone x environment interactions, heritability and genetic gains for seven morphological and physical characters. Genetic and environmental correlations between characters, and efficiency of indirect selection is reported in a separate paper.

Materials and Methods

The clonal materials used in this study were chosen from the willow clonal screening tests of the Forest Genetics Laboratory, University of Toronto, in which most of the clones were produced by Mosseler (1987) in a hybridization study. The parents of these clones were collected from natural populations within a 100-km-radius of Toronto, Ontario, Canada. The tests were established in the spring of 1987 at the Howard Ferguson Nursery, Kemptville, and at the Petawawa National Forestry Institute, Chalk River, both in eastern Ontario, Canada. The trees were planted via unrooted cuttings in a completely randomized block design with a three-ramet, non-contiguous-plot in each of 3 blocks within each of the 2 sites.

In the winter of 1990, the above-ground part of the tree was harvested, and measurements were taken for the following characters: stem height (cm), stem basal diameter (cm), number of stems/stool, number of branches/stool, wood moisture content (%), wood specific gravity (g/cm^3) and oven-dry biomass/stool (g). Number of stems/stool measures the coppicing ability of the stool after harvest, and can be one of the important characters under consideration in a breeding program for biomass production.

Prior to data analysis, a W-test (see Anderson and McLean, 1974) was used to test normality, and a Bartlett's test (see Sokal and Rohlf, 1981) was used to test the homogeneity of variances of the samples. Number of branches (NB) and biomass (WT) did not satisfy the assumptions ,and therefore were transformed using a square-root transformation before the analysis.

The linear model for the analysys of variance (ANOVA) was: —(1)

$$Y_{ijkl} = \mu + S_i + B_{j(i)} + C_k + SC_{ik} + BC_{j(i)k} + e_{(ijk)l} \quad (1)$$

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where Y_{ijkl} is the performance of lth ramet of kth clone growing in jth block of ith site; μ is the overall mean; S_i is the effect of ith test site ($i=1,2$); $B_{j(i)}$ is the effect of jth block (replicate) within ith site ($j=1,2,3$); C_k is the effect of kth clone ($k=1,..,20$); SC_{ik} is the interactive effect of ith test side and kth clone$_i$ $BC_{j(i)k}$ is the interactive effect of kth clone and jth block (within ith site); and $e_{(ijk)l}$ is the random error associated with ramets within plot ($l=1,2,3$).

All effects were considered random. No test of significance of sites or blocks was possible, due to the restriction error associated with these sources of variation (ANDERSON and MCLEAN, 1974).

The expected mean squares (EMS) and the denominators for the F-test are presented in *table 1*. Note that the coefficients are not integers due to the unequal number of ramets (missing ramets) among plots. These coefficients were calculated using the General Linear Model (GLM) of SAS (SAS Institute Inc., 1988). A pseudo-mean square was calculated by the GLM to conduct the F-test for the effect due to site x clone interaction according to HICKS (1982).

The broad sense heritability or the degree of genetic determination of individual ramets (H^2_i) was calculated as (PARK and FOWLER, 1987): —(2)

$$H_i^2 = \frac{\sigma_G^2}{\sigma_{ph.i}^2} = \frac{\sigma_C^2}{\sigma_S^2+\sigma_B^2+\sigma_C^2+\sigma_{SxC}^2+\sigma_{BxC}^2+\sigma_e^2} \qquad (2)$$

where σ^2_G is the total genetic variance; $\sigma_C{}^2$ is clonal component of variance; $\sigma^2_{ph.i}$ is the phenotypic variance of individual ramets.

The broad sense heritability of clonal means (H^2_c) was calculated as: —(3)

$$H_c^2 = \frac{\sigma_G^2}{\sigma_{ph.c}^2} = \frac{\sigma_C^2}{\sigma_S^2/2+\sigma_B^2/6+\sigma_C^2+\sigma_{SxC}^2/2+\sigma_{BxC}^2/6+\sigma_e^2/18} \qquad (3)$$

where $\sigma^2_{ph.c}$ is the phenotypic variance of clonal means.

Variance components due to sites and blocks were included in the denominator of the heritability formula, since the omission of these components from the denominator may result in the overestimation of heritability and consequent gain (COTTERILL, 1987).

Genetic gains in clonal selection for the characters were calculated according to FALCONER (1989, Chapter 11), using the respective phenotypic standard deviation and heritability for individual ramets (ΔG_i) and clonal means (ΔG_c). These are presented in the unit of the measurement and as a percentage of the mean for the characters.

Results and Discussion

The phenotypic measurements and, where applicable, the transformed data are summarized in *table 2*. The coefficients of variation show that large phenotypic variation was obvious in stem height, stem basal diameter, number of stems, number of branches and biomass, while limited phenotypic variation was found in moisture content and specific gravity.

Clonal variation

Table 3 indicates that there was no significant clonal variation detected in stem height and biomass, although large phenotypic variation was evident in these 2 characters (*Table 2*). The clonal component accounted for just 0.8% and 6.1% of the total variation in stem height and biomass, respectively. Since *Salix eriocephala* is a shrub-type willow, the clonal variation in height presented here is not comparable to those of tree-type species in which significant clonal variation in height is often detected (e. g., BENTZER *et al.*, 1989; FARMER *et al.*, 1988).

Table 1. — Expected mean squares for the analysis of variance.

Source[a]	DF	Expected Mean Squares	Denominator for F-test
S_i	1	$\sigma_e^2 + 2.1176\ \sigma_{BxC}^2 + 6.3529\ \sigma_{SxC}^2 + 42.3530\ \sigma_B^2 + 127.0588\ \sigma_S^2$	
$B_{j(i)}$	4	$\sigma_e^2 + 2.2329\ \sigma_{BxC}^2 + 44.6576\ \sigma_B^2$	
C_k	19	$\sigma_e^2 + 2.1630\ \sigma_{BxC}^2 + 6.4890\ \sigma_{SxC}^2 + 12.9779\ \sigma_C^2$	MS_{SxC}
SC_{ik}	19	$\sigma_e^2 + 2.1630\ \sigma_{BxC}^2 + 6.4890\ \sigma_{SxC}^2$	$0.9183\ MS_{BxC} + 0.0817\ MS_e$
$BC_{j(i)k}$	76	$\sigma_e^2 + 2.3554\ \sigma_{BxC}^2$	MS_e
$e_{(ijk)l}$	170	σ_e^2	
Total	289		

[a]) Source of variation: S_i, site; $B_{j(i)}$, block within site; C_k, clone; SC_{ik}, site x clone; $BC_{j(i)k}$, block x clone $e_{(ijk)l}$, within plot error.

Table 2. — **Summary of the simple statistics for the characters or the transformation of the characters.**

Characters[a]	Range	Mean ± S.E.	C.V.%
HT (cm)	43.3 - 307.7	172.3 ± 2.38	23.5
BD (cm)	0.49 - 2.59	1.15 ± 0.02	26.2
NS	1 - 32	13.2 ± 0.36	47.0
NB	2 - 159	58.6 ± 1.83	53.0
MC (%)	40.60 - 57.59	51.66 ± 0.14	4.7
SG (g/cm^3)	0.39 - 0.50	0.44 ± 0.001	5.7
WT (g)	26.5 - 4667.0	744.1 ± 31.47	72.0
$\sqrt{NB}$	1.41 - 12.61	7.36 ± 0.12	28.8
$\sqrt{WT}$	5.34 - 58.63	24.90 ± 0.50	34.5

[a]) Characters: HT, stem height; BD, stem basal diameter; NS, number of stems/stool; NB, number of branches/stool; MC, wood moisture content; SG, wood specific gravity; WT, oven-dry biomass/stool.

Mosseler *et al.* (1987) found that there is significant genetic variation among *Salix* species in biomass, and that considerable gain can be achieved through selection at the species level. The non-significant 6.1% clonal variation detected in biomass (*Table 3*) in this study indicates that the chance for improvement in this character through clonal selection within a species could be limited. On the other hand, it suggests that under similar environmental conditions, silvicultural and management activities can exercise a major impact on biomass growth.

Significant clonal variation was detected for stem basal diameter, number of stems, number of branches, wood moisture content and specific gravity (*Table 3*). The clonal component made up a large proportion of the total variation in these characters, ranging from 9% in stem basal diameter to 42.2% in specific gravity.

The significant clonal variation detected in moisture content and specific gravity (*Table 3*) was comparable to those reported in another clonal test of *Salix eriocephala* by Kenney (1990), who recorded a 19% and 40% clonal variation in these 2 characters, respectively.

Clone-environment interactions

Table 3 shows that there was no significant variance due to the interactions between clones and sites for any of the characters examined. The proportion of variance due to this component in the total variation was small in all the characters (0.0% to 5.8%, respectively). The clone x block interaction, however, was highly significant in stem height, stem basal diameter, biomass, number of branches, and specific gravity. This interaction contributed a large proportion to the total variation in stem height, stem basal diameter, biomass, and number of branches, ranging from 8.6% to 21.3%, respectively, and a small proportion to the total variation in specific gravity (2.4%).

Although the clone x site interaction was not significant statistically, it should not be concluded that all the characters were stable and selection for these characters need not to be site specific, because the number of sites included in the tests was small. With a larger number of sites, it is possible we might have detected statistically significant genotype x site interactions.

On the other hand, the non-uniformity of soil conditions (i.e., depth, amount of coarse fragments) within blocks was inevitable, especially at the Petawawa site.This might be large enough to cause a significant clone x block interaction. There were also missing ramets due to mortality in both test sites. These allow more growing space for the remaining neighbor trees, and could be partially responsible for the large clone x block interaction, particularly in growth characters, such as stem height, stem basal diameter, biomass, and number of branches.

Shelbourne (1972) suggested that if the variance component due to the GE interaction is greater than one half of that due to genotypes (i.e., $\sigma^2_{GxE}/\sigma^2_G > 0.5$), then one must consider the interaction in a breeding program. According to this criterion, the clone x block interaction in specific gravity does not have to be taken into consideration. On the other hand, the clone x block interaction in other characters does appear to have practical importance in a breeding program, but any inference should be drawn with caution because of the non-significance of clone x site interaction. A further test with a larger number of sites and more uniform soil conditions within blocks is necessary if the interactions detected in this study are to be utilized in a practical willow breeding program.

Heritabilities

Table 4 summarizes the broad sense heritabilities calculated from clonal selection for the characters evaluated in the study. Heritabilities estimated for stem height and stem basal diameter were low ($H^2_i=0.01$, $H^2_c=0.05$ for height, $H^2_i=0.09$, $H^2_c=0.30$ for basal diameter). These are lower than the estimates for height and DBH in tree-

form species. Notably, heritability estimated for biomass was also low (H^2_i=0.06, H^2_c=0.31).

The results indicate that number of branches in *Salix eriocephala* is under moderate genetic control (H^2_i=0.15, H^2_c=0.51). These are lower than similar estimates for eastern cottonwood (WILCOX and FARMER, 1967), but higher than estimates for number of branches/whorl in white spruce (MERRILL and MOHN, 1985). Number of stems was also found to be moderately heritable (H_i^2=0.13, H^2_c=0.37).

The heritability estimates for moisture content in the current study (H^2_i=0.26, H^2_c=0.62) correspond well to those of KENNEY (1990) who recorded a heritability of 0.19 and 0.63, respectively, based on individual ramets and clonal means. ANDERSON and ZSUFFA (1982) suggested that willow clones with similar productivity (yield) but different moisture contents may reflect differences in water use-efficiency and thus a different adaptability to drier sites. If this was the case, moisture content could be one of the target characters in selection of superior clones to be used in arid areas.

The heritability for specific gravity was the highest for the characters studied (H^2_i=0.42, H^2_c=0.65). It was similar to that recorded for other species such as *Populus deltoides* (FARMER, 1970; OLSON *et al.*, 1985), but was somewhat lower than that reported by KENNEY (1990).

Estimates of heritability have rather large standard deviations (*Table 4*), therefore, the ratios obtained are only a relative indication of genetic control and should not be interpreted as absolute or invariant values.

Genetic gains

Very little gain in stem height (0.3% and 0.7%, respectively, based on individual ramets and clonal means) is achievable, since the heritability in stem height was low. Moderate gain is obtainable from selection for stem basal diameter.

Gains for moisture content and specific gravity were not large (1.7% and 3.8%, respectively, based on individual ramets and 2.7% and 4.7% based on clonal means). This is because phenotypic variation was low in these 2 characters (*Table 2*), even though heritabilities were high (*Table 4*). The gain values were somewhat lower than those reported by KENNEY (1990) who recorded a 8.5% and 7.3% gain (based on a selection intensity of 20%), respectively, expected from selection based on clonal means.

Genetic gains obtainable in number of stems and number of branches were quite high (9.6% and 16.2% in number of stems, 6.4% and 11.7% in number of branches based on individual ramets and clonal means, respectively). These large expected gains resulted from sufficiently high heritability estimates combined with high phenotypic variation in these two characters.

Notably, only moderate improvement could be expected from selection for higher biomass (3.2% and 7.4% based on individual ramets and clonal means, respectively). Since biomass is the most important character in energy fuel production, the little gain achievable in it could become an obstacle to the success of a breeding program.

However, WRIGHT (1976) and ZOBEL and TALBERT (1984) pointed out that tree breeders can influence gain from selection by controlling the environment to maximize heritability. Genetic gain could also possibly be increased by employing an appropriate method of indirect selection (WRIGHT, 1976; FALCONER, 1989). We will discuss this aspect elsewhere (LIN and ZSUFFA, 1993).

Conclusions

The objective of this study was to estimate the quantitative genetic parameters in morphological and physical characteristics of *Salix eriocephala* to provide information for practical selection and breeding programs appropriate for SRIC plantations.

The results indicate that significant clonal variation exists in stem basal diameter, number of stems, number of branches, wood moisture content, and wood specific gravity (*Table 3*), and can be utilized in a breeding program. However, the improvement that can be achieved from clonal selection for these characters depends upon their heritabilities and their phenotypic variation. While

Table 3. — Variance components due to various sources of variation for 7 characters of *Salix eriocephala*.

Source	HT		BD		NS		NB		MC		SG		WT	
	MS[a,b]	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
S_i	0.00	0.0	0.0268	24.0	18.7	37.5	0.74	14.5	1.50	23.0	0.00034	41.0	0.00	0.0
$B_{j(i)}$	856.90	42.0	0.0246	22.0	0.5	1.1	0.00	0.0	0.17	2.6	0.00002	2.4	27.06	29.1
C_k	15.54	0.8	0.0100*	9.0	6.5**	13.1	0.77*	15.0	1.68**	25.7	0.00035**	42.2	5.69	6.1
SC_{ik}	92.37	4.5	0.0056	5.0	0.0	0.0	0.19	3.6	0.10	1.6	0.00001	1.2	5.44	5.8
$BC_{j(i)k}$	283.96**	13.9	0.0096**	8.6	1.3	2.6	0.80**	15.6	0.33	5.0	0.00002**	2.4	19.78**	21.3
$e_{(ijk)l}$	791.75	38.8	0.0351	31.4	22.8	45.8	2.64	51.3	2.74	42.1	0.00009	10.8	35.03	37.7
Total	2040.51	100.0	0.1117	100.0	49.8	100.0	5.15	100.0	6.51	100.0	0.00083	100.0	93.0	100.0

[a]) MS, mean squares. Negative variance components were taken as zero.
[b]) *), significant at 0.05 level; **), significant at 0.01 level.

Table 4. — Broad sense heritabilities (± standard deviation) and genetic gains (expressed in the units of measurement and as a percentage of the mean of the character) estimated from clonal selection in *Salix eriocephala*.

	HT	BD	NS	NB	MC	SG	WT
H_i^2	0.01±0.14	0.09±0.16	0.13±0.16	0.15±0.17	0.26±0.19	0.42±0.20	0.06±0.15
H_c^2	0.05±0.15	0.30±0.19	0.37±0.20	0.51±0.20	0.62±0.18	0.65±0.18	0.31±0.19
ΔG_i	0.47	0.04	1.27	0.47	0.90	0.017	0.81
%	0.3	3.6	9.6	6.4	1.7	3.8	3.2
ΔG_c	1.24	0.07	2.14	0.86	1.40	0.021	1.83
%	0.7	6.5	16.2	11.7	2.7	4.7	7.4

Notes: H^2_i, H^2_c: heritability of individual ramets and clonal means, respectively. ΔG_i, ΔG_c: genetic gain based on individual ramets and clonal means, respectively, assuming a 20% selection intensity or i=1.372. Standard deviations of heritabilities were calculated according to Falconer (1989, Chapter 10).

large gains are possible from selection for higher number of stems and branches, only small to moderate improvement can be obtained from clonal selection for greater height, diameter or biomass growth, or for higher moisture content and specific gravity. An alternative selection strategy, such as indirect selection (Lin and Zsuffa, 1993), is needed if significant improvement in some important characters, e. g. biomass, is to be achieved.

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Isozyme Studies of New Zealand Nothofagus Species (Southern Beech) Using Leaf Extracts

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Summary

Leaf extracts of the New Zealand species of *Nothofagus* (southern beech) were subjected to horizontal starch gel electrophoresis and stained gel slices were scored for 12 enzyme systems segregating into 22 putative loci. Results of isozyme analysis are supportive of the present taxonomic concept and reveal a major genetic divergence between *N. menziesii* and the remaining four species which belong to the *'fusca'* group and form natural hybrids among each other. Genetic variation in *N. menziesii* is distinctly higher than in any of the *'fusca'* group species where *N. fusca* shows the lowest levels of variation. Hierarchical analysis of gene diversity for each species shows that 84% to 100% of the total gene diversity resides within populations while only 0% to 16% are due to variation between populations. In the *'fusca'* group as a whole, the between-population component of gene diversity amounts to 52.0% and is much higher than in any single species including *N. menziesii* (12.3%). Comparison of observed and expected frequencies of heterozygotes gave no significant deviation from HARDY-WEINBERG equilibrium indicating predominant cross-pollination in both *N. menziesii* and the *'fusca'* group species.

Key words: *Nothofagus*, southern beech, genetic variation, isozyme analysis, electrophoresis, leaf tissue, New Zealand, Fagaceae.

Introduction

The native species of *Nothofagus* (southern beech) are New Zealand's most common and widespread forest trees and a large amount of information on their ecology, distribution, and physiology has already been published (WARDLE, 1984). There has been one study on provenance variation of *Nothofagus* seedling growth and morphology (WILCOX and LEDGARD, 1983), but at the biochemical level, genetic analyses of provenance variation, breeding system, and taxonomical relationships are lacking so far. The present taxonomic concept (WARDLE, 1984; POOLE, 1987) distinguishes four native species, the crenate to dentate-leaved *N. menziesii* (HOOK. f.) OERST. (silver beech), *N. fusca* (HOOK. f.) OERST. (red beech), and *N. truncata* (COL.) CKN. (hard beech), and the entire-leaved *N. solandri* with the recognized varieties *solandri* (HOOK. f.) OERST. (black beech), a lowland form with a small-leaved, divaricating juvenile growth habit, and the montane to subalpine *cliffortioides* (HOOK. f.) POOLE (mountain beech) with generally smaller leaves and lacking the juvenile characteristics of the former. All species except *N. menziesii* form natural hybrids among each other and are collectively classified as the *'fusca'* group. Species and varieties are both referred to as 'species' in the present study.

A comparatively quick and inexpensive method for preliminary investigations of the breeding system, genetic diversity, and provenance variation is isozyme analysis via starch gel or polyacrylamide gel electrophoresis. Isozyme analysis has been widely employed to investigate genetic variation of native forest trees in North America (e. g. WHEELER and GURIES, 1982; CHELIAK and PITEL, 1984) and Australia (e. g., MORAN and HOPPER, 1983; COATES and SOKOLOWSKI, 1989; PETERS et al., 1990), and there have been similar studies on European beech (*Fagus sylvatica* L.) (THIEBAUT et al., 1982; MÜLLER-STARCK, 1985), but in New Zealand research on genetic variation of native trees has only recently been initiated (HAWKINS and SWEET, 1989; HAWKINS et al., 1991; BILLINGTON, 1991).

Imbibed or germinating seeds are the preferred source material for isozyme studies because they are easily stored and extracted and usually contain high concentrations of enzymes. Seed collection of New Zealand *Nothofagus* presents a major problem in this respect because all species flower and reproduce only intermittently and not always throughout their range rendering provenance studies difficult and time-consuming. Since all native species are evergreen, foliage provides an alternative source material which is available throughout the year and easily collected. As in many other species, however, phenolic compounds in the foliage are rapidly oxidized to form quinones, condensed tannins, and brown pigments which inactive most enzymes when the tissue is homogenized during conventional extraction procedures (browning effect; ANDERSON, 1968). This problem has been overcome in recent years by adding a range of chemical agents to the extraction buffer which inhibit phenol oxidase activity and reduce or bind the products of the reaction (quinones).

This paper describes the extraction and assay of enzymes from *Nothofagus* foliage and discusses the results of isozyme analysis of 12 enzyme systems segregating into 22 putative loci.

Materials and Methods

Three populations each of all 5 native *Nothofagus* species were sampled from various provenances in the South

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