Genetic parameters and age–age correlations in a clonally replicated test of black spruce after 10 years

Article *in* Canadian Journal of Forest Research · December 1994

DOI: 10.1139/x94-301

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Estimating genetic gains from alternative breeding strategies for clonal forestry

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Received December 20, 1990
Accepted July 29, 1991

MULLIN, T.J., and PARK, Y.S. 1992. Estimating genetic gains from alternative breeding strategies for clonal forestry. Can. J. For. Res. **22**: 14–23.

Concepts and procedures are presented for the analysis of progeny trials that incorporate clonal replication as a means to resolve variance arising from nonadditive gene effects. Components of variance from the linear model may be expressed in terms of expected covariances among relatives, and these, in turn, may be used to derive approximations of additive, dominance, and epistatic components of genetic variance. In addition to the usual assumptions applied to conventional progeny trials, the use of this expanded genetic model in the analysis of tests with clonal replicates assumes that the greatest portion of the total epistasis is due to interactions involving groups of more than two or three loci. If this assumption is not satisfied, estimates of additive and dominance variance, including those from trials without clonal replicates, will be contaminated by a large fraction of epistasis, and total epistasis will be underestimated by a corresponding amount. Heritability and gain formulae for alternative selection and deployment schemes are developed and illustrate the use of genetic parameters in the comparison of seedling and clonal reforestation strategies.

MULLIN, T.J., et PARK, Y.S. 1992. Estimating genetic gains from alternative breeding strategies for clonal forestry. Can. J. For. Res. **22** : 14–23.

Des concepts et procédures sont présentés afin d'analyser des tests de descendances impliquant des répétitions clonales comme moyen de résoudre la variance résultant d'effets génétiques non additifs. Les composantes de la variance d'un modèle linéaire peuvent être exprimées en termes de covariances espérées entre individus apparentés et ces demières, à leur tour, peuvent être utilisées afin de dériver des approximations des composantes additive, de dominance et d'épistasie de la variance génétique. En plus des pré-requis et règles habituels s'appliquant à l'analyse des tests de descendances conventionnels, l'utilisation de ce modèle génétique élargi dans l'analyse des tests à répétitions clonales présume que la plus grande partie de l'épistasie totale est causée par des interactions impliquant des groupes de plus de deux ou trois loci. Si cette présomption n'est pas remplie, les estimations de variance additive et de dominance, incluant celles des tests sans répétitions clonales, seront contaminées par une large part d'épistasie, et l'épistasie totale sera sous-estimée d'une quantité correspondante. Les formules d'héritabilité et de gain pour la sélection alternative et les stratégies de déploiement sont développées. Elles illustrent l'utilisation des paramètres génétiques dans la comparaison des stratégies de reboisement clonal versus celles impliquant des semis.

[Traduit par la rédaction]

Introduction

Breeding strategies are normally evaluated in terms of the genetic gain expected for traits of importance, usually over a period of time. Expected gain is a function of variation of the trait in the breeding population and the degree to which this trait is under a form of genetic control, which can be utilized by a proposed selection and propagation system. Obviously, meaningful calculations of gain require reliable estimates of these parameters.

Most improvement strategies currently used in forestry have adopted some version of a recurrent selection system to generate successive generations of breeding materials. In each generation, selected materials are managed in conventional seed orchards to produce seeds for planting-stock production. The genetic parameters necessary for the estimation of genetic gain from these strategies are obtained by evaluating the performance of progeny from an appropriate mating design. Genetic gain realized by sexual offspring of selected parents results almost entirely from the additive component of genetic variation, while selected clones retain the effects of allele interactions in addition to the additive effects. Procedures for the calculation of genetic gain in these situations are well documented (Namkoong *et al.* 1966; Shelbourne 1969).

Recently, breeding strategies utilizing vegetative propagation to produce improved planting stock, i.e., clonal forestry, have been promoted as an alternative to conventional seed-orchard procedures. There are many advantages of clonal forestry (Carson 1986a; Libby and Rauter 1984; Libby 1990), including the opportunity to utilize both additive and non-additive variances, which could result in larger gains from selection than are possible using sexual offspring. To predict genetic gains from clonal strategies, it is necessary to obtain estimates of both additive and nonadditive variances. One possibility is to establish a clonal progeny trial in which individuals within families are replicated vegetatively. Several investigators have used clonal replicates of open-pollinated families to obtain estimates of additive and nonadditive genetic variances (e.g., Foster 1985; Foster *et al.* 1984; Park and Fowler 1987). Only a few have cloned individuals from control-pollinated families to further resolve components of nonadditive genetic variance in forest trees (Foster and Shaw 1988; Stonecypher and McCullough 1986), although the value of clonal replicates for such purposes has long been recognized (Burdon and Shelbourne 1974; Comstock *et al.* 1958; Wricke and Weber 1986).

Printed in Canada / Imprimé au Canada

The production population for a clonal strategy is markedly different from the wind-pollinated orchard trees used in conventional seed-based programs. Collections of juvenile donor plants, clone hedges, or frozen embryo cultures become the source of nursery propagation material. Advanced generation breeding populations must still be managed with breeding groups or sublines (Burdon and Namkoong 1983; van Buijtenen and Lowe 1979) to control inbreeding while progress is made in each generation through controlled mating and recurrent selection. The management of the breeding population continues as the main source of progressive improvement, while clonal production populations accelerate the deployment of improved materials and permit additional genetic gain to be realized in each generation (Burdon 1982). Because of the possible importance of nonadditive variance to clonal breeding strategies, large effective population sizes are required to retain favourable dominant alleles and epistatic loci combinations (Namkoong 1982). Multiple-population breeding is an option to maintain a large effective population size and is consistent with selecting clones for specific uses (Hood 1985). Clearly, the design of clonal strategies will benefit from detailed information about genetic variance structure of clonal populations, including the resolution of nonadditive variance due to dominance and epistatic gene effects.

In this paper, the procedures for estimating genetic parameters appropriate for the evaluation of clonal breeding strategies are developed and illustrated using a field test model based on a diallel cross with clonal replicates. The procedures derived are applied to actual data from a black spruce (*Picea mariana* (Mill.) B.S.P.) clonal test in a subsequent paper (Mullin *et al.* 1992).

Describing variation in populations

The observed value of a trait for an individual is known as its phenotype (P), and is the product of the individual's genetic constitution, or genotype (G), and environmental factors (E), so that

$$P = G + E$$

It follows that for a given trait, phenotypic variation in a population may be partitioned into genetic and environmental components of variance:

$$\sigma^2_{ph} = \sigma^2_G + \sigma^2_E$$

This simple model may be extended to further partition genetic variance into components reflecting different modes of gene action that affect the way a trait is passed on to offspring. It is particularly useful to breeders to consider the total genetic variance (σ^2_G) to be the sum of variances arising from additive, dominance, and epistatic gene effects (Cockerham 1963; Dudley and Moll 1969):

$$\sigma^2_G = \sigma^2_A + \sigma^2_D + \sigma^2_I$$

The additive variance (σ^2_A) is due to variation caused by the average effect of substituting one allele at a locus for another, commonly known as the breeding value. Dominance variance (σ^2_D) results from within-locus variation remaining after subtracting the variance due to additive effects, i.e., intralocus interactions between alleles. Both additive and dominance variances depend on gene frequency of the population, and they are, therefore, partly properties of the population (Falconer 1981); thus, prediction of genetic variances requires knowledge of both gene action and gene frequency. If a trait is polygenic, i.e., influenced by alleles at two or more gene loci, interloci interactions among gene effects give rise to epistatic variance (σ^2_I). The total epistatic variance may be further partitioned in terms of the interactions between genes at different loci, such that

$$\sigma^2_I = \sigma^2_{AA} + \sigma^2_{AD} + \sigma^2_{DD} + \sigma^2_{AAA} + \sigma^2_{AAD} + \sigma^2_{ADD} + \sigma^2_{DDD} + \ldots$$

where σ^2_{AA}, σ^2_{AD}, and σ^2_{DD} indicate the total additive × additive, additive × dominance, and dominance × dominance interaction variances involving two loci, respectively. Similarly, the remaining terms indicate the numbers of loci and types of higher order epistatic interactions (Kempthorne 1969).

In genetic experiments, groups of relatives provide sources of experimental variance that can be controlled and analyzed. Genetic effects may be studied by measuring the similarity, or covariance (cov), of group members (Cockerham 1963; Namkoong 1979). Members of a full-sib family having both parents in common will be more closely related, and therefore will exhibit a higher covariance, than half-sibs, which only have one common parent. When family groups are produced by crossing unrelated diploid parents from a random mating population, the among-group variation is a measure of the family covariance. If regular Mendelian behaviour and linkage equilibrium at gene loci are assumed, these family covariances may be expressed as a function of genetic components of variance (Cockerham 1954):

$$\text{cov(full sibs)} = \frac{1}{2}\sigma^2_A + \frac{1}{4}\sigma^2_D + \frac{1}{4}\sigma^2_{AA} + \frac{1}{8}\sigma^2_{AD} + \frac{1}{16}\sigma^2_{DD} + \frac{1}{8}\sigma^2_{AAA} + \frac{1}{16}\sigma^2_{AAD} + \frac{1}{32}\sigma^2_{ADD} + \frac{1}{64}\sigma^2_{DDD} + \ldots$$

$$\text{cov(half-sibs)} = \frac{1}{4}\sigma^2_A + \frac{1}{16}\sigma^2_{AA} + \frac{1}{64}\sigma^2_{AAA} + \ldots$$

Among-group variances estimated from this linear model may be translated into covariances of relatives. When the family structure produces two or more levels of relationship, the covariance equations may be solved to estimate two or more components of total genetic variance. Experimental designs with complex family structures may produce several covariances of relatives that will provide greater resolution of the components of total genetic variance (Wricke and Weber 1986).

General linear model

Relatives may be produced and genetic parameters estimated using any of a number of mating designs (see reviews by Heaman 1988; Namkoong and Roberds 1974; van Buijtenen 1976; van Buijtenen and Namkoong 1983; van Buijtenen and Tuskan 1986). The diallel cross was originally proposed for use with homozygous parents (Hayman 1954), but has been recommended for use in forest tree breeding where the production of inbred lines is not feasible (Hinkelmann and Stern 1960). The design used here for illustration is referred to by van Buijtenen and Namkoong (1983) as a modified half-diallel, in which each parent tree is crossed with all other parents, resulting in a complete set of crosses, excluding reciprocals and selfs (Table 1).

TABLE 1. Modified half-diallel mating design for p parents

Seed parent	Pollen parent							
	1	2	3	4	...	$p-2$	$p-1$	p
1		x	x	x	...	x	x	x
2			x	x	...	x	x	x
3				x	...	x	x	x
4					...	x	x	x
.						.	.	.
.						.	.	.
.						.	.	.
$p-3$						x	x	x
$p-2$							x	x
$p-1$								x
p								

TABLE 2. Analysis of variance for modified half-diallel mating design with clonal replicates

Source	df*	Expected mean squares†
Family	$\frac{p(p-1)}{2} - 1$	$\sigma_E^2 + r\sigma_C^2 + cr\sigma_F^2$
GCA	$p - 1$	$\sigma_E^2 + r\sigma_C^2 + cr\sigma_s^2 + cr(p-2)\sigma_g^2$
SCA	$\frac{p(p-3)}{2}$	$\sigma_E^2 + r\sigma_C^2 + cr\sigma_s^2$
Clone in family	$\frac{p(p-1)}{2}(c-1)$	$\sigma_E^2 + r\sigma_C^2$
Error	$\frac{cp(p-1)}{2}(r-1)$	σ_E^2

*p, c, and r are the numbers of parents, clones within families, and ramets within clones, respectively.
†σ_E^2, σ_C^2, σ_g^2, σ_s^2, and σ_F^2 are variance components due to ramet within clone error, clone within family, GCA, SCA, and full-sib family, respectively.

The family structure produced by the modified half-diallel permits the evaluation of both general and specific combining ability. General combining ability (GCA) is the average performance of progeny of a given parent produced when that parent is crossed with several other individuals. Specific combining ability (SCA) refers to the difference in the average performance of the progeny of two specific parents from what would be expected based on their individual GCAs alone (Zobel and Talbert 1984). Replicating the progeny in the test by cloning imposes another level of relationship in the family structure. Ramets of a given clone are genetically identical and thus are the closest possible group of relatives.

In this example, complications of site and block effects, and genotype–environment interactions, are ignored, focussing attention on the genetic interpretation of family and clonal variances. The analysis of linear models for diallel mating designs was discussed in detail by Griffing (1956). With the addition of clonal replication, the general linear model for the modified half-diallel becomes

$$Y_{ijkl} = \mu + g_i + g_j + s_{ij} + C_{(ij)k} + E_{ijkl}$$

where

Y_{ijkl} is the observed performance of the lth ramet of the kth clone within the ijth full-sib family

μ is the overall mean

g_i, g_j are the GCA effects of the ith and jth parents $(i, j = 1, 2, ..., p)$

s_{ij} is the SCA effect of the ith and jth parents

$C_{(ij)k}$ is the effect of the kth clone within the ijth family $(k = 1, 2, ..., c)$

E_{ijkl} is the error term $(l = 1, 2, ..., r)$

p, c, r are the numbers of parents, clones within families, and ramets within clones, respectively

If the data are balanced and all of the model effects are considered to be random, the analysis of variance is carried out with degrees of freedom and expected mean squares as given in Table 2. The mean squares are equated to the expected mean squares and solved to derive estimates of the variance components for each term in the linear model. The use of analysis of variance to estimate variance components requires the usual assumptions that (*i*) model effects are additive and (*ii*) experimental errors are random and uncorrelated, with a mean of zero (Steel and Torrie 1980). Additional assumptions that experimental errors are independent and normally distributed with a common variance are not required, as these only affect the validity of the F-test and are not applicable to estimates of variance components (Hallauer and Miranda 1981).

Derivation of genetic variances

The family (σ_F^2), GCA (σ_g^2), SCA (σ_s^2), and clone within family (σ_C^2) variance components derived from the linear model may be expressed in terms of expected covariances among relatives (Becker 1984):

$$\sigma_F^2 = \text{cov(full sibs)}$$

$$\sigma_g^2 = \text{cov(half-sibs)}$$

$$\sigma_s^2 = \text{cov(full sibs)} - 2\,\text{cov(half-sibs)}$$

$$\sigma_C^2 = \sigma_G^2 - \text{cov(full sibs)}$$

Translating the expected covariances in terms of genetic components of variance, these may be expressed:

$$\sigma_F^2 = \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_D^2 + \frac{1}{4}\sigma_{AA}^2 + \frac{1}{8}\sigma_{AD}^2 + \frac{1}{16}\sigma_{DD}^2 + \frac{1}{8}\sigma_{AAA}^2 + \frac{1}{16}\sigma_{AAD}^2 + \frac{1}{32}\sigma_{ADD}^2 + \frac{1}{64}\sigma_{DDD}^2 + \cdots$$

$$\sigma_g^2 = \frac{1}{4}\sigma_A^2 + \frac{1}{16}\sigma_{AA}^2 + \frac{1}{64}\sigma_{AAA}^2 + \cdots$$

$$\sigma_s^2 = \frac{1}{4}\sigma_D^2 + \frac{1}{8}\sigma_{AA}^2 + \frac{1}{8}\sigma_{AD}^2 + \frac{1}{16}\sigma_{DD}^2 + \frac{3}{32}\sigma_{AAA}^2 + \frac{1}{16}\sigma_{AAD}^2 + \frac{1}{32}\sigma_{ADD}^2 + \frac{1}{64}\sigma_{DDD}^2 + \cdots$$

$$\sigma_C^2 = \frac{1}{2}\sigma_A^2 + \frac{3}{4}\sigma_D^2 + \frac{3}{4}\sigma_{AA}^2 + \frac{7}{8}\sigma_{AD}^2 + \frac{15}{16}\sigma_{DD}^2 + \frac{7}{8}\sigma_{AAA}^2 + \frac{15}{16}\sigma_{AAD}^2 + \frac{31}{32}\sigma_{ADD}^2 + \frac{63}{64}\sigma_{DDD}^2 + \cdots$$

Using these relationships, the total genetic variance is given by

$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$$
$$= 2\sigma_g^2 + \sigma_s^2 + \sigma_C^2$$

It can be seen from these equations that a fraction of the epistatic variance is expressed by σ_g^2 and σ_s^2, derived primarily from interactions involving groups of two or three loci. The remainder of the epistasis expressed by σ_C^2 includes virtually all of the variance derived from interloci interactions involving larger groups of loci. Thus, we have approximated genetic variance components:

$$\sigma_{A'}^2 = 4\sigma_g^2 = \sigma_A^2 + \frac{1}{4}\sigma_{AA}^2 + \frac{1}{16}\sigma_{AAA}^2 + \cdots$$

$$\sigma_{D'}^2 = 4\sigma_s^2 = \sigma_D^2 + \frac{1}{2}\sigma_{AA}^2 + \frac{1}{2}\sigma_{AD}^2 + \frac{1}{4}\sigma_{DD}^2 + \frac{3}{8}\sigma_{AAA}^2 + \frac{1}{4}\sigma_{AAD}^2 + \frac{1}{8}\sigma_{ADD}^2 + \frac{1}{16}\sigma_{DDD}^2 + \cdots$$

$$k\sigma_I^2 = \sigma_C^2 - 2\sigma_g^2 - 3\sigma_s^2$$
$$= \frac{1}{4}\sigma_{AA}^2 + \frac{1}{2}\sigma_{AD}^2 + \frac{3}{4}\sigma_{DD}^2 + \frac{9}{16}\sigma_{AAA}^2 + \frac{3}{4}\sigma_{AAD}^2 + \frac{7}{8}\sigma_{ADD}^2 + \frac{15}{16}\sigma_{DDD}^2 + \cdots$$

where $\sigma_{A'}^2$ and $\sigma_{D'}^2$ are approximated additive and dominance variances, respectively, which are contaminated by a portion of the epistasis caused primarily by groups of two or three interacting loci; and $k\sigma_I^2$ is a fraction of epistasis contained by the clone variance component. Although $\sigma_{A'}^2$ and $\sigma_{D'}^2$ are contaminated by fractions of the total epistatic variance, these estimates are identical with those obtained from progeny trials without clonal replicates (Foster and Shaw 1988).

The exact order and magnitude of epistatic interaction components cannot be determined without additional information about gene action, allele frequencies, and numbers of loci involved. It is important to note that if the epistasis is limited to additive × additive interactions involving pairs of gene loci, $k\sigma_I^2$ will estimate only one-quarter of the total epistatic variance, and the remaining epistatic variance will be contained by $\sigma_{A'}^2$ and $\sigma_{D'}^2$. However, a complex metric trait such as growth is due to many physiological responses, controlled by a great many genes. Although low-order interactions might be expected to contribute substantially to the epistatic variance, it is possible that a large portion of the variance results from the many possible interactions among larger numbers (i.e., greater than two or three) of gene loci. In this case, the sum of the lowest order interactions involving groups of two or three loci may account for a small fraction of the total epistasis. Thus, even when epistatic variances constitute a large portion of the total genetic variance, estimates of σ_g^2 and σ_s^2 may be contaminated by a minute portion of the total interaction variance, and $k\sigma_I^2$, the fraction of epistasis estimated by σ_C^2, will then account for most of the total epistatic variance. Under this scenario, reasonable estimates of genetic variances may be calculated as

$$\hat{\sigma}_A^2 = 4\hat{\sigma}_g^2$$

$$\hat{\sigma}_D^2 = 4\hat{\sigma}_s^2$$

$$\hat{\sigma}_I^2 = \hat{\sigma}_C^2 - 2\hat{\sigma}_g^2 - 3\hat{\sigma}_s^2$$

When progeny trials are established with seedling material, no estimate of epistasis is available and estimates of additive and dominance variances for metric traits require the assumption that epistasis is absent (Cockerham 1954). When clonal replicates are used, the assumption could be relaxed somewhat to state that the greatest portion of the total epistasis is due to interactions involving groups of more than two or three loci. If this is actually the case, the contamination of additive and dominance variance estimates will be very small, and the estimate of epistatic variance will be reasonable. However, if epistasis is in fact derived only from lowest order loci interactions, estimates of additive and dominance variance will be seriously contaminated by interloci interaction variance and $\hat{\sigma}_I^2$ will estimate as little as one-quarter of the total epistasis.

Selection schemes, heritability, and gain estimation

These estimates of genetic variances can be used to calculate heritabilities for a variety of selection strategies. Heritability is broadly defined as the proportion of the total phenotypic variance for a given trait that is due to genotypic variation (Falconer 1981). When selection and deployment schemes are considered, heritability assumes a predictive role and is more specifically defined as the ratio of genetic variance recovered by the breeding method divided by the phenotypic variance of the selection unit: individuals, family mean, clone mean, etc. (Hanson 1963; Jacquard 1983).

If estimates of phenotypic variance and heritability are available, genetic gain that might be achieved under a given breeding strategy may be calculated using the generalized formula:

$$\Delta G_n = h_n^2 \, i_n \sqrt{\hat{\sigma}_n^2}$$

where

ΔG_n is the genetic gain for the nth strategy

h_n^2 is the applicable heritability

i_n is the standardized selection differential

$\hat{\sigma}_n^2$ is the phenotypic variance of the nth selection unit

The procedures demonstrated by Namkoong *et al.* (1966) have been widely employed to construct gain formulae for typical tree-breeding strategies. A similar approach is used here to derive gain formulae applicable to five simple breeding strategies, using information from the clonal test described previously. The examples described cover both conventional and clonal breeding options. In all cases, heritability is calculated by dividing the recoverable genetic variance(s) by the phenotypic variance of the selection unit. The calculation of genetic gain then follows the generalized formula given above.

Mass selection and grafted orchards

In this commonly employed scheme, individuals are selected from a population based on their phenotype and established by grafting into a conventional seed orchard. Planting stock is deployed as seedlings raised from orchard seeds. Since sexual recombination occurs in the production of offspring, only the additive portion of genetic variance and small fractions of σ_{AA}^2, σ_{AAA}^2, etc. are recovered, estimated in the field test by the GCA variance. The narrow-sense individual heritability (h^2) is applicable and calculated as

$$h^2 = \frac{\sigma_A^2 + \frac{1}{4}\sigma_{AA}^2 + \frac{1}{16}\sigma_{AAA}^2 + \ldots}{\sigma_{\text{ph}}^2}$$

$$= \frac{4\hat{\sigma}_g^2}{2\hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \hat{\sigma}_C^2 + \hat{\sigma}_E^2}$$

where σ_{ph}^2 is the phenotypic variance of individuals.

The genetic gain achieved by this scheme (ΔG_1) is estimated as

$$\Delta G_1 = h^2 \, i \sqrt{\hat{\sigma}_{\text{ph}}^2}$$

Some important assumptions apply to this gain estimate, in addition to those normally associated with the estimation of genetic and phenotypic variances. Mating in the orchard is assumed to be panmictic, and each genotype in the orchard contributes equally to the male and female gamete pool, with no inbreeding and no contamination from outside the orchard. In practice, it is easy to see that these assumptions are violated to some degree by most, if not all wind-pollinated seed orchards. Where violations are serious, estimates of genetic gain may indeed be very misleading.

Mass selection and cloning

In this strategy, mass selection is used as in the first case, but planting stock is produced and deployed as clones. Tissue is collected from the selected trees and cloned by rooted cuttings or some other vegetative propagation system. Clonal hedges would normally be established to provide the tissue material for operational production of clonal planting stock. A broad-sense individual heritability (H^2) is calculated with the expectation that all of the genetic components of individual phenotypic variance will be recovered. The heritability formula applicable to our sample field test is constructed:

$$H^2 = \frac{\sigma_A^2 + \sigma_D^2 + \sigma_I^2}{\sigma_{\text{ph}}^2}$$

$$= \frac{2\hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \hat{\sigma}_C^2}{2\hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \hat{\sigma}_C^2 + \hat{\sigma}_E^2}$$

If at least some of the genetic variance is nonadditive, this broad-sense heritability will be greater than the narrow-sense heritability calculated in the first scheme, and the genetic gain (ΔG_2) will also be correspondingly larger:

$$\Delta G_2 = H^2 \, i \sqrt{\hat{\sigma}_{\text{ph}}^2}$$

The assumptions described for the previous scheme (ΔG_1) regarding seed-orchard efficiency do not apply in this case. However, deployment as clones requires the assumption that vegetative propagules will grow like seedlings, although the effects of increasing maturation state may make this impossible to accomplish. The important assumption applied to this gain estimate is that the rejuvenation procedures used to clone the selected trees will also eliminate cumulative "C effects" (nonrandom environmental variances common to members of subgroups within a population (Lerner 1958)).

Backward GCA selection and polycross

The breeding value of parents can be determined by evaluating the performance of their half-sib progeny, produced by the diallel cross. The best GCA parents might be left in a seed orchard after a roguing; hence the term *backward GCA selection*. Alternatively, limited quantities of seeds from the best GCA parents could be produced by controlled crossing with other selected GCA parents, perhaps in a breeding hall or container orchard. Simple vegetative propagation techniques using juvenile seedlings could then produce steckling stock for deployment to plantations. A portion of the additive genetic variance will be recovered from the phenotypic variance of family means. The appropriate half-sib family mean heritability ($h_{\overline{\text{HS}}}^2$), is calculated as

$$h_{\overline{\text{HS}}}^2 = \frac{\frac{1}{4}\sigma_A^2 + \frac{1}{16}\sigma_{AA}^2 + \frac{1}{64}\sigma_{AAA}^2 + \ldots}{\sigma_{\overline{\text{HS}}}^2}$$

$$= \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \frac{cr\hat{\sigma}_s^2}{cr(p-2)} + \frac{r\hat{\sigma}_C^2}{cr(p-2)} + \frac{\hat{\sigma}_E^2}{cr(p-2)}}$$

where $\sigma_{\overline{\text{HS}}}^2$ is the phenotypic variance of half-sib family means and p, c, r are the numbers of parents, clones within family, and ramets within clone, respectively.

The genetic gain resulting from backward GCA selection (ΔG_3) is calculated in the usual fashion, but since both seed and pollen parents have been selected based on their tested GCA, the expected genetic gain is multiplied by 2:

$$\Delta G_3 = 2h_{\overline{\text{HS}}}^2 \, i \sqrt{\hat{\sigma}_{\overline{\text{HS}}}^2}$$

If seed production is from GCA-selected parents in a rogued orchard, all of the assumptions stated earlier for wind-pollinated orchards will of course apply. These assumptions

are more likely to be met if seeds are produced by controlled pollination or, alternatively, in a breeding hall – container orchard where a polycross is performed using an unrelated pollen mix composed of other best-GCA parents. If deployment is to be accomplished using vegetative propagules such as rooted cuttings, the assumptions regarding maturation and C effects also apply. However, as vegetative multiplication will be from juvenile seedlings, these assumptions require a smaller leap of faith than if older materials were used. Recent advances in biotechnology indicate that somatic embryogenesis may also become a promising technique for mass vegetative propagation, particularly in some northern conifers.

Backward SCA selection and repeat crossing

Selection of full-sib families can utilize the SCA of pairs of parents in addition to their individual GCAs; hence the term *backward SCA selection*. Nursery production will require that the selected families be regenerated by means of repeat controlled pollination. Large quantities of seeds will be both difficult and expensive to produce, so vegetative propagation will most likely be used to grow steckling stock for planting programs. The full-sib family mean heritability ($h^2_{\overline{FS}}$) indicates that fractions of additive, dominance, and epistatic genetic variance will be recovered:

$$h^2_{\overline{FS}} = \frac{\frac{1}{2}\sigma^2_A + \frac{1}{4}\sigma^2_D + \frac{1}{4}\sigma^2_{AA} + \frac{1}{8}\sigma^2_{AD} + \frac{1}{16}\sigma^2_{DD} + \frac{1}{8}\sigma^2_{AAA} + \frac{1}{16}\sigma^2_{AAD} + \frac{1}{32}\sigma^2_{ADD} + \frac{1}{64}\sigma^2_{DDD} + \ldots}{\sigma^2_{\overline{FS}}}$$

$$= \frac{\hat{\sigma}^2_F}{\hat{\sigma}^2_F + \frac{r\hat{\sigma}^2_C}{cr} + \frac{\hat{\sigma}^2_E}{cr}}$$

where $\sigma^2_{\overline{FS}}$ is the phenotypic variance of full-sib family means.

The genetic gain from backward SCA selection is estimated by

$$\Delta G_4 = h^2_{\overline{FS}}\, i \sqrt{\hat{\sigma}^2_{\overline{FS}}}$$

Production of large quantities of seeds for selected full-sib families could be accomplished by means of a biclonal seed orchard (Shelbourne 1969), which would introduce assumptions regarding gamete recombination under field pollination conditions. More likely, small quantities of seeds from repeat controlled crossing would produce donor plants for vegetative multiplication by rooted cuttings or somatic embryogenesis. If crosses were repeated frequently and donor plants maintained in a common nursery area and replaced, say every other year, problems of maturation state ageing and presumably C effects would be minimized. This "family forestry" approach has developed recently in New Zealand (Carson 1986*b*; Shelbourne *et al.* 1989) where vegetative multiplication was combined with the idea of control-pollinated orchards, proposed earlier by Sweet and Krugman (1977).

Clone selection and cloning

In this scheme, clones are field tested and selection is based on ranked clone means. The genetic portion of the clone-mean variance contains the total genetic variance, estimated in this example by the sum of the family and clone within family variances. The selected clones would then be mass propagated for operational deployment. The broad-sense clone-mean heritability ($H^2_{\overline{C}}$) is calculated as

$$H^2_{\overline{C}} = \frac{\sigma^2_A + \sigma^2_D + \sigma^2_I}{\sigma^2_{\overline{C}}}$$

$$= \frac{\hat{\sigma}^2_F + \hat{\sigma}^2_C}{\hat{\sigma}^2_F + \hat{\sigma}^2_C + \frac{\hat{\sigma}^2_E}{r}}$$

where $\sigma^2_{\overline{C}}$ is the phenotypic variance of clone means.

The genetic gain resulting from clone mean selection is estimated by

$$\Delta G_5 = H^2_{\overline{C}}\, i \sqrt{\hat{\sigma}^2_{\overline{C}}}$$

Of the strategies discussed in this paper, clone selection and cloning will likely produce the greatest gain. In practice, a portion of the ramets from each of the clones being field tested may be retained in the juvenile state in hedges (DeWitt 1989; Libby and Hood 1976; Libby *et al.* 1972; Wise *et al.* 1986), or by repeated cycles of rooting (Kleinschmit and Schmidt 1977; St. Clair *et al.* 1985; von Wühlisch 1984). For most conifer species, however, maintaining such material in a juvenile state is almost impossible (Greenwood 1987). The practical application of this strategy may depend on cryopreservation of somatic embryos or embryogenic cultures (Cheliak 1989; Gupta *et al.* 1987; Kartha *et al.* 1988). A portion of the embryogenic cultures may be stored in liquid nitrogen during clonal testing, thus eliminating the problem of changing maturation state.

Discussion

Throughout this paper, the estimation of genetic parameters has referred to several assumptions. The most important of these were given by Comstock *et al.* (1958) and are summarized below, with modifications for vegetatively propagated materials:

(1) Parents are randomly sampled from a random mating population.
(2) There is regular Mendelian diploid behaviour at meiosis.
(3) Linkage equilibrium exists such that gene loci affecting an observed character segregate independently, or where linkage does exist, the distribution of genotypes is as expected in the absence of linkage (Cockerham 1956).

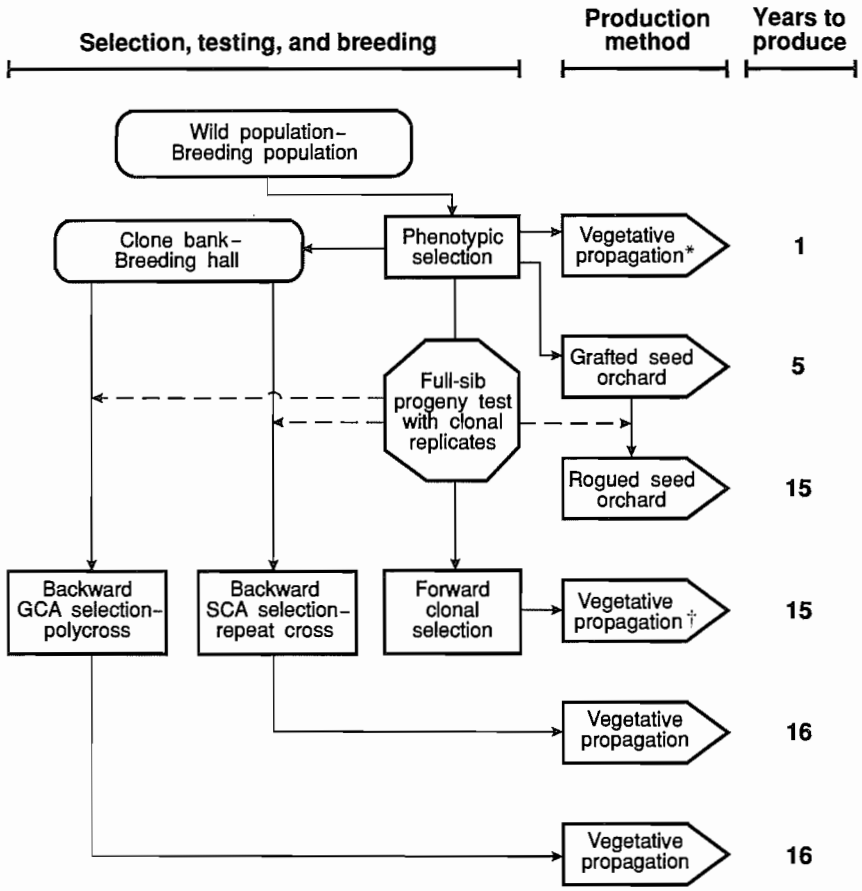

FIG. 1. Possible flowchart of breeding–cloning strategies. Solid and broken lines indicate material and information flow, respectively. Time required for flowering is assumed to be 5 years, and there are 10 years for testing. *, Vegetative propagation may not be possible from mature donor trees. †, True to type vegetative propagation may be difficult to achieve due to maturation of the donor plants during the test period. Cryopreserved embryonic culture may circumvent this problem.

(4) When present, epistasis is due primarily to interactions involving groups of more than two or three loci; otherwise, estimates of additive and dominance variances will be seriously contaminated, and epistasis underestimated by a corresponding amount.
(5) C effects are absent.

The introduction of C effects by cloning may bias estimates of variance components and reduce the efficiency of selection (Libby and Jund 1962; Burdon and Shelbourne 1974). C effects have been documented in several species, but generally account for a small portion of the total clonal variation (Cannell *et al.* 1988; Farmer *et al.* 1988, 1989; Foster *et al.* 1984; Wilcox and Farmer 1968). Libby (1976) recognized three important sources of C effects in tree breeding: (*i*) the "maternal effect," which influences the performance of progeny of the same female parent; (*ii*) environmental covariance, which occurs when groups of trees are raised in the same part of the nursery or test site; and (*iii*) a special kind of environmental variance due to differences in propagule conditions within clones. This last effect, which may arise from variation in size of cutting, position on the ortet, propagation cycle, ortet vigour, etc., contributes to variance within clones but does not affect variance among clones and therefore does not result in inflated estimates of genetic gain (Foster *et al.* 1984; 1989).

Beyond the technical problems involved in cloning trees, the practice of clonal forestry is contingent on the growth and form of vegetative propagules compared with that of seedling planting stock (Brix and van den Driessche 1977; Thompson 1984). Field comparisons of growth and morphology of stecklings and seedlings have produced apparently conflicting results. Some studies have indicated that stecklings are inferior to seedlings (Shelbourne and Thulin 1974; Sweet and Wells 1974), some have found cuttings superior (Burdon and Bannister 1985; Roulund 1974, 1977, 1978), and others have found little difference (Copes 1976; Fielding 1970; Foster 1988). The maturation state of the donor plant is recognized as having a profound effect on the performance of stecklings (Foster *et al.* 1987; Greenwood 1984; Greenwood and Nussbaum 1981; Menzies and Klomp 1988; Roulund 1979; Sweet 1973), as does the quality of the induced root system (Foster *et al.* 1984, 1985; Greenwood 1984; Struve *et al.* 1984) and the overall vigour of the propagule (Foster *et al.* 1987).

Currently, operational clonal programs in northern conifers rely on rooting of cuttings from juvenile plants, and several cycles of propagation are often carried out to meet stock requirements. Micropropagation, particularly by somatic embryogenesis, holds the future promise of cloning systems with greater efficiency. Since 1985, rapid progress has been made in the development of procedures for somatic

embryogenesis using tissue from mature and immature embryos for several northern conifer genera, e.g., *Larix* (Nagmani and Bonga 1985), *Pinus* (Gupta and Durzan 1986, 1987*a*), *Picea* (Attree *et al.* 1989; Hakman and Fowke 1987; Hakman and von Arnold 1988; Lu and Thorpe 1987; Nagmani *et al.* 1987; von Arnold 1987; Tremblay 1990), and *Pseudotsuga* (Gupta and Durzan 1987*b*). Recently, plantlet regeneration has even been achieved using somatic embryos induced from cultured shoots and cotyledons of young black spruce seedlings (Attree *et al.* 1990). "Emblings" will, of course, require all of the tests of developmental fidelity that have proved to be so important for stecklings (see Ahuja and Libby 1991).

The gain formulae presented here illustrate comparative gains that might be achieved in a given selection stage; however, the choice of overall breeding strategy is influenced by several factors beyond gain achieved during any one step. As illustrated in the papers by Namkoong *et al.* (1966) and Shelbourne (1969), a typical tree-improvement plan will consist of several generations, each with multiple stages of selection. Choice will be influenced by time schedules for breeding operations, rotation length, seed-orchard productivity, stumpage prices, cost, and practical considerations (Cotterill 1986; Lindgren 1977; Paques 1989; Pepper and Namkoong 1978). When estimates of genetic variances and heritabilities are available and when operational time schedules known, gains over time from complex strategies combining both recurrent selection and clonal testing, such as those proposed by Fowler (1986, 1987, 1988), can be modelled using the general approach given in the classic paper by Namkoong *et al.* (1966).

A simplified illustration of possible breeding and cloning strategies is presented in Fig. 1. In this example, we assume that the time required for flowering and field testing is 5 and 10 years, respectively. In the context of second-generation improvement, and as an alternative to conventional seed-orchard procedures, these strategies can begin in clone banks or breeding halls by performing controlled pollinations on first-generation selections. Although the mating design discussed in this paper is a modified half-diallel, different mating designs may be used depending on the breeding–cloning strategy used. For example, if a breeder opts for backward GCA selection and polycross, the mating design can be a simple polycross. To utilize some of the nonadditive variance, it is necessary to use a full-sib mating design, such as single-pair matings, or factorial, nested, or diallel crosses. Using clonal replicates with any of these mating designs is likely to increase the efficiency of estimating genetic parameters and selection (Shaw and Hood 1985).

The breeding strategies, backward GCA and backward SCA selection, can be practiced currently using the serial rooted-cutting procedure from juvenile plants, albeit at higher nursery production cost. Even so, it is possible that the efficiencies gained by using small breeding halls instead of large soil-based orchards, the elimination of large seed-extraction plants, as well as additional gain and flexibility will more than offset the higher cost of stock production. When biotechnology such as somatic embryogenesis becomes an operational tool, the efficiency of cloning will be increased. Coupled with cryopreservation of embryogenic cultures, strategies such as forward clonal selection will become more effective as juvenility of superior clones will be maintained in storage during the test period. The next-generation breeding population may be formed by selecting the best clones from the best backward GCA- or SCA-selected families, and the cycle shown in Fig. 1 repeated.

Acknowledgments

We are grateful to Dr. E.K. Morgenstern, University of New Brunswick; Dr. D.P. Fowler, Forestry Canada; Dr. G.C.C. Tai, Agriculture Canada; and particularly Dr. K. Hinkelmann, Virginia Polytechnic Institute and State University for helpful comments on an early draft; and Dr. W.J. Libby, University of California at Berkeley for his detailed review and suggestions, which significantly improved the final manuscript. This paper is based on work conducted while T.J. Mullin was in residence as a Ph.D. candidate and receiving financial assistance from the University of New Brunswick.

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