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# Maximizing gain per effort by using clonal replicates in genetic tests

D. V. Shaw[1] and J. V. Hood[2,*]

[1] International Forest Seed Company, P.O. Box 290, Odenville, AL 35120, USA
[2] Ontario Ministry of Natural Resources, Ontario Tree Improvement and Forest Biomass Institute, Maple, Ontario, Canada

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**Summary.** Models for predicting cummulative genetic gain from recurrent selection applicable to predominantly outcrossing plant species are derived to include the effect of observations on clonal replicates (ramets) in addition to observations on individuals and family means. Such models are discussed with special reference to forest trees. The consequence of redistributing effort from individuals to ramets is investigated for several conditions with a fixed number of families and fixed total test size. Factors that affect the distribution of variance among sources and factors that affect individual selection intensity are the primary determinants of the optimum distribution of effort. The optimum number of ramets ranged from 1 to 6 for the conditions tested and the efficiency of redistribution (ratio of gain for the optimum distribution to the gain for the single-ramet, or non-clonal case) ranged from 1.00 to 1.20. Using clonal replicates in genetic tests usually results in increased cummulative genetic gain relative to non-clonal tests, without an increase in test effort.

**Key words:** Selection efficiency – Clonal replication – Genetic gain – Forest trees

## Introduction

Genetic tests for cross-fertilized plant species are usually structured to include a hierarchy of families and individuals within families. Each individual's genetic contribution is coupled with a unique environmental effect to form the resulting individual phenotype. Because environmental effects are usually large and because genetic variance among individuals (genotypes) is confounded with environmental effects, individual genotypic values are difficult to assess. The confounding of genetic and environmental effects is troublesome when individual selections must be made to initiate the following generations; such is the case for our area of specialization, forest tree improvement. Consequently, most recurrent selection programs designed for forest trees depend heavily on evaluations and selections based on family performance. The genetic variation that exists within families remains largely unused. When clonal replicates of each individual (ramets) can be produced, each individual's genotypic contribution can be observed in combination with a number of unique environmental effects (i.e. genotypic value and environment are no longer confounded). Intuitively, such repeated observations should allow additional precision in ranking individuals with regard to their genetic value and consequently allow better utilization of the genetic variability that exists within families.

Most of the published investigation relating to the optimum number of ramets in genetic tests are aimed at accurate evaluation of individual (clone) values in the absence of family data. Also, such investigations usually start with empirical data and infer an optimum as the number of ramets necessary to evaluate a clone mean with a specified degree of precision. Our approach has been to include clonal replication as a component in a testing scheme that utilizes several levels of variation. Libby (1969) presented a treatment with a similar intent to ours by modifying an efficiency formula originally developed for index selection based on a nested mating design (Osborn 1957). The optimum number of ramets per individual was one in all cases; we will compare Libby's results to our own in the following sections.

* Current address: Forest Research Branch, 99 Wellesley Street West, Toronto, Ontario M7A 1W3, Canada

Our objectives for this paper are as follows:

1) To provide models for predicting genetic gain when genetic tests include observations on clonal replicates of individuals in addition to family structure.
2) To provide a method for predicting the optimum number of ramets per individual when both the number of families available for selection and the total test size (number of plants tested) are fixed.
3) To provide demonstrative examples based on a range of population, genetic and environmental parameters realistic for forest tree improvement.

Although we use our own specialization (forest tree improvement) as an example, our results are generally applicable to cross fertilizing species for which clonal propagation is an option.

## Material and methods

The consequences of using clonal replicates of genotypes in genetic tests were investigated for three selection methods: 1) two-stage selection based on full-sib families and individuals within families, 2) three-stage selection based on half-sib families, full-sib families selected within half-sib families, and individuals selected within full-sib families, and 3) combined index selection. For all methods, formulas were derived for predicting genetic gain and applied to a method for optimizing the distribution of effort between observations on individuals and ramets. Optimization was performed for a variety of conditions.

*Prediction of genetic gain*

Formulas for predicting genetic gain from multistage selection are derived below. The following model is appropriate when each individual may be represented in a genetic test by multiple ramets:

$$Y_{ijklm} = u + G_{j(i)} + G_{k(i)} + S_{jk(i)} + C_{l(ijk)} + E_{m(ijkl)} \quad (1)$$

in which

u = population mean
$G_j$ = the average deviation from the mean of families containing the $j^{th}$ parent
$G_k$ = the average deviation from the mean of families containing the $k^{th}$ parent
$S_{jk}$ = the deviation of family $jk$ from the average of the $j^{th}$ and $k^{th}$ family means
$C_l$ = the deviation of individual $l$ from the $jk$ family mean
$E_m$ = a random error effect

The subscript $i$ denotes the $i^{th}$ breeding group; variation that exists among breeding groups has been ignored in our initial treatment. Taking expectations for the above model (see Kempthorne 1969) yields the following variances:

V (half-sib (hs) family means)
$$= (1 + 1/n_x)\,\sigma_g^2 + \sigma_s^2/n_x + \sigma_c^2/n_x n_c + \sigma_e/n_x n_c n_r \quad (2)$$

V (full-sib (fs) family means)
$$= 2\,\sigma_g^2 + \sigma_s^2 + \sigma_c^2/n_c + \sigma_e^2/n_c n_r \quad (3)$$

V (individual means)
$$= 2\,\sigma_g^2 + \sigma_s^2 + \sigma_c^2 + \sigma_e^2/n_r \quad (4)$$

in which

$n_x$ = number of full-sib families per parent
$n_c$ = number of individuals per cross (full-sib family)
$n_r$ = number of ramets per individual

Recalling that:

V (full-sib family deviations) = V (fs family means) − V (hs family means), and V (individual deviations) = V (individual means) − V (fs family means) and substituting $V_E$ for $\sigma_e^2$, $\frac{1}{4} V_A$ for $\sigma_g^2$, $\frac{1}{4} V_D$ for $\sigma_s^2$ and $\frac{1}{2} V_A + \frac{3}{4} V_D$ for $\sigma_c^2$ heritabilities were obtained for half-sib family means (hs), full-sib family means (fs), full-sib family deviations (fsd) and individual deviations (ind)[1]. Genetic gain formulas were obtained from the formula:

$$G = i\,\sigma_p\,h^2 \quad \text{(Falconer 1960)} \quad (5)$$

in which, $i$, $\sigma_p$ and $h^2$ are the selection intensity, phenotypic standard deviation, and the heritability for the appropriate selection unit (e.g. half-sib family mean, full-sib family deviation, etc.). The following gain predictors were obtained for two-stage selection:

$$G_{fs} = i_{fs}\,h^2_{fs}\,\sigma_{fs} = \frac{i_{fs}\,(V_A/2 + V_A/2n_c)}{(V_A/2 + V_D/4 + V_A/2n_c + 3V_D/4n_c + V_E/n_c n_r)^{1/2}} \quad (6)$$

$$G_{ind} = i_{ind}\,h^2_{ind}\,\sigma_{ind} = \frac{i_{ind}\,[(1 - 1/n_c)\,V_A/2]}{((1 - 1/n_c)\,(V_A/2 + 3V_D/4 + V_E/n_r))^{1/2}} \quad (7)$$

and for 3 stage selection:

$$G_{hs} = i_{hs}\,h^2_{hs}\,\sigma_{hs} = \frac{i_{hs}\,[((1 + 1/n_x)\,V_A/4) + (1/n_x n_c\,V_A/2)]}{((1 + 1/n_x)\,(V_A/4 + V_D/4n_x + V_A/2n_x n_c + 3V_D/4n_x n_c + V_E/n_c n_r n_x))^{1/2}} \quad (8)$$

$$G_{fsd} = i_{fsd}\,h^2_{fsd}\,\sigma_{fsd} = \frac{i_{fsd}\,[(1 - 1/n_x)\,(V_A/4 + V_A/2n_c)]}{((1 - 1/n_x)\,(V_A/4 + V_D/4 + V_A/2n_c + 3V_D/4n_c + V_E/n_c n_r))^{1/2}}. \quad (9)$$

Gain for individual selection in the three-stage method is identical to that for the two-stage method (7). Note that the only consequence of including clonal replicates on the above formulas is the inclusion of the factor $1/n_r$ in some components of the heritability denominator. When $n_r = 1$, the above formulas are equivalent to formulas for predicting genetic gain in the absence of clonal propagation.

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1 $V_A$, $V_D$ and $V_E$ are the additive genetic, dominance genetic and environmental variance, respectively

Selection intensities for half-sib family means, full-sib family means and for individual deviations were calculated directly from tables given in Becker (1975). Selection intensities for full-sib family deviations present a more complex problem when three-stage selection is performed. Factorial and diallel breeding designs generate full-sib families that are members of two half-sib families. When half-sib families are selected first in the three-stage series, it is possible to select the same full-sib family from two previously selected half-sib families. When this occurs, an alternative full-sib family must be selected to achieve the desired population size. The result would be a reduction in the effective full-sib family selection intensity. Squillace (1973) noted this problem and compensated by selecting twice the number of half-sib families necessary, a method that is likely to over-compensate for the problem. We calculated the probability that two, three, four, or more, half-sib families would be selected from a single breeding group and the conditional probability that a single full-sib family would be selected twice. The average number of full-sib families selected twice was calculated and selection intensities were adjusted to reflect selection of additional families as compensation. One caveat to our adjustment is that all half-sib family means were considered independent and the rank of full-sib families within two overlapping half-sib families assumes no covariance. Additive genetic covariance due to sharing of common parents is likely to invalidate the above to some extent and cause our full-sib family selection intensities to be somewhat larger than appropriate.

Gains were calculated for each stage of selection and the total gain for multistage selection is the sum of the gains for each stage.

Gains for combined index selection were predicted using the formula:

$$G = i\,(b'\,C)^{1/2} \qquad (11)$$

in which G is the genetic gain, $i$ is the selection intensity, $b$ is the vector of optimum weights for the phenotypic records indexed (e.g. clone mean, family mean) and $C$ is the vector of expected covariances between the phenotypic record and the true genetic value of the individual. Methods for obtaining indexes and predicting gain for any mating design are available (e.g. Van Vleck 1979) and will not be discussed further here. The advantage of using clonal replicates in index selection is realized through indexing on a clone mean (average over several ramets) rather than a single phenotypic value.

*Method of optimization*

Our method for optimizing the distribution of effort among genotypes and ramets is similar to that used by Squillace (1973) for distributing effort among families and individuals. First, we fix both the total effort (number of "plants" in the test) and the number of families available for selection in each generation. As a result the number of plants per family is also fixed. We vary the number of unique genotypes tested ($n_c$) and the number of ramets per genotypes ($n_r$) such that $n_c \times n_r$ always equals the fixed number of plants per family. Gains are calculated for each combination of $n_c$ and $n_r$, for a specific set of population parameters (variance components, mating designs, selection intensities). The optimum distribution of effort for a set of conditions is defined as that combination of $n_c$ and $n_r$ which yields the largest gain.

The assumptions necessary for applying the formulas derived and the above method of optimization are as follows:

1) Only additive and dominance genetic effects are important to the total genetic variance.

2) Either the results are for a single environment or there are no genotype × environment interactions. Also, within-family and between family environmental variances are equal – this is valid for tests established in a non-contiguous plot design.

3) No additional variance is created by the vegetative propagation process.

4) Coancestry control is limited: no coancestry control is practiced for index selection and half-sib family structure is ignored when doing two-stage selection.

Each of these assumptions is likely to be invalid, at least to some extent, in most tree improvement programs. However, all of the controlling assumptions are testable and their magnitude to some degree estimable.

*Conditions*

The conditions are given in Table 1. We use a recurrent selection program in which 120 full-sib families are created and 144 plants per family are tested each generation. The mating design controls both the number of genotypes that must be selected and crossed each generation, and the number of full-sib families per half-sib family. For example, a $2 \times 2$ factorial scheme requires the mating of 120 selected genotypes to produce 120 full-sib families and provides 120 overlapping half-sib families for selection. A $4 \times 4$ factorial scheme requires the mating of 60 selected genotypes to produce 120 full-sib families and provides 60 half-sib families for selection. The lower order mating designs ($2 \times 2$ factorial and $3 \times 3$ diallel schemes) also differ from their higher order ($4 \times 4$ and $5 \times 5$) counterparts in the number of full-sib families per half-sib family; this affects both the selection intensity for full-sib families and the gain formulas presented above. Table 2 summarizes our selection scenarios. For two stage selection, half-sib relationships are ignored and mating design affects only full-sib family and individual selection intensities. When three-stage selection is performed, the mating design affects selection intensities at all three levels. For index selection the mating design affects both the selection intensity and the precision of estimating each individual's true genetic value.

Variance parameters were chosen to represent a character with low to moderate heritability and to cover a range of possible genetic-environment variance combinations that could result in each heritability. The consequence of fixing both the total variance and the heritability for each set is

**Table 1.** Conditions used to generate sample gain estimates for evaluating the optimum distribution of effort between individuals and ramets

| Parameters | Conditions |
|---|---|
| 1. $\sigma_p$: | 3 |
| 2. $h^2$: | 0.11, 0.22 |
| 3. $\sigma_A^2/\sigma_D^2$: | 10, 1, 1/2 |
| 4. No. families | 120 |
| 5. No. plants/family: | 144 |
| 6. No. ramets/genotype: | 1, 2, 3, 4, 6, 8 |
| 7. Mating design: | $2\times2$ and $4\times4$ disconnected factorials<br>$3\times3$ and $5\times5$ disconnected half diallels |

$\sigma_p$ = phenotypic standard deviation; $h^2$ = narrow sense heritability; $\sigma_A^2$ = additive genetic variance; $\sigma_D^2$ = dominance genetic variance

important and will be discussed further in the results section. Gain predictions are presented in phenotypic standard deviation units. With proper scaling they are applicable to a range of characters having similar heritabilities.

## Results

The more apparent consequences of redistributing effort from individuals to ramets for multistage selection can be predicted from examination of equations (6)–(9):

1) Only the components of variance that occur within families are affected by redistribution of effort. These are the components that include $n_c$ (the number of individuals per family) as a divisor. As more effort is directed towards ramets, $n_c$ becomes smaller and the number of ramets per individual ($n_r$) becomes larger. Components divided by a smaller $n_c$ assume larger values and become more important to the gain formulas. Because components affected by $n_c$ occur in both the numerator and denominator of all gain equations, the effect of altering $n_c$ will depend on the relative size of variance components and will be difficult to predict a priori.

2) The only factor affect by $n_r$ is the environmental component of variance ($V_e$). When $V_e$ appears in family selection gain formulas (6), (8), and (9), it is always divided by the constant value $n_c \times n_r$. Increases in gain due to family selection cannot result from a reduction of the contribution of $V_E$ to the gain denominator. Conversely, the individual gain formula (7) has the component $V_E$ divided by $n_r$ alone. Reducing the gain denominator will increase the precision of selection within families, and thus act to increase gain.

3) Conversely to (2), redistributing effort from individuals to ramets also reduces the number of unique genotypes within each family from which selections can be made. When a fixed number of individuals must be selected from each family, the result is a reduction in individual selection intensity and a consequent reduction in gain.

Because redistribution of effort can result in both gains and losses, an optimum allocation scheme must exist for any set of conditions.

### *Multistage selection*

The results for two-stage and three-stage selection demonstrate similar principals and will be discussed together. Table 3 provides a typical example of the optimization process for three-stage selection. A 4×4 disconnected factorial is shown, 50% of the available half-sib families and one full-sib family per half-sib family are saved; the heritability = 0.11 and $\sigma^2_A/\sigma^2_D = 1.0$. As mentioned above $n_c \times n_r$ is always 144. Note (column 3) that the individual selection intensity ($i_{ind}$) decreases with decreasing $n_c$. Redistribution of effort affects gain at all levels of selection, with the most

**Table 2.** The number of half-sib families, full-sib families, and individuals selected for 4 different mating designs[a]

| Two-stage selection | | | | | |
|---|---|---|---|---|---|
| 2×2 factorial or 3×3 diallel | | | 4×4 factorial or 5×5 diallel | | |
| Full-sib families | Individuals | | Full-sib families | Individuals | |
| 60 | 2 | | 60 | 1 | |
| 40 | 3 | | 30 | 2 | |
| 30 | 4 | | 20 | 3 | |
| 24 | 5 | | 15 | 4 | |
| **Three-stage selection** | | | | | |
| 2×2 factorial or 3×3 diallel | | | 4×4 factorial or 5×5 diallel | | |
| Half-sib families | Full-sib families | Individuals | Half-sib families | Full-sib families | Individuals |
| 60 | 1 | 2 | 30 | 1 | 2 |
| 40 | 1 | 3 | 20 | 1 | 3 |
| 30 | 1 | 4 | 15 | 1 | 4 |
| 24 | 1 | 5 | 12 | 1 | 5 |
| 20 | 1 | 6 | 30 | 2 | 1 |
| | | | 15 | 2 | 2 |

[a] Full-sib family selection intensities for 3-stage selection were calculated using these numbers after correction for the probability of selecting a given full-sib family from two overlapping (previously selected) half-sib families; see text for details. Selection intensities for 2-stage selection were calculated directly from the values above

**Table 3.** Predicted gains in standard deviation units for a 4×4 disconnected factorial mating design: 30 of 60 available half-sib families selected ($i_{hs} = 0.79$), $i_{fs}$ (adjusted) = 0.99; $h^2 = 0.11$, $\sigma^2_A/\sigma^2_D = 1$

| $n_c$ | $n_r$ | $i_{ind}$ | $G_{ind}$ | $G_{fs}$ | $G_{hs}$ | $G_t$ |
|---|---|---|---|---|---|---|
| 144 | 1 | 2.46 | 0.142 | 0.097 | 0.132 | 0.371 |
| 72 | 2 | 2.20 | 0.166 | 0.098 | 0.133 | 0.397 |
| 48 | 3 | 2.04 | 0.177 | 0.098 | 0.133 | 0.408 |
| 36 | 4 | 1.91 | 0.182 | 0.098 | 0.132 | 0.412 |
| 24 | 6 | 1.73 | 0.182 | 0.100 | 0.132 | 0.414 |
| 16 | 8 | 1.53 | 0.168 | 0.102 | 0.131 | 0.402 |

$n_c$ = number of genotypes per full-sib family; $n_r$ = number of ramets per genotype; $i_{ind}$ = within-family selection intensity; $G_{ind}$ = within-family selection gain; $G_{fs}$ = full-sib within half-sib family selection gain; $G_{hs}$ = half-sib family selection gain; $G_t$ = total gain

dramatic changes evident for individual gain. Further decreasing $n_c$ by increasing $n_r$ will eventually reduce family selection gains by increasing the contribution of within-family genetic variance components to the family gain denominators. However, in the example presented, there is no appreciable loss in family selection gain even when $n_c$ has been reduced to 16. Total gain is maximum for these conditions when 24 genotypes and 6 ramets of each genotype are used. The efficiency of redistribution (gain for the optimum distribution divided by the gain for the single ramet case) is 1.12.

The optimum number of ramets and maximum genetic gain for two-stage selection (Table 4) and for three-stage selection (Tables 5, 6 and 7) were investigated for the conditions listed in Table 1. For these conditions, the optimum number of ramets per individual varied from 1 to 6 and the efficiency varied from 1.0 to 1.20. The effect of varying conditions is similar between the two selection methods and can be summarized as follows:

1) Small ratios of additive to dominance variance favor optimum distributions with few ramets per individual and yield less total gain than cases for which the ratio is large.

2) Cases with higher heritabilities have optimum distributions with fewer ramets per individual and larger genetic gains than cases with lower heritabilities.

3) Conditions that allow more intensive family selection have optimums with fewer ramets per individual than when family selection is less severe. More intensive family selection always yielded greater total gains than less intensive selection.

4) Mating designs that include more parents per set (i.e. 4×4 factorial vs. 2×2 factorial) favor larger numbers of ramets, although this effect is very small. Genetic gains were larger when sets including more parents were used and little difference in gain was noticable between mating designs that had the same number of full-sib families per half-sib family.

Our model allows allocation of the total variance among only additive genetic, dominance genetic, and environmental sources. Because the total variance is fixed by assumption, alteration of conditions that affect genetic components of variance will also affect the amount of variance that is allocated to environmental effects. Recall that the benefit of clonal replication is realized by reduction of the contribution of the environmental variance component to the individual gain denominator. Reduction of this contribution is of little consequence when the environmental variance is already a small proportion of the total (e.g. high heritability, large dominance genetic component). Both family selection intensities and mating design have their ultimate effect on individual selection intensities. When few families are selected, a larger number (and proportion) of the individuals available within each selected family must be retained to maintain a constant breeding population size, reducing individual selection intensity. Mating designs that include more parents per set require selection of fewer individuals each generation to produce the designated 120 full-sib families,

**Table 4.** Optimum number of ramets and predicted gain in phenotypic standard deviation units (in parentheses) for two-stage selection. All mating designs provide 120 full-sib families for selection

| | Optimum no. of ramets (predicted gain) | | | | | |
|---|---|---|---|---|---|---|
| | $h^2 = 0.11$ | | | $h^2 = 0.22$ | | |
| | $\sigma_A^2/\sigma_D^2$ | | | $\sigma_A^2/\sigma_D^2$ | | |
| Proportion of families saved | 10 | 1 | 0.5 | 10 | 1 | 0.5 |
| 2×2 factorial or 3×3 half diallel | | | | | | |
| 0.50 | 6 (0.38) | 6 (0.33) | 3–4 (0.30) | 6 (0.63) | 3 (0.54) | 2 (0.50) |
| 0.33 | 6 (0.43) | 4 (0.37) | 3 (0.33) | 4 (0.69) | 2 (0.60) | 2 (0.55) |
| 0.25 | 6 (0.45) | 4 (0.39) | 3 (0.35) | 4 (0.73) | 2 (0.63) | 1 (0.58) |
| 0.20 | 4–6 (0.47) | 3 (0.41) | 3 (0.36) | 3–4 (0.75) | 2 (0.65) | 1 (0.60) |
| 0.166 | 4 (0.48) | 3 (0.42) | 2 (0.37) | 3 (0.76) | 2 (0.67) | 1 (0.61) |
| 4×4 factorial or 5×5 half diallel | | | | | | |
| 0.50 | 6 (0.41) | 6 (0.35) | 4 (0.32) | 6 (0.68) | 3–4 (0.57) | 2 (0.52) |
| 0.25 | 6 (0.49) | 6 (0.42) | 3–4 (0.37) | 6 (0.78) | 3 (0.67) | 2 (0.61) |
| 0.166 | 6 (0.52) | 4 (0.45) | 3 (0.40) | 4 (0.82) | 2 (0.71) | 2 (0.64) |
| 0.125 | 6 (0.53) | 4 (0.46) | 3 (0.41) | 4 (0.85) | 2 (0.73) | 1 (0.66) |

$h^2$ = narrow sense heritability; $\sigma_A^2$ = additive genetic variance; $\sigma_D^2$ = dominance genetic variance

**Table 5.** Optimum number of ramets and predicted gain in phenotypic standard deviation units (in parentheses) for three-stage selection[a]

| | Optimum no. of ramets (predicted gain) | | | | | |
|---|---|---|---|---|---|---|
| | $h^2 = 0.11$ | | | $h^2 = 0.22$ | | |
| | $\sigma_A^2/\sigma_D^2$ | | | $\sigma_A^2/\sigma_D^2$ | | |
| Proportion of hs families saved | 10 | 1 | 0.5 | 10 | 1 | 0.5 |
| 2×2 factorial | | | | | | |
| 0.50 | 6 (0.41) | 4–6 (0.36) | 3 (0.32) | 6 (0.77) | 3 (0.68) | 2 (0.64) |
| 0.33 | 6 (0.45) | 4 (0.40) | 3 (0.36) | 4 (0.78) | 2 (0.78) | 2 (0.73) |
| 0.25 | 6 (0.47) | 4 (0.42) | 3 (0.38) | 4 (0.91) | 2 (0.83) | 1 (0.78) |
| 0.20 | 6 (0.49) | 3–4 (0.43) | 3 (0.39) | 3 (0.94) | 2 (0.86) | 1 (0.81) |
| 0.167 | 4 (0.50) | 3 (0.44) | 2 (0.40) | 3 (0.97) | 2 (0.89) | 1 (0.84) |
| 3×3 half-diallel | | | | | | |
| 0.50 | 6 (0.41) | 6 (0.36) | 3–4 (0.32) | 6 (0.77) | 3 (0.69) | 2 (0.64) |
| 0.33 | 6 (0.45) | 4 (0.40) | 3 (0.36) | 4 (0.86) | 2 (0.78) | 2 (0.73) |
| 0.25 | 6 (0.47) | 4 (0.42) | 3 (0.38) | 4 (0.91) | 2 (0.83) | 1 (0.78) |
| 0.20 | 6 (0.49) | 3 (0.43) | 3 (0.39) | 3–4 (0.95) | 2 (0.86) | 1 (0.81) |
| 0.167 | 4 (0.50) | 3 (0.44) | 2 (0.40) | 3 (0.97) | 2 (0.89) | 1 (0.84) |
| 2×2 factorial of 3×3 half diallel with adjusted full-sib selection intensity | | | | | | |
| 0.50 | 6 (0.42) | 6 (0.37) | 3 (0.33) | 6 (0.78) | 3 (0.69) | 2 (0.64) |
| 0.33 | 6 (0.46) | 4 (0.40) | 3 (0.36) | 4 (0.77) | 2 (0.77) | 2 (0.73) |
| 0.25 | 6 (0.48) | 3–4 (0.42) | 3 (0.38) | 4 (0.92) | 2 (0.83) | 1 (0.78) |
| 0.20 | 4–6 (0.40) | 3 (0.43) | 3 (0.39) | 3 (0.95) | 2 (0.87) | 1 (0.82) |
| 0.167 | 4 (0.50) | 3 (0.44) | 2 (0.40) | 3 (0.97) | 2 (0.89) | 1 (0.84) |

[a] Mating designs each produce a total of 120 families and one full-sib family was selected from each selected half-sib family. Full-sib family selection intensities were: 0.53 for adjusted 2×2 factorial, 0.54 for adjusted 3×3 half-diallel and 0.56 for both unadjusted designs
$h^2$ = narrow sense heritability; $\sigma_A^2$ = additive genetic variance; $\sigma_D^2$ = dominance genetic variance

**Table 6.** Optimum number of ramets and predicted gain in phenotypic standard deviation units (in parentheses) for three-stage selection[a]

| | Optimum no. of ramets (predicted gain) | | | | | |
|---|---|---|---|---|---|---|
| | $h^2 = 0.11$ | | | $h^2 = 0.22$ | | |
| | $\sigma_A^2/\sigma_D^2$ | | | $\sigma_A^2/\sigma_D^2$ | | |
| Proportion of hs families saved | 10 | 1 | 0.5 | 10 | 1 | 0.5 |
| 4×4 factorial | | | | | | |
| 0.50 | 6 (0.48) | 6 (0.41) | 3–4 (0.37) | 6 (0.86) | 3 (0.75) | 2 (0.70) |
| 0.33 | 6 (0.51) | 4 (0.45) | 3 (0.41) | 4 (0.93) | 2 (0.84) | 2 (0.78) |
| 0.25 | 6 (0.53) | 4 (0.47) | 3 (0.42) | 4 (0.98) | 2 (0.88) | 1 (0.83) |
| 0.20 | 6 (0.54) | 3 (0.48) | 3 (0.44) | 3 (1.01) | 2 (0.91) | 1 (0.86) |
| 5×5 half diallel | | | | | | |
| 0.50 | 6 (0.48) | 6 (0.41) | 3–4 (0.37) | 6 (0.86) | 3 (0.75) | 2 (0.70) |
| 0.33 | 6 (0.51) | 4 (0.45) | 3 (0.41) | 4 (0.93) | 2 (0.84) | 2 (0.78) |
| 0.25 | 6 (0.53) | 4 (0.47) | 3 (0.43) | 4 (0.98) | 2 (0.88) | 1 (0.83) |
| 0.20 | 6 (0.54) | 3 (0.48) | 3 (0.44) | 3 (1.01) | 2 (0.91) | 1 (0.86) |
| 4×4 factorial or 5×5 half diallel with unadjusted full-sib family selection intensity | | | | | | |
| 0.50 | 6 (0.48) | 6 (0.42) | 3–4 (0.38) | 6 (0.87) | 3 (0.76) | 2 (0.70) |
| 0.33 | 6 (0.52) | 4 (0.45) | 3 (0.41) | 4 (0.94) | 2 (0.84) | 2 (0.78) |
| 0.25 | 6 (0.53) | 4 (0.47) | 3 (0.43) | 4 (0.98) | 2 (0.88) | 1 (0.83) |
| 0.20 | 4–6 (0.54) | 3 (0.48) | 3 (0.44) | 3–4 (1.01) | 2 (0.91) | 1 (0.86) |

[a] Mating designs each produce 120 full-sib and 60 half-sib families; one full-sib family was selected from each selected half-sib family. Full-sib family selection intensities were: 1.02 for adjusted 4×4 factorial, 1.02 for adjusted 5×5 half diallel and 1.03 for both designs when unadjusted
$h^2$ = narrow sense heritability; $\sigma_A^2$ = additive genetic variance; $\sigma_D^2$ = dominance genetic variance

**Table 7.** Optimum number of ramets and predicted gain in phenotypic standard deviation units (in parentheses) for three-stage selection using a 4×4 factorial mating design; two full-sib families are saved for each selected half-sib family. Full-sib family selection intensity is 0.59 when adjusted and 0.66 when adjusted

| | Optimum no. of ramets (predicted gain) | | | | | |
|---|---|---|---|---|---|---|
| | $h^2 = 0.11$ | | | $h^2 = 0.22$ | | |
| | $\sigma_A^2/\sigma_D^2$ | | | $\sigma_A^2/\sigma_D^2$ | | |
| Proportion of hs families saved | 10 | 1 | 0.5 | 10 | 1 | 0.5 |
| 4×4 factorial | | | | | | |
| 0.50 | 6 (0.45) | 6 (0.40) | 4 (0.36) | 6 (0.83) | 3–4 (0.72) | 2 (0.67) |
| 0.25 | 6 (0.52) | 6 (0.45) | 3–4 (0.41) | 6 (0.96) | 3 (0.86) | 2 (0.81) |
| 4×4 factorial with unadjusted full-sib family selection intensity | | | | | | |
| 0.50 | 6 (0.46) | 6 (0.40) | 4 (0.36) | 6 (0.84) | 3–4 (0.74) | 2 (0.86) |
| 0.25 | 6 (0.52) | 6 (0.46) | 3–4 (0.42) | 6 (0.97) | 3 (0.87) | 2 (0.81) |

$h^2$ = narrow sense heritability; $\sigma_A^2$ = additive genetic variance; $\sigma_D^2$ = dominance genetic variance

**Table 8.** Predicted genetic gain for combined index selection using factorial and half diallel mating designs. The optimum number of ramets was 1 in all cases of index selection

| | Predicted gain | | | | | |
|---|---|---|---|---|---|---|
| | $h^2 = 0.11$ | | | $h^2 = 0.22$ | | |
| | $\sigma_A^2/\sigma_D^2$ | | | $\sigma_A^2/\sigma_D^2$ | | |
| Mating design | 10 | 1 | 0.5 | 10 | 1 | 0.5 |
| 2×2 factorial | 0.64 | 0.54 | 0.52 | 0.95 | 0.85 | 0.78 |
| 3×3 half diallel | 0.64 | 0.56 | 0.51 | 0.95 | 0.84 | 0.78 |
| 4×4 factorial | 0.70 | 0.64 | 0.60 | 1.03 | 0.95 | 0.89 |
| 5×5 half diallel | 0.69 | 0.63 | 0.58 | 1.02 | 0.93 | 0.88 |

$h^2$ = narrow sense heritability; $\sigma_A^2$ = additive genetic variance; $\sigma_D^2$ = dominance genetic variance

resulting in larger individual selection intensities. The relationship between factors affecting selection intensity and the optimum distribution of effort will not be simple because selection intensity is not a linear function of the proportion of families or individuals saved.

Reducing the full-sib family selection intensity, by selecting more than one full-sib family per half-sib family (Table 7), increases individual selection intensity. Comparison of data in Table 7 with corresponding data in Table 6 indicates that such a redistribution of selection intensity favors optimum distributions with more ramets per individual, but results in smaller gains. However, when the total number of full-sib families selected was the same (15 of 60 half-sib families with two full-sib families each versus 30 of 60 half-sib familes with one full-sib family each) the optimum number of ramets was identical and gains were greater with more intensive half-sib selection. Distribution of selection intensity between kinds of families would have little effect on individual selection intensity and thus would not have much effect on the optimum distribution of individuals and ramets.

The predicted gains require little discussion. As expected, high heritabilities, large ratios of additive to dominance variance and conditions that allow high selection intensities – for both families and individuals – resulted in the largest genetic gains. Gains were very similar for mating designs that have the same number of full-sib families per half-sib family. Adjustment of full-sib family selection intensities had a small effect on gains; higher order designs were less affected due to lower probabilities of selecting the same full-sib family twice.

*Combined index selection*

The optimum number of ramets for index selection was one for all cases tested (Table 8). Gains are greater for index selection than for multistage selection, but follow the same trends. Factorial mating designs yielded slightly larger expected gains than diallel designs when the number of full-sib families per half-sib family was the same, although the difference was very small.

## Discussion

The most commonly cited benefit of clonal propagation is the potential for using non-additive genetic

variance in a production population. With the exception of reciprocal recurrent selection, most selection schemes are not designed to accumulate such gain over generations. Our results indicate that the use of clonal replicates in genetic tests can increase the cumulative or additive genetic gain that is obtained at each cycle of breeding, relative to that expected from the single ramet, or non-clonal case. The use of multiple ramets to improve cumulative genetic gain does not preclude the use of combined family-individual-ramet tests to select superior genotypes for propagation. Our own investigations (data not presented) indicate that selection for both additive and non-additive effects will yield substantial, although partially noncumulative, gains over selection for additive effects alone.

The relative advantage of using multiple versus single ramets for each tested individual was highly dependent on the conditions invoked and the single ramet case was rarely optimal for multistage selection, over the range of conditions that we tested. Further, a substantial portion of the total gain is usually obtained from family selection even when clonal replication is advantageous, regardless of the value of clonal replication. This demonstrates the importance of considering clonal propagules as a component of a breeding and testing scheme that incorporates several levels of variation.

Combined index selection yielded the largest expected gains, and always gave optimum gains with a single ramet. This result agrees with that published by Libby (1969) for a model based on the hierarchical mating design. The difference between index and multistage selection results may best be explained by a consideration of the individuals likely to be selected when using each method. Index selection imposes no coancestry control. As a consequence, a large proportion of the selected individuals often originate from a few of the best families, a factor that is certain to reduce the effective individual selection intensity. Our results indicate that cases with conditions that cause low individual selection intensity tend to favor distributions with few ramets per individual. Multistage selection is in fact a method of coancestry control. A restricted index selection scheme with some coancestry control imposed might yield a pattern similar to that observed for multistage selection.

In many forest tree breeding programs, the amount of material available at a specified level of improvement and/or the necessity for avoidance of inbreeding will control the intensity of family selection. When family selection intensity must be sacrificed to avoid relatedness within the breeding population, substitution of ramets for individuals can partially compensate for this loss in intensity. As programs mature, more emphasis must be placed on within-family selection and the use of clonal propagules in genetic tests should become more valuable.

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