Efficiency of Selection Based on Phenotype, Clone and Progeny Testing in Long-term Breeding

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Abstract

The overall goal for long-term breeding was formulated as maximising annual progress in group merit (GMG/Y) at a given annual budget. Group merit is a weighted average of breeding value and gene diversity. Breeding strategies based on testing of *phenotypes, clones* or *progeny* for selection of parents for next breeding cycle were optimised as regards testing time and test entry size. The dependence of GMG/Y on genetic parameters, cost and time components was investigated. Numeric values were chosen with long-term breeding of Norway spruce in mind.

The highest GMG/Y under the most likely parameter values for *clone, phenotype* and *progeny* strategies was 0.250%, 0.152% and 0.139%, respectively. The *clone* strategy was the best over the whole range of considered cases, except for the scenario with high narrow-sense heritability, for which the *phenotype* strategy was the most efficient. Except for low narrow-sense heritability, the *phenotype* strategy was the second best, but superiority of the *phenotype* strategy over *progeny* strategy was usually small. If reproductive maturity of the test parents could be shortened to below about 12 years, the *progeny* strategy may be better than the *phenotype* strategy. Comparably high costs (per parent) seem to be acceptable for promoting early sexual maturity.

Narrow-sense heritability, additive variance at mature age, rotation age, plant-dependent cost and the time needed to produce the test plants had the strongest effect on GMG/Y. The *clone* strategy became less superior at high dominance variance. Short rotation age favoured the *clone* and *phenotype* strategies. Reduction of cost per test plant was especially beneficial for the *clonal* and *progeny* strategies.

Key words: genetic gain, gene diversity, group merit, testing method, Norway spruce, *Picea abies*, tree improvement.

1. Introduction

Long-term plant breeding is repeated cycles of recombination and selection. For selection the material needs to be tested. Candidates for the next breeding population can be tested on three different levels: *phenotype*s, clones or *progeny*. Therefore, a key problem is to consider the relative efficiency of these three methods. Breeding can be formulated as management of the breeding population by simultaneously considering breeding value, gene diversity, time, cost and technique, but it is not clear how these key factors can be combined into an efficient breeding program.

Advance in breeding value and the associated loss of gene diversity (increase in group coancestry) shall be considered simultaneously as a joint index known as Group Merit to be maximised in long-term breeding (LINDGREN and MULLIN, 1997; ROSVALL *et al.*, 1998). Time factor shall also be included in this joint index to estimate the benefit per unit of time (WEI and LINDGREN, in press, 2002).

Long-term breeding based on balanced within-family selection and equal parent contribution minimises loss of gene diversity per breeding cycle, i.e. it maintains the greatest possible gene diversity in the breeding population (ROSVALL, 1999). Thus, the gain per unit of gene diversity lost is not far from the optimal (ROSVALL, 1999). Under a balanced breeding plan, group coancestry in a breeding population of 50 individuals would rise by 0.005 per breeding cycle. Thus, an appropriate level of gene diversity will be maintained for at least 10 breeding cycles (ROSVALL, 1999).

Following this breeding plan, a problem is to find the most optimum testing strategy for balanced within-family selection of the parents for the following breeding cycle in a breeding population by considering variation in genetic parameters, time and cost components simultaneously. In theory, the advantage of clonal testing is a higher precision in predicting the genetic gain than by progeny testing or phenotypic selection and at a shorter time than by *progeny* testing (e.g. BURDON, 1986; MATHESON and LINDGREN, 1985). The interest, however, is to assess the response of clonal testing to variable values of genetic parameters, cost and time components. For instance, high heritability and low budget of a breeding programme may favour selection based on phenotype rather than on clonal or progeny test. Furthermore, if cloning is not biologically possible, comparison between selection based on phenotype and progeny test at variable values of the parameters is of interest. Most of the theoretical studies on benefit by different testing methods for selection in breeding populations did not consider gain, diversity, cost and time simultaneously (e.g. COTTERILL, 1984; SHAW and HOOD, 1985; BURDON, 1986; SHELBOURNE and JORDAAN, 1991; FOSTER, 1992; MULLIN and PARK, 1992; RUSELL and LOO-DINKINS, 1993; WEI, 1995; MEUWISSEN and SONESSON, 1998).

Objective of this study is to evaluate the efficiency (in terms of Group Merit Gain per year) for breeding strategies based on testing of *phenotype*s, clones or *progeny* for selection of parents for the subsequent breeding cycle as a function of cost and time components, genetic parameters and annual budget.

2. Material and Methods

2.1. Breeding plan, testing alternatives and basic assumptions

The outline of the breeding strategies evaluated and the values of the parameters considered were chosen with the Swedish long-term breeding program for Norway spruce in mind (DANELL, 1993 a, b; ROSVALL, 1999; KARLSSON, 2000). The long-term breeding plan is to maintain a meta-population made up from a number of unrelated breeding populations of 50 members each. Within each breeding population, the breeding will be carried out by the means of double-pair mating among 50 members and balanced within family selection of one individual per full-sib family as a parent for the following breeding cycle.

The following breeding strategies to generate material for ranking of breeding values in a target trait or an index and the subsequent selection of one individual per full-sib family were compared:

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• *Phenotype* strategy: full-sibs are planted in a field test, the best *phenotype* is selected according to its phenotypic performance.

• *Clone* strategy: full-sibs are vegetatively propagated and ramets of each clone are planted in a field test. The best clone based on its clonal average is selected.

• *Progeny* strategy: progeny from the full-sibs (open-pollinated or polycross) are planted in a field test and the parent of the best progeny is selected.

Testing was assumed to be carried out in a single constant environment (no G x E interaction). No C-effects (non-genetic causes of variation, e.g. maternal or cloning effects) or epistatic variance were considered. Breeding value of selected founders was set to zero (used as reference for the gain).

2.2. Simulation model

The infinitesimal genetic model was assumed (infinite number of unlinked loci each with a small effect). The simulations were based on the main and the alternative scenarios (*Table 1*). While testing an alternative value of a parameter, all the other parameters were kept at the values for the main scenario. A deterministic simulator BREEDING CYCLE ANALYZER based MS Excel workbook was used (available on the WEB at www.genfys.slu.se/staff/dagl).

Group Merit Gain per year (*GMG*/*Y*) (WEI and LINDGREN, 2002) was chosen as the parameter to be maximised when searching for the best breeding strategy at a given total cost of one complete breeding cycle:

$$GMG = G - c\Theta, \quad [1]$$

where, GMG is group merit gain obtainable from selection, *G* is estimated additive genetic gain at rotation age (average breeding value of the individuals selected), c is a weighting factor between loss of genetic diversity and genetic gain and also converting gain and diversity to the same scale, Θ is raise in group coancestry per breeding cycle, which, assuming that each parent contributes two offspring to be used as the parents in the next breeding cycle, was estimated as:

$$\Theta = 0.25 / n, \quad [2]$$

where, *n* is number of the individuals selected.

Loss of gene diversity (raise in group coancestry) per breeding cycle is dependent on breeding population size only. However, cycling time may vary depending on the breeding strategy and, thus, diversity loss per unit of time may be variable.

The genetic gain at rotation age from within family selection following each breeding strategy was predicted according to the following formulas (LINDGREN and WERNER, 1989):

Phenotype strategy (selection based on *phenotype*):

$$G = \frac{\sigma_{Am}\, r_{j-m}\, i\sigma_A}{\sqrt{\sigma_A^2 + \sigma_D^2 + \sigma_E^2}} \quad [3]$$

Clone strategy (selection based on clonal test):

$$G = \frac{\sigma_{Am}\, r_{j-m}\, i\, \sigma_A}{\sqrt{\sigma_A^2 + \sigma_D^2 + \frac{\sigma_E^2}{n}}} \quad [4]$$

Progeny strategy (selection based on half-sib *progeny* test):

$$G = \frac{\sigma_{Am}\, r_{j-m}\, i0.5\sigma_A}{\sqrt{0.25\sigma_A^2 + \frac{0.75\sigma_A^2 + \sigma_D^2 + \sigma_E^2}{n}}} \quad [5]$$

where: *G* is additive genetic gain in percentage, σ^2_A is additive variance, σ^2_D is dominance variance, σ^2_E is environmental variance, *n* is number of plants per family, σ_{Am} is standard deviation in breeding value of the selected individuals for a target trait at rotation age and is given as percentage of the average breeding value of the unimproved individuals for this trait (one standard deviation is equal to 10%), *i* is selection intensity estimated in the units of standard deviation of the mean of the selected individuals from the family mean by the aid of an approximation by BURROWS (1975), r_{j-m} is juvenile-mature (J-M) genetic correlation estimated according to the formula by LAMBETH (1980) with an adjustment for the ratio of selection age to rotation age (*Q*) being close to 0 or 1:

if $0 < Q < 0.1$, then $r_{j-m} = Q * 3.108$

if $0.1 <= Q <= 0.9$, then $r_{j-m} = 1.02 + 0.308 * \mathrm{Log}(Q)$ [6]

if $0.9 < Q <= 1$, then $r_{j-m} = 0.988 + (Q - 0.9) * 0.012 / 0.1$

The scenarios at short rotation age may be interpreted as simulation of high J-M genetic correlation.

The variable parameters to find maximum *GMG* per year at a given cost for each of the scenarios in *Table 1* were the following: (1) age of selection in the selection test, (2) family size for testing (*phenotype* strategy), family size and number of ramets for clonal testing of each family member (*clone* strategy), family size and number of half-sib *progeny* for testing of each family member (*progeny* strategy).

2.3. Reasoning of the values for the main and the alternative scenarios

Values used as the "input" in the model were chosen to be suitable for breeding of northerly Norway spruce. A most likely value was identified as the main scenario value (for some parameters main scenario used different values for different testing strategies). Then an upper and a lower reasonable bound for each value were identified (i.e. the highest and the lowest values, which seem likely to be compatible with the actual circumstances).

2.3.1 Additive variance at mature age and loss of gene diversity

Additive variance at mature stage (σ_{Am}) was set to 10% for the main scenario and to 5% and 20% for the alternative scenarios. If it is at the lower value, genetic gain is given less importance versus gene diversity and, if its on the higher value, vice versa.

For the main scenario, the weighting factor for loss of diversity (*c*) was set to 100 to make it compatible with genetic gain. This means that a 1% change in genetic gain is regarded as equally important as 1% change in gene diversity. This results in 0.5% loss of diversity per cycle. For the alternative scenarios, the weighting factor was set to result to a lower and to a greater penalty for loss of diversity (i.e. emphasising the importance of gene diversity), leading to a loss of diversity of 0.25% and 1%, respectively.

2.3.2 Variance components

Genetic parameters for the main scenario were chosen to represent height growth traits of northerly Norway spruce based on results of many experiments (ROSVALL, 1999). The initial additive variance within family was set constant to 1 (this makes 2 in the breeding population) and the initial dominance and environmental variances were expressed as ratios of the additive variance. Additive variance within family is only dependent of the inbreeding of the parents (FOULLEY and CHEVALET, 1981). As inbreeding is low for all important scenarios, the additive variance within family can be considered a constant. For the main scenario, the dominance variance was

Table 1. – Parameters for the main and alternative scenarios. When an alternative value was tested, all other values were kept at the main scenario. BP means breeding population. If at a given scenario different values were given for different breeding strategies, the breeding strategy is indicated in the parentheses. All the costs are expressed per breeding population member.

Parameters	Main scenario	Alternative scenarios
Additive variance (σ_A^2)	1	
Dominance variance, % of the additive variance in BP (σ_D^2)	25	0; 100
Narrow-sense heritability (h^2) (obtained by changing σ_E^2)	0.1	0.05; 0.5
Additive standard deviation at mature age (σ_{Am}), %	10	5; 20
Diversity loss per cycle, %	0.5	0.25;1
Rotation age, years	60	10; 120
Time before establishment of the selection test (T_{BEFORE}), years	1 (*phenotype*)	3; 5 (*phenotype*)
	5 (*clone*)	3; 7 (*clone*)
	17 (*progeny*)	5; 7 (*progeny*)
Recombination cost (C_{RECOMB}), $	30	15; 50
Cost per genotype (Cg), $	0.1 (*clone*),	1; 5 (*clone*),
	1 (*progeny*)	0.1; 5 (*progeny*)
Cost per plant (Cp), $	1	0.5; 3
Cost per year and parent (constraint)	10	5; 20
Group Merit Gain per year (GMG/Y)	To be maximized	

set to make up 25% of the additive variance in the breeding population. In northerly conifers, growth rhythm traits are mainly controlled by the genes with additive effects, while for growth traits, the dominance variance may have an import effect (review by HANNERZ, 1998). Considering this, the dominance variance was set to alternative values of 0% and 100% of the additive variance in the breeding population. Presence of epistatic variance was ignored. In northerly conifers, narrow-sense heritability usually varies at about 0.5 for adaptive traits (review by HANNERZ, 1998) and between 0.1 and 0.2 for height growth traits (DANELL, 1991; ROSVALL, 1999). In our study, narrow-sense heritability in the breeding population was set to 0.1 for the *main scenario* and to 0.05 and 0.5 for the alternative scenarios.

2.3.3 Cost components

The cost components within one breeding cycle were expressed per breeding population member. The total cost per cycle and breeding population member was calculated as:

$$C_{PER\ CYCLE} = C_{RECOMB} + C_{INIT} + n\ (C_G + m\ C_P), \quad [7]$$

where, C_{RECOMB} is cost for recombination among the founders, C_{INIT} is cost for initiation of the test, C_G is cost per genotype, i.e. cost dependent on the type of reproductive material (genotype-dependent cost), C_P is cost per test plant (plant-dependent cost), n is number of genotypes (ortets for clonal test of female parents for *progeny* test) and m is number of plants (number of ramets per clone in clonal test or number of seedlings per family in *progeny* test).

Genotype-dependent costs were assumed to cover the following steps: for the *clone* strategy, production of ortets in nursery for 4 years, or, alternatively, treatment to get tissue-culture and growth of culture; for the *progeny* strategy, production of female parents for 15 years, polycross or open-pollination, seed collection, seed extraction.

Plant-dependent costs were assumed to cover the following steps: for the *phenotype* strategy, (a) seeding production in the nursery (seeding, labelling, watering, fertilising, lifting out), and (b) establishment and maintenance of the selection test (transportation, soil preparation, planting, maintenance of test plantation, assessments, account for field mortality or severe damage by refilling or by planting more plants than evaluated); for the *clone* strategy, (a) ramet production in nursery (cutting and rooting of ramets, labelling, watering and fertilising), (b) establishment and maintenance of the selection test (as in *phenotype* strategy), and for *progeny* strategy: (a) seedling production in nursery (seeding, labelling, watering, fertilising, lifting out), and (b) establishment and maintenance of the selection test (as in *phenotype* strategy).

Costs were expressed in "$" as "cost-units". The principle for setting the costs for different operations within a breeding cycle was the following: cost per test plant (plant-dependent cost) was set to 1$ and all the other costs were expressed as the ratios from 1$. Additional cost associated with age of the selection test was not considered, e.g. tall trees may be more expensive to measure than short trees or long-lived experiments may cost more to establish and maintain that short-lived and less trees will remain to measure in the end. Cost settings were the following: (1) cost for recombination among breeding population members was set to 30$ in the main scenario and to 15$ and 50$ in the alternative scenarios, (2) cost for initiation of the selection test (planning and localization of the test and the associated travelling costs) was set to 0 for all the three alternatives, (3) genotype-dependent cost for *clone* strategy was assumed to make 1/10 of the cost per plant, i.e. was set to 0.1$ per one ortet for the main alternative; genotype-dependent cost for the *progeny* strategy was assumed to be 10 times higher than that for the *clone* strategy and was set to 1$ per one female parent for the main scenario; alternative values of genotype-dependent cost were 1$ and 5$ for the *clone* strategy and 0.1$ and 5$ for the *progeny* strategy, (4) plant-dependent cost was fixed to 1$ per test plant for all the strategies; there may be some difference in plant-dependent cost between the strategies as regards type of the test plants (seedlings or ramets), however, we consider it as insignificant, as establishment, maintenance and assessment of the selection test make up the major part of plant-dependent cost.

The simulations were run with the annual budget constraint of 10$ per breeding population member for the main scenario and 5$ and 20$ per breeding population member for the alternative scenarios.

2.3.4 Time components

The time of one breeding cycle was subdivided into the following components:

$$T_{CYCLE} = T_{RECOMB} + T_{BEFORE} + T_{TEST} + T_{AFTER,} \quad [8]$$

where, T_{RECOMB} is the time needed for recombination (crossing and seed production), T_{BEFORE} is the time needed to produce plants for the selection test (i.e. time from seeding in nursery to planting in the field test), T_{TEST} is the time needed for testing and selection of individuals as the parents for the subsequent breeding cycle, $T_{AFTER,}$ is the time from selection of the new parents to harvest of their seeds for the next breeding cycle.

For the main scenario, the timing for certain operations was chosen to be in agreement with the present-day practical experience with Norway spruce: the time for recombination was set to 3 years for all the alternatives; selection test may be established with 1-year-old seedlings (for the *phenotype* strategy, T_{BEFORE} was set to 1 year); a 4-year-old seedling of Norway spruce is large enough to provide up to 20 cuttings and in 1 year, the cuttings may develop an appropriate root system (for the clonal strategy, T_{BEFORE} was set to 5 years); *progeny* of Norway spruce reach the reproductive maturity at the age of 15 years and it takes 2 years to get open-pollinated or polycross seeds (for the *progeny* strategy, T_{BEFORE} was set to 17 years). T_{AFTER} was set to 2 years for all the strategies (assuming that the test species is Norway spruce and that the crossing archive is established at the same time as seeding of full-sib families in nursery). Effect T_{AFTER} or T_{RECOMB} was not studied but as the effect of change in T_{AFTER} or T_{RECOMB} is equivalent to the effect of change in T_{BEFORE}, it is sufficient to include that in the alternative settings.

Alternative settings for T_{BEFORE} were the following: (1) 3 and 5 years for *phenotype* strategy, assuming that it may take longer time to produce seedlings or larger seedlings may better survive the establishment phase (2) 3 and 7 years for the *clone* strategy, assuming that, if less than 20 ramets per ortet are needed, 2-or 3- year-old ortets may be of sufficient size, or alternatively, if more than 20 ramets per ortet are needed, we may need 6-year-old ortets (3) 5 and 7 years for the *progeny* strategy, assuming that new techniques for induction of early flowering or tissue culture may bring the first seed crop at the age of 5 or 7 years.

3. Results

3.1 Comparison between the strategies

According to the main scenario, ranking between the strategies was the following: *clone* strategy, *phenotype* strategy and *progeny* strategy with *GMG*/*Y* of 0.250%, 0.152% and 0.139%, respectively (*Table 2*). The main scenario estimates of J-M genetic correlation for *clone*, *phenotype* and *progeny* strategies were 0.60 (age 15 to 60), 0.68 (age 20 to 60) and 0.84 (age 33 to 60), respectively.

The *clone* strategy was the best breeding strategy in maximising *GMG*/*Y*, except of the alternative scenario with high initial narrow-sense heritability (*Table 2*, *Figures 1* and *2*).

3.2 Effect of genetic parameters

Increase in dominance variance reduced *GMG*/*Y* from the *clone* strategy and had no significant effect on *GMG*/*Y* from the *phenotype* as well as *progeny* strategies (*Figure 1a*). However, even if dominance variance would make 200% of the additive variance for the target trait in the breeding population, *GMG*/*Y* obtainable from the *clone* strategy would still be for 24% and for 27% greater than *GMG*/*Y* from the *phenotype* and *progeny* strategies, respectively.

Increase in initial narrow-sense heritability markedly improved *GMG*/*Y* for all the strategies and changed the ranking between the *clone* strategy and *phenotype* strategies, which became superior over the *clone* strategy at heritabilities exceeding 0.4 (*Figure 1b*). In response to changing heritability, *GMG*/*Y* from the *progeny* strategy changed least, but the response was similar to the response by the *clone* strategy.

As expected, a higher value of genetic variance at mature age (σ_{Am}) markedly improved *GMG*/*Y* (*Figure 1c*). The raise was slightly faster than suggested by the quotient between the σ_{Am} extremes (=4): the highest for the *phenotype* strategy and about the same for the *clone* and *progeny* strategies. Thus, at high mature genetic variance, the *phenotype* strategy was more favourable than the *progeny* strategy. If genetic gain is zero, thus given no importance (σ_{Am} = 0), *GMG*/*Y* would attain a negative value depending on the cycling time, thus, different for the different strategies (*Figure 1c*).

A higher loss of gene diversity (i.e. a greater penalty given to loss of gene diversity) had a minor effect on *GMG*/*Y* from all the breeding strategies (*Figure 1d*). With increasing importance of gene diversity, *GMG*/*Y* from the *phenotype* strategy decreased somewhat faster than *GMG*/*Y* from the other *breeding* strategies. When gene diversity loss reached 1%, the *progeny* strategy provided a greater *GMG*/*Y* than the *phenotype* strategy (*Figure 1d*).

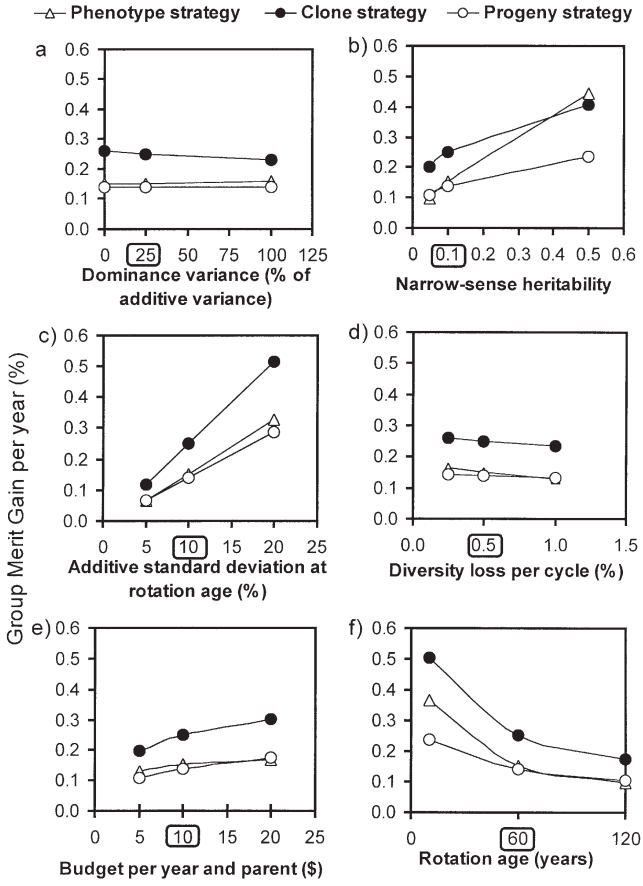

Figure 1. – Ranking between the breeding strategies in Group Merit Gain per year at the main and alternative values for genetic parameters (plots a, b, c, d), total budget as the constraint (e) and rotation age (f). Scenarios at short rotation age may be interpreted as simulation for high juvenile-mature genetic correlation. The outlined labels on the X-axis show the values for the main scenario.

Table 2. – Group Merit Gain per year (*GMG/Y* in %) from simulations according to the main and alternative scenarios is given separately for each of the breeding strategies. Optimum number of test plants and optimum selection age (counted from establishment of the selection test) as regards *GMG/Y* are given for each of the 50 full-sib families. While testing an alternative value of a parameter, all the other parameters were kept at the values for the main scenario. 1.

Parameter	Value	*Phenotype* strategy			*Clone* strategy				*Progeny* strategy			
		GMG/Y	Age to select	Family size	*GMG/Y*	Age to select	Ortet no.	Ramet no. per ortet	*GMG/Y*	Age to select	Female parent no.	Progeny no. per parent
σ_D^2, % of σ_A^2	0	0.150	15	182	0.259	26	16	17	0.138	34	11	47
	25*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	100	0.157	15	181	0.229	19	17	15	0.141	33	11	46
h^2	0.05	0.098	17	196	0.201	23	13	23	0.107	39	8	71
	0.1*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	0.5	0.442	14	165	0.406	16	46	5	0.236	26	30	14
σ_{Am}, %	5	0.065	19	215	0.117	24	19	16	0.065	37	11	50
	10*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	20	0.328	14	168	0.517	20	18	15	0.287	32	11	45
Diversity loss, %	0.25	0.164	14	168	0.258	20	18	15	0.144	32	11	45
	0.50*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	1	0.128	15	184	0.234	24	19	16	0.130	37	11	50
Rotation age, years	10	0.365	6	91	0.504	9	13	12	0.236	9	8	34
	60*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	120	0.097	25	275	0.175	31	22	17	0.104	47	13	50
Time before establishment of selection test, years	1^{Ph}	0.152	15	182								
	3	0.141	17	215	0.263	19	17	14				
	5^{Cl}	0.132	18	247	0.250	20	18	15	0.164	27	9	37
	7				0.239	22	19	16	0.159	28	9	40
	17P								0.139	34	11	47
Recombination cost, $	15	0.153	15	195	0.254	20	19	15	0.140	32	11	47
	30*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	50	0.149	16	167	0.245	22	18	15	0.137	35	11	46
Cost per test genotype, $	0.1^{Cl}				0.250	20	18	15	0.139	38	12	47
	1.0^{Pr}				0.246	22	17	16	0.139	34	11	47
	5.0				0.233	23	13	18	0.135	35	10	49
Cost per test plant, $	0.5	0.167	15	351	0.297	19	26	20	0.171	30	11	68
	1*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	3	0.127	17	66	0.180	23	11	9	0.087	37	4	46
Total budget, $	5	0.132	17	86	0.198	24	10	10	0.106	40	8	34
	10*	0.152	15	182	0.250	20	18	15	0.139	34	11	47
	20	0.168	14	376	0.301	19	21	21	0.173	30	15	66

The symbol in the superscript of the parameter values indicates the main scenario value for: (*) all breeding strategies, (Ph) *phenotype* strategy, (Cl) *clone* strategy, (Pr) *progeny* strategy.

3.3 Effect of time and cost components

Increase of annual budget of the breeding programme (per breeding population member) raised the *GMG/Y*, but not dramatically (*Figure 1e*). Actually, the return on the investment seemed to fall rather fast with increasing budget. At low budget, the *phenotype* strategy generated slightly higher *GMG/Y* than the *progeny* strategy, while, at high budget, vice versa. However, the relations between the breeding strategies were not dramatically dependent on the total budget.

Shortening of rotation age affects juvenile-mature (J-M) genetic correlation, i.e. the higher is the rotation age the less precise is the prediction at a given juvenile age. The response by the *clone* and *phenotype* strategies to the increase in J-M genetic correlation (decrease in rotation age) was stronger than the response by the *progeny* strategy (*Figure 1f*). At rotation age of 10 years (high J-M genetic correlation), the *phenotype* strategy gave a markedly greater *GMG/Y* than the *progeny* strategy. However, at rotation age over 60 years, there was no large difference in *GMG/Y* between the *phenotype* and *progeny* strategies (*Figure 1f*).

Change in recombination cost had no evident effect on *GMG/Y* nor on ranking between the breeding strategies (*Figure 2a*). Recombination cost is fixed, so a cheaper recombination favours short cycling time.

Raise in cost per test genotype for 50 times had a little effect on *GMG/Y* from the *progeny* strategy and resulted in a minor drop in *GMG/Y* from the *clone* strategy (*Figure 2b*). Whereas, increase in cost per test plant resulted in a marked reduction of *GMG/Y* from all the breeding strategies and affected the ranking in *GMG/Y* between the *phenotype* and *progeny* strategies

(*Figure 2c*). With increasing cost per test plant, *GMG*/*Y* from the *phenotype* strategy was decreasing more slowly than *GMG*/*Y* from the other breeding strategies and, at the cost per test plant of 3$, resulted in a markedly greater *GMG*/*Y* than the *progeny* strategy (*Figure 2c*). Decrease in *GMG*/*Y* from the *clone* strategy was especially pronounced when cost per test plant increased from 0.5$ to 1$. At a very high cost per test plant (>4-5$), the *phenotype* strategy may give a higher *GMG*/*Y* than the *clone* strategy (*Figure 2c*).

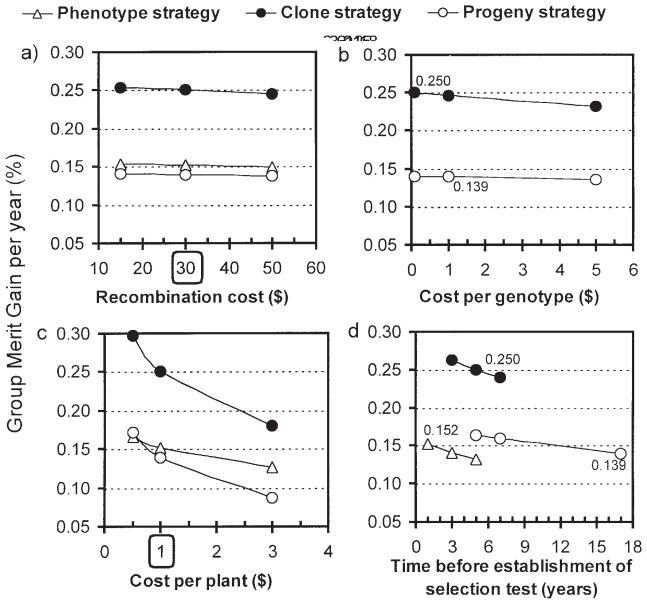

Figure 2. – Ranking between the breeding strategies in *GMG*/*Y* at the main and alternative values for cost components (plots a, b, c) and time before establishment of the selection test (i.e. time needed to produce seeds and plants for testing) (plot d). In the plots a) and c), the main scenario values are the same for all the test alternatives and are outlined on the X-axis. In the plots b) and d), the main scenario values are shown on the plot at the marker for a corresponding test alternative.

Shortening the time before establishment of selection test (T_{BEFORE}) may change ranking in *GMG*/*Y* between the *phenotype* and *progeny* strategies (*Figure 2d*). At T_{BEFORE} for the *phenotype* strategy equal to 1 year and T_{BEFORE} for the *progeny* strategy less than 11 years, *GMG*/*Y* obtainable from the *progeny* strategy was greater than *GMG*/*Y* from the *phenotype* strategy with the other parameters at the main scenario values (*Figure 2d*).

3.4 Optimum number of test plants and age for selection

Following the main scenario, *GMG*/*Y* from the *clone* strategy increased with increasing number of ortets and decreasing number of ramets per ortet in the selection test until a threshold value was reached. With all the parameter values at the main scenario, the highest *GMG*/*Y* can be obtained by testing 18 ortets per family with 15 ramets each and selection shall be made 20 years after establishment of the selection test. A similar tendency was observed for the *progeny* strategy, only, the rate of decrease in number of seedlings per female parent with increasing number of female parents was much less: at the main scenario, the optimum family size was 11 individuals with 47 seedlings each and the optimum age of selection was 34 years (*Table 2*). The higher was the heritability the more genetic units with less plants from each unit were needed to obtain the maximum *GMG*/*Y* (*Table 2*). Effect of the other parameters on optimal number of test plants per genetic unit was the following: increase in σ_{Am} resulted in a lower number of test plants for the *phenotype* strategy and had a little effect on optimum number of test plants for the other breeding strategies; short rotation age markedly reduced optimum number of test plants for all the breeding strategies (*Table 2*).

Given the fixed budget constraint per breeding population member and year, a costly strategy required a longer testing time in the selection test to achieve maximum *GMG*/*Y* than a cheap breeding strategy. Thus, a relatively higher cost of a breeding strategy was penalised by a relatively longer testing time to achieve maximum *GMG*/*Y*. The optimum age for selection in the test averaged over all the scenarios for *phenotype*, *clone* and *progeny* strategies was 15 years (standard deviation was 3), 21 years (standard deviation was 4) and 33 years (standard deviation was 7), respectively. As regards the effect of the parameters within each testing alternative, optimum selection age was mainly influenced by initial narrow-sense heritability and rotation age (*Table 2*). The dependence on rotation age reflected the strength of J-M genetic correlation and its and development over time. Increase of initial narrow-sense heritability markedly reduced the optimum selection age for the *clone* and *progeny* strategies, but had only a minor effect on optimum selection age for the *phenotype* strategy. Shortening of rotation age led to a shorter optimum selection age for all the breeding strategies (*Table 2*).

4. Discussion

4.1 Comparison between the breeding strategies

If available, the *clone* strategy is best to maximise *GMG*/*Y* under balanced within family selection, except for the traits with a high heritability (*Table 1*, *Figures 1, 2*). This may be expected considering the efficiency of clonal testing, where each candidate carries two sets of the tested genes while in case of *progeny* testing it is just one set. However, an interesting finding is that *GMG*/*Y* obtainable from *clone* strategy remained high under most of the parameter values simulated, including high cost for cloning and high dominance variance (*Figures 2b* and *1b*). The negative effect of dominance variance seemed to be minor if compared with the advantage in prediction of breeding value by clonal testing. Owing to a comparably shorter testing time, *clone* strategy would especially be beneficial in the presence of high J-M genetic correlation (*Figure 1f*). Shortening of the time to produce ortets for clonal test from 5 to 2 years resulted in 3% increase in *GMG*/*Y*, and the effect of variation in cost for production of ortets was even less (increase in *Cg* for 5 times reduced *GMG*/*Y* for 2% only) (*Figure 2b* and *d*). Thus, the cheapest method to *clone* the individuals such as production of cuttings from hedges would be the most profitable and investment in to the propagation techniques to shorten the time for production of *clone*d test plants may not be of the first priority, especially, if considering the irregular growth characteristics of micropropagated plantlets (HÄGGMAN *et al.*, 1996).

If narrow-sense heritability for the target trait is greater than 0.4–0.5, the *phenotype* strategy is a better choice than the *clone* and *progeny* strategies (*Figure 1b*). Whereas, for the traits with low narrow-sense heritability, the *phenotype* strategy is the most inferior breeding strategy (*Figure 1b*). If resemblance among phenotype and genotype is high, there is no need for a large number of test plants to test this resemblance. In northerly conifers, narrow-sense heritability for adaptive traits (e.g. growth rhythm) usually ranges between 0.4–0.5 (review by HANNERZ, 1998). Thus, for improvement of adaptive traits, the *phenotype* strategy may be the best among the three alternatives, but, on the other hand, advanced gene-

tic materials are unlikely to be selected only for adaptedness, that will just be a part of a selection index. If breeding and production populations share the same adaptive target (as assumed in our simulation), a strong G x E interaction for adaptive traits is unlikely to occur. Thus, high to medium J-M correlations for adaptive traits may be expected. Consequently, *phenotype* test is the best strategy to test for superiority in adaptive traits.

As later selection in practice often makes the per (measured) plant cost higher, and as the strategies differ in optimal selection age (*Table 2*), the relations among the strategies obtained in this study may underestimate the benefits of the *phenotypic* strategy and overestimate the benefits of the *progeny* strategy.

The *progeny* strategy resulted in a similar or lower GMG/Y than the *phenotype* strategy, except for the scenarios with high narrow sense heritability (*Figure 1b*), short rotation (*Figure 1f*) and high cost per plant (*Figure 2c*). The main disadvantage of the *progeny* strategy is comparably long time for production of the test plants. Thus, it would especially be advantageous to shorten the time for production of female parents for *progeny* testing (T_{BEFORE}). Increase of genotype-depended cost had a minor effect on efficiency of *progeny* testing (*Figure 2b*). Thus, T_{BEFORE} can be shortened at a high cost (e.g. in indoor seed orchards or through tissue culture) as soon as the technique will be operatively available to shorten the T_{BEFORE}. Shortening of T_{BEFORE} to 11 years will already make *progeny* strategy superior over the *phenotype* strategy with the other parameters at the main scenario values (*Figure 2d*). Shortening of T_{BEFORE} would also lead to a lower total cost for the *progeny* strategy. Reduction of cost per plant by half may make the *progeny* strategy superior over the *phenotype* strategy with the other parameters at the main scenario values (*Figure 2c*). Speeding up the reproductive maturity by the means of flowering stimulation in indoor seed orchards or through tissue culture may be an interesting possibility (e.g. HÄGGMAN *et al.*, 1996).

4.2 Effect of the parameters

Narrow-sense heritability, mature additive variance (weight for genetic gain), rotation age (J-M genetic correlation), cost per test plant and time before establishment of the selection test had the strongest effect on GMG/Y obtainable from all the breeding strategies. Whereas, the effects of dominance variance, gene diversity loss, annual budget, recombination cost and cost per genotype on GMG/Y were comparably weaker.

The presence of dominance seemed to improve the *phenotype* and *progeny* strategies (*Figure 1a*). This somewhat contra-intuitive observation was obtained because, under a constant narrow-sense heritability, a higher dominance variance forced a lower environmental variance, and, for the *phenotype* and *progeny* strategies, this was more favourable than dominance was unfavourable. Whereas, for *clone* strategy, on the contrary, the increase in dominance variance was less favourable than decrease in environmental variance. The reason for this is that (1) prediction of breeding values by clonal test is less sensitive to environmental variation than by *progeny* and, particularly, *phenotype* tests and (2) dominance variance causes a greater bias in prediction of breeding values by clonal and *phenotype* tests than by *progeny* test (BURDON and SHELBOURNE, 1974). For the *phenotype* strategy, this bias is compensated by the decrease in environmental variance, while for *clone* strategy is not. As long as dominance variance makes up less than 300% of the additive variance, *clone* strategy is the best breeding strategy to maximise GMG/Y, while there is no marked difference on whether *phenotype* or *progeny* strategy is used. However, such a high proportion of dominance variance is unlikely for the target traits in breeding of northerly conifers (growth rhythm and height growth) (e.g. EKBERG *et al.*, 1979; HANNERZ 1998).

Owing to a higher accuracy in prediction of breeding value with less resources (less test plants per genetic unit), increase in initial narrow-sense heritability led to a higher GMG/Y from all the breeding strategies.

In comparison with the *progeny* strategy, decrease of rotation age from 60 to 10 (corresponding to a sharp increase in J-M genetic correlation) was more advantageous for the *phenotype* and *clone* strategies, which led to a higher GMG/Y from the *phenotype* and *clone* strategies (*Figure 1f*). The *progeny* strategy is comparably more cost-demanding which means that less number of test plants can be tested, which, in turn, leads to a longer selection age. Considerations about the test time are strongly dependent on the J-M genetic correlation development suggested by LAMBETH (1980) based on observations on phenotypic correlations. The J-M genetic correlation may increase faster, and thus our prediction is a bit "conservative" (GWAZE *et al.*, 1997).

Gene diversity in a small breeding population is lost faster than in a large breeding population. Here the loss of gene diversity by selecting a single individual from a family is assumed to be constant (we assume that inbreeding and relatedness are kept constant), but over the whole breeding population, the size matters. Thus, the alternative scenario with high loss of gene diversity equal to 1% (instead of 0.5% at the main scenario) may also be interpreted as simulation of a breeding population with 25 individuals (instead of 50 individuals at the main scenario) (*Figure 1d*) (cf. ROSVALL, 1998).

The relations between the strategies referring to gene diversity loss (*Figure 1d*) depend on their cycling time. As the *phenotype* strategy involves more frequent cycles, it becomes less favourable at a higher diversity loss per cycle. As the *progeny* strategy involves longer and less frequent cycles it becomes more favourable with higher diversity loss per cycle and, thus, for relatively smaller breeding populations.

Making just a minor part of the overall cost per cycle, cost per genotype had a little effect on GMG/Y (*Figure 2b*). Thus, an expensive genotype production technique may be of interest only if it would markedly shorten the time for production of genetic entries for the test. With lower cost per plant, more plants can be tested, which would lead to a higher precision of the prediction of breeding values and could shorten the optimum selection age in the test. Reduction of the cost per test plant would especially be beneficial for the *clone* and *progeny* strategies as these strategies are more cost demanding than the *phenotype* strategy (*Figure 2c*, *Table 2*).

4.3 Concluding remarks

The *clone* strategy is the best breeding strategy to maximise Group Merit Gain per unit of time at all the parameter values common for northerly Norway spruce. If cloning of test plants is not operational, the *phenotype* strategy is the best choice, except for (1) the traits with low narrow-sense heritability (2) high budget of the breeding programme, and (3) if seeds for *progeny* testing can be obtained at age 11 and earlier. However, at most of the parameters, superiority of the *phenotype* strategy over the *progeny* strategy is minor, and thus additional considerations may be important. The *progeny* strategy may be an alternative to the *phenotype* strategy in case of shortening the reproductive maturity of the test parents at least to the age of 11 years. An expensive technique can be used for this purpose, as effect of genotype-depended cost on Group Merit Gain from *progeny* testing is minor.

As regards future improvement, a study on benefit of combination of breeding strategies in a stage-wise manner would be of value, e.g. phenotypic selection for growth rhythm traits followed by reselection by clonal testing (e.g. COTTERILL 1984).

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5. References

BURDON, R.: Clonal forestry and breeding strategies- a perspective. In: Proc. IUFRO Meeting of Working Parties on Breeding Theory, Progeny Testing, Seed Orchards, Oct. 13–17, Williamsburg, Virginia. N. C. State University- Industry Coop. Tree Imp. Prog., pp. 645–659 (1986). — BURDON, R. and SHELBOURNE, C. J. A.: The use of vegetative propagules for obtaining genetic information. N. Z. J. For. Sci. **4**: 418–425 (1974). — BURROWS, P.: Variance of selection differentials in normal samples. Biometrics **31**: 125–133 (1975). — COTTERILL, P. P.: A plan for breeding radiata pine. Silvae Genet. **33**: 84–90 (1984). — DANELL, Ö.: Survey of past, current and future Swedish forest tree breeding. Silva Fennica **25**: 241–247 (1991). — DANELL, Ö.: Breeding programmes in Sweden. 1. General approach. In: S. J. Lee (ed.). Progeny testing and breeding strategies. Proceedings of the Nordic group of tree breeding, Oct. 1993, Edinburgh. Forestry Commission. 128 (i-v) (1993a). — DANELL, Ö.: Tree breeding strategy: are we too concerned conservationists but inefficient breeders. In: S. J. Lee (ed.). Progeny testing and breeding strategies. Proceedings of the Nordic group of tree breeding, Oct. 1993, Edinburgh. Forestry Commission. 128, pp. 80–94 (1993b). — EKBERG, I., ERIKSSON, G. and DORMLING, I.: Photoperiodic reactions in conifer species. Holarctic Ecology **2**: 255–263 (1979). — FOSTER, G. S.: Selection and breeding for extreme genotypes. In: AHUJA, M. R. and LIBBY, W. J. (eds.). Clonal Forestry I, Genetics and Biotechnology. Springer-Verlag, pp. 50–67 (1992) — FOULLEY, J.-L and CHEVALET, C.: Méthode de prise en compte de la consanguinité dans un modèle simple de simulation de performances. Ann. Génét. Sél. Anim. **13**: 189–196 (1981). — GWAZE, D. P., WOOLIAMS, J. A. and KANOWSKI, P. J.: Optimum selection age for height of *Pinus taeda* L. in Zimbabwe. Silvae Genet. **44**: 358–365 (1997). — HANNERZ, M.: Genetic and seasonal variation in hardiness and growth rhythm in boreal and temperate conifers- a review and annotated bibliography. Forest Research Institute of Sweden, Report No. 2, 140 p. (1998). — HÄGGMAN, H. M., ARONEN, T. S. and STOMP, A.-M.: Early flowering Scots pines through tissue culture for accelerating tree breeding. Theor. Appl. Genet. **93**: 840–848 (1996). — KARLSSON, B.: Clone testing and genotype x environment interaction in *Picea abies*. Ph. D. thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, Sylvestria 162 (2000). — LAMBETH, C. C.: Juvenile-mature correlation in *Pinaceae* and implications for early selection. Forest Science **26**: 571–580 (1980). — LINDGREN, D. and WERNER, M.: Gain generating efficiency of different Norway spruce seed orchard designs. The Institute for Forest Improvement. Rep. No. **11**: 189–207 (1989). — LINDGREN, D. and MULLIN, T. J.: Balancing gain and relatedness in selection. Silvae Genet. **46**: 124–129 (1997). — MATHESON, A. C. and LINDGREN, D.: Gains from the clonal and the clonal seed-orchard options compared for tree breeding programmes. Theor. Appl. Genet. **71**: 242–249 (1985). — MEUWISSEN, T. H. E. and SONESSON, A. K.: Maximising the response of selection with a predefined rate of inbreeding: overlapping generations. Journal of Animal Science **76**: 2575–2583 (1998). — MULLIN, T. J. and PARK, Y. S.: Stochastic simulator of population management strategies for tree breeding: a new decision-support tool for personal computers. Silvae Genet. **44**: 133–141 (1995). — ROSVALL, O., LINDGREN, D. and MULLIN, T. J.: Sustainability robustness and efficiency of a multi-generation breeding strategy based on within family clonal selection. Silvae Genet. **47**: 307–321 (1998). — ROSVALL, O.: Enhancing gain from long-term forest tree breeding while conserving genetic diversity. Ph. D. thesis, Swedish University of Agricultural Sciences, Umeå, Sweden, Sylvestria 109 (1999). — RUSSELL, J. H. and LOO-DINKINS, J. A.: Distribution of testing effort in *clone*d genetic tests. Silvae Genet. **42**: 98–104 (1993). — SHAW, D. V. and HOOD, J. V.: Maximizing gain per effort by using clonal replicates in genetic tests. Theor. Appl. Genet. **71**: 392–399 (1985). — SHELBOURNE, C. J. A and JORDAAN, J. V.: Genetic gains from different kinds of breeding population and seed or plant production population. South African Forestry Journal **160**: 49–65 (1992). — WEI, R.-P.: Predicting genetic diversity and optimising selection in breeding programmes. Ph. D. thesis, Swedish University of Agricultural Sciences, Umeå, Sweden, (1995). — WEI, R.-P. and LINDGREN, D.: Optimum breeding generation interval considering build-up of relatedness. Theor. Appl. Genet. (in press).

Landscape Genetic Structure of *Pinus banksiana:* Seedling Traits

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Abstract

The extent and patterning of genetic diversity at a landscape scale (30 km x 30 km) was investigated using seedlings from 47 stands of *Pinus banksiana* LAMB. collected in a pine-oak barrens in west-central Wisconsin, USA. Seedlings grown for six months in a greenhouse were evaluated for the number of cotyledons, the length of the longest cotyledon, the number of early needle fascicles, seedling height, timing of bud set, and the dry weight of roots, foliage, stem and total seedling, shoot:root ratio and foliage:root ratio. A pronounced genetic structure exists for most traits, with stands showing significant differentiation at geographic distances up to 25 km. Seedlings originating from trees growing on sandy sites were larger than those from sandy-loam sites. The scale and pattern of differentiation for several traits parallels the scale and pattern of soil variation on the landscape, supporting the hypothesis that stand genetic differentiation corresponds to a gradient of environmental differences. The combined effect of soil texture, drainage and ground-water influence, apparently are the primary selective forces influencing among-stand genetic differentiation for the traits under study within this landscape. The results could be useful in a program of genetic resource management.

Key words: Landscape, genetic variation, genetic differentiation, quantitative traits, *Pinus banksiana*, spatial statistics, autocorrelation, kriging, forest gene conservation.

Introduction

Ecological factors help shape genetic architecture in plant populations, with correlations between environmental and

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