TWO-STAGE SELECTION STRATEGIES IN TREE BREEDING CONSIDERING GAIN, DIVERSITY, TIME AND COST

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ABSTRACT

Methods for calculating Group Merit (an average of genetic gain and gene diversity) Gain per year (GMG/Y) were developed for a number of breeding strategies involving recurrent cycles of balanced within-family selection. Optima were sought, considering resource deployment and age of selection, with the aid of a deterministic computer simulator. The breeding strategies compared comprised a single stage of selection (based on phenotype, clone or progeny testing) or two selection stages per breeding cycle. The latter involved a first stage of selection based on phenotype followed by a second stage based on clonal or progeny tests of those selected at the first stage. Genetic variance components, the number of plants deployed, cost and time components were all variable. When heritability is high, phenotypic selection is a superior breeding strategy, but at moderate or low heritability, it is inferior. Strategies including clonal testing were superior and it made little difference if the clone test was preceded by a phenotypic selection or not. A strategy based on progeny testing was improved by a first stage phenotypic selection. In the two-stage strategy, it seemed favourable to delay the phenotypic selection until about canopy-closure, at least if juvenile-mature correlation develops as for growth characters.

Key-words: group merit, long-term breeding, Norway spruce, optimisation, stage-wise selection.

INTRODUCTION

In long-term breeding programmes based on repeated cycles of recombination and selection, it is important to exploit all available possibilities to maximise genetic gain per unit time and gene diversity lost (LINDGREN & MULLIN 1997, ROSVALL 1999). Under a conventional single-stage selection based on progeny or clonal tests, the time before establishment of the selection test yields no gain. It may be advantageous to utilise this time by selecting in a stage-wise manner, i.e. selecting phenotypically at first, followed by a second stage involving more thorough testing based on replicated tests of the individuals selected in the first stage (ROULUND *et al.* 1986, COTTERILL & JACKSON 1989, ADAMS & JOYCE 1990, BORRALHO *et al.* 1992). Where juvenile heritability is reasonably high, first stage phenotypic selection is an effective method to reduce the costs of the subsequent field progeny tests and increase the overall genetic gain (NAMKOONG 1970, COTTERILL & JAMES 1981, WU 1998, ADAMS *et al.* 2001).

On the other hand, two-stage selection is a more complex strategy that may incur higher costs and require longer cycles. Furthermore, phenotypic selection may be much less efficient than selection based on replicated tests. Thus, the problem is to identify under which conditions (if any) a two-stage selection strategy is advantageous over a single-stage selection strategy by considering genetic gain, gene diversity, cost and time.

Long-term breeding should consider genetic gain as well as gene diversity (coancestry), cf. LINDGREN & MULLIN (1997). Relatedness (expressed as group coancestry) and average breeding value of the breeding population can be merged into a weighted average, "group merit" and the annual increase of group merit can be used as a criterion of breeding progress (WEI & LINDGREN 2001). A long-term breeding plan based on recurrent cycles of within-family selection with equal parent contributions allows the greatest possible gene diversity to be maintained in a breeding population of a fixed size (ROSVALL 1999).

Stage-wise selection is used in a number of forest tree breeding programmes (e.g. COTTERILL 1984, KLEIN 1998, HAAPANEN *et al.* 1999, MIKOLA 2002). However,

to our knowledge, there have been no studies on the relative benefit and optimisation of two-stage selection strategies under long-term tree breeding based on balanced within-family selection in which genetic gain, gene diversity, cost and time have been simultaneously considered. An earlier study we published on the optimisation of single-stage breeding strategies under recurrent cycles of balanced within-family selection showed that a strategy based on clonal testing was superior at most of the parameter values common in breeding forest trees except for high heritability (DANUSEVICIUS & LINDGREN 2002).

The objectives of this study were (1) to analyse and optimise a two-stage selection strategy based on phenotypic pre-selection followed by clonal or progeny testing and (2) to compare two-stage selection strategies with selection in a single stage.

MATERIAL AND METHODS

The breeding programme and strategies

The inputs and strategic alternatives considered in this study are in line with the Swedish breeding programme for Norway spruce (DANELL 1993a & b, KARLSSON 2000). The long-term goal of this programme is to maintain a meta-population composed of a number of unrelated breeding populations of 50 members each. Within each breeding population, the breeding is to be carried out by double-pair mating among the 50 members and balanced within-family selection of the single best individual from each of the 50 full-sib families as a parent for the following breeding cycle. This is planned to be repeated over many generations, and this study deals with one of those cycles. The selection of the best individual is based on a single value formed as an index weighting different traits with the aim to maximise the correlation with the target economical benefit. It may be simpler to just visualise it as the target character per area volume production at mature age as a function of height, diameter and survival at young age.

Within this general strategy, the following breeding strategies for a long-term breeding population were compared (Fig. 1):

- Two-stage *Phenotype/Clone* selection strategy. Stage 1: Phenotypic selection of equal numbers of candidates from each of the 50 full-sib families in a test. Stage 2: Vegetative propagation of the selected clones and planting of the clonal copies in a new test, from which a single best candidate is selected from each of the 50 families based on its clonal average (Fig. 1a).
- Two-stage *Phenotype/Progeny* selection strategy. Stage 1: Phenotypic selection of an equal number of candidates from each of the 50 full-sib families in a test. Stage 2: Sexual propagation of the selected candidates and planting of the progenies in a new test, from which a single best candidate is selected from each of the 50 families based on the performance of its progeny (Fig. 1b).
- Single-stage *Phenotype* selection strategy. Planting of equal numbers of candidates from each family in a test, from which the single best phenotype from each of the 50 full-sib families is selected according to its phenotypic performance (Fig. 1c).
- Single-stage *Clone* selection strategy. Production of an equal number of candidates from each family, vegetative propagation of the candidates and planting of their clonal copies in a test, from which a single best candidate is selected from each of the 50 full-sib families based on its clonal average (Fig. 1d).
- Single-stage *Progeny* strategy. Production of an equal number of candidates from each family, planting of the progeny of the candidates (open-pollinated or polycross) in a test from which a single best candidate is selected from each of the 50 families based on the performance of its progeny (Fig. 1d).

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

The simulation model

The infinitesimal genetic model was assumed (i.e. that for each trait there is an infinite number of unlinked loci, each with a small effect) and the simulations were based on a series of main and alternative scenarios (Table 1). While testing an alternative value of a parameter, all the other parameters were kept at the values applied in the main scenario. In these analyses an MS Excel-based deterministic simulator called BREEDING CYCLE ANALYZER was used (available on the internet at www.genfys.slu.se/staff/dagl). It was assumed that selection would be performed on the same trait or index at stages 1 and 2. Group Merit Gain per year (*GMG/Y*) (WEI & LINDGREN 2001) 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:

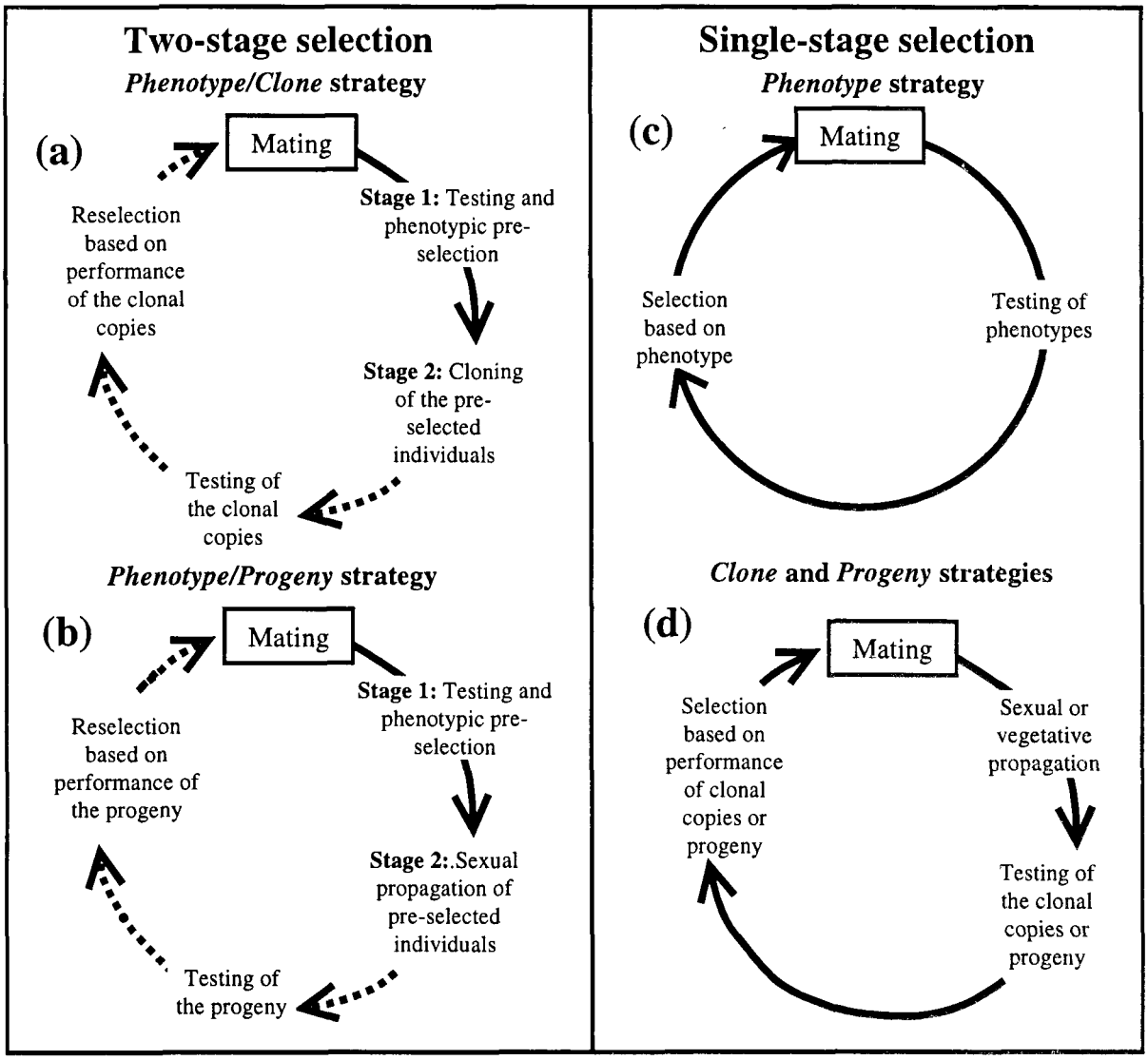

Figure 1. Outline of the breeding strategies. The two-stage selection strategies are shown in (a) and (b) while the single-stage selection strategies are shown in (c) and (d). For the two-stage strategies, the solid arrows refer to the first stage of selection and the dotted arrows to the second stage of selection.

$$GMG/Y = (G - c\Theta)/CT, \quad [1]$$

where, GMG is Group Merit Gain obtainable from selection, G is estimated additive genetic gain at rotation age (%) (cumulative for two stages), c is a weighting factor between loss of genetic diversity and genetic gain that converts gain and diversity to the same scale, CT is cycle time, Θ is the rise in group coancestry per breeding cycle, which, assuming that each parent contributes two offspring for use as parents in the next breeding cycle, was estimated as:

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

where, n is breeding population size.

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

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

Selection based on phenotype:

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

Selection based on clonal test:

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

Selection based on half-sib progeny test:

$$G = \frac{\sigma_{Am} r_{j-m} i 0.5 \sigma_A}{\sqrt{0.25\sigma_A^2 + \frac{0.75\sigma_A^2 + \sigma_D^2 + \sigma_E^2}{n}}} \quad [5]$$

where: G is additive genetic gain (%); σ_A^2 is additive variance; σ_D^2 is dominance variance; σ_E^2 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, given as a percentage of the average breeding value of the unimproved individuals for this trait (one standard deviation is equal to 10 %); and i is selection intensity estimated in units of standard deviation of the mean of the selected individuals from the family mean by using an approximation by BURROWS (1975). Finally, 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, applied to make the r_{j-m} function more linear if the trait is measured at a very young age or an age close to rotation:

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

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

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

The variable parameters used to find the maximum possible *GMG* per year at a given cost for each of the scenarios in Table 1 were as follows. For the two-stage strategies, the required variables were: the selection ages at stages 1 and 2; the family size for testing and the number of phenotypically selected candidates at stage 1; and the number of ramets per ortet or number

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
Environmental vaiance, % of total variance (σ_E^2)	88	0; 38; 94
Additive standard deviation at mature age (σ_{Am})	10	5; 20
Diversity loss per cycle, %	0.5	0.25; 1; 5
Rotation age, years	60	10; 20; 120
Time before establishment of the selection test (T_{BEFORE}), years	1 (*phenotype*)	3; 5 (*phenotype*)
	5 (*clone; phenotype/clone*)	3; 7 (*clone; phenotype/clone*)
	17 (*progeny; phenotype/progeny*)	5; 7 (*progeny; phenotype/progeny*)
Recombination cost (C_{RECOMB}), $	30	–
Cost per genotype (Cg), $	0.1 (*clone*),	1; 5 (*clone*)
	1 (*progeny*)	0.1; 5 (*progeny*)
Cost per plant (Cp), $	1	0.5; 3
Budget per year and parent (the constraint)	10	5; 20; 50
Group Merit Gain per year (*GMG/Y*)	To be maximized	

of progenies per individual for the stage 2 selection test. For the single-stage strategies the variables required were: age of selection in the test, family size for testing, and the number of plants for clonal or progeny testing of each family member.

The input values used in the main and alternative scenarios

Values used as the "inputs" in the model were chosen for their relevance to breeding northerly Norway spruce. A most likely value was identified as the main scenario value (for some parameters the main scenario used different values for different testing strategies). Then reasonable upper and lower limits for each value were selected (i.e. the highest and lowest values likely to be compatible with the actual circumstances). For many genetic parameters the values could be based on reviews from field tests (e.g. HANNERZ 1998, ROSVALL 1999).

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

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

where, C_{RECOMB} is the cost for recombination among the founders, C_{INIT} is the cost for initiation of the test, C_G is cost per genotype, i.e. cost dependent on the type of reproductive material used (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 production of the genetic entries (ortets or female parents). Plant-dependent costs were assumed to cover production of the test plants in the nursery (seedlings or cuttings), establishment, maintenance and assessment of the selection test.

Costs were expressed in "$", which can be interpreted as "cost-units". The basis for setting the costs for different operations within a breeding cycle was as follows: cost per test plant (plant-dependent cost) was set to 1$ and all the other costs were expressed in ratios of 1$ (Table 1). Cost was assumed to be independent of the age of the selection test, but if comparisons cover very different ages this assumption will be unrealistic. Only a few cost estimates are available from the literature, and it is not straightforward to estimate valid cost components because there are many variables and issues to consider (e.g. the extent to which costs are fixed, or flexible, the analytical procedures and measurement methods).

The simulations were run with an annual budget constraint of 10$ per breeding population member for the main scenario and 5$, 20$ and 50$ per breeding population member for the alternative scenarios.

The time per breeding cycle was subdivided into the following components:

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

where, T_{RECOMB} is the time needed for recombination among breeding population members (crossing and seed production), T_{BEFORE} is the time needed to produce plants for the selection test (i.e. time from seeding in the 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, and T_{AFTER} is the time from selection of the new parents to harvesting their seeds for the next breeding cycle.

The time for recombination was set to three years for all the alternatives. The criteria for setting the time before establishment of the selection test were as follows. The selection test may be established with 1-year-old seedlings. A 4-year-old seedling of Norway spruce is large enough to provide up to 25 cuttings, and in one year the cuttings may develop an appropriate root system for planting out. The progeny of northerly conifers reach sexual maturity at 15 years of age and it takes two years to get open-pollinated or polycross seeds. T_{AFTER} was set to two 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 nurseries).

T_{AFTER} for the single-stage *Phenotype* strategy depends on the following two alternatives to complete a breeding cycle: (1) to make crosses in a crossing archive, i.e. to top graft the selected phenotypes (time after would be five years) or to graft into a root stock (time after would be 10 years), (2) to pre-select some percentage of best performing individuals within each family at some 5 years before the final selection and to top graft their copies in the crossing archive, followed by flower stimulation to induce early flowering on the top-grafted copies at the time of final selection in the test (ALMQVIST & EKBERG 2001). The selections could also be complemented by pollen harvest on good phenotypes in the test, which were not top grafted earlier. In our study, we assume that the second alternative is performed.

In simulations for the two-stage selection strategies, the lower limit for the age at the phenotypic selection was set to four years for the *Phenotype/Clone* strategy and 15 years for the *Phenotype/Progeny* strategy (to

ensure that the selected candidates are reproductively mature).

RESULTS

Ranking of the strategies and effect of the parameters

For the main scenario, the GMG/Y ranking of the strategies was as follows (actual values within parentheses): single-stage *Clone* (0.250 %) gave the best results, followed by two-stage *Phenotype/Clone* (0.247 %), two-stage *Phenotype/Progeny* (0.181 %), single-stage *Phenotype* (0.152 %) and single-stage *Progeny* (0.139 %).

The single-stage *Clone* strategy and the two-stage *Phenotype/Clone* strategy provided the highest GMG/Y for all scenarios, except those with high narrow-sense heritability (low environmental variance), for which the single-stage *Phenotype* strategy was best (Figures 2 and 3, Tables 2 and 3).

Environmental variance (heritability), additive variance at mature age, and rotation age (J–M genetic correlation) had the strongest effects on GMG/Y (Figure 2). Heritability of the target trait and rotation age may affect the choice between the single-stage and two-stage selection strategies (Figure 2b and f). Thus, values of these parameters should be considered first when choosing the breeding strategy.

A detailed discussion on the effect of the parameters is given in our previous comparison of single-stage breeding strategies (DANUSEVICIUS & LINDGREN 2002). Therefore, we will not place much emphasis on the single-stage selection strategies, except when they are compared to the corresponding two-stage selections.

Two-stage *Phenotype/Clone* strategy versus single-stage *Clone* selection strategy

There was no marked difference in GMG/Y between the two-stage *Phenotype/Clone* and the single-stage *Clone* strategies, the latter being slightly superior at all the parameter values, except for the scenarios with short rotation age and low environmental variance (Table 2), for which single-stage phenotypic selection can be superior.

In most of the scenarios for the two-stage *Phenotype/Clone* strategy, the optimum age for the first-stage phenotypic selection was four years, which was set as the lowest age for the phenotypic selection since a selected individual has to be large enough to produce sufficient cuttings for the subsequent clonal test (Table 2).

Two-stage *Phenotype/Progeny* strategy versus single-stage *Progeny* strategy

In all the scenarios, the two-stage *Phenotype/Progeny* strategy generated higher GMG/Y values than the single-stage *Progeny* strategy (Figures 2 and 3, Table 3). Owing to a higher benefit from the first-stage phenotypic selection, the relative advantage of the two-stage *Phenotype/Progeny* strategy was markedly greater under low environmental variance (Figure 2b) and short rotation age (Figure 2f), but then, single-stage *Phenotype* strategy was superior to both the *Phenotype/Progeny* and *Progeny* strategies.

For most of the scenarios, the optimum age for the first-stage phenotypic selection was 15 years, which was set as the lower limit for the selection age, assuming that the candidates should be reproductively mature at the time of the first-stage phenotypic selection (Table 3).

Using the main scenario values for the genetic parameters and cost components, the two-stage *Phenotype/Progeny* strategy with the time before establishment of the selection test set to 17 years yielded higher GMG/Y values than the single-stage *Progeny* strategy with the time before establishment of the selection test set to five years (Figure 3b).

Analysis of the variation in GMG/Y obtained from the two-stage *Phenotype/Progeny* strategy with age at the first-stage phenotypic selection (when the other parameters were set at the main scenario values) showed that the optimum age for the first-stage phenotypic selection is 12 years (Figure 4).

DISCUSSION

Our model did not consider the possibility that the costs may increase with the age of the test plants. However, as the response of the breeding strategies to a rather extreme variation in the cost components was comparably robust (Figure 3), this should probably not bias the main findings of this study.

Our base-line breeding strategy normally minimises the loss of gene diversity per generation as the selection is completely balanced. This is not usually the best strategy for selections with a more immediate goal, e.g. selecting the founders for long-term breeding programmes (ROUTSALAINEN & LINDGREN 1998) or selection for seed orchards, when gene diversity can be sacrificed. However, for long-term breeding, balanced within-family selection seems to be a near-optimal strategy, even considering possible gain in multiplication populations drawn from the breeding population (ROSVALL 1999). Optimisation of long-term breeding with unbalanced selection is more complex and requires

Table 2. Comparison of Group Merit Gain per year (GMG/Y in %) yielded in the main and alternative scenarios of the two-stage *Phenotype/Clone* and single-stage *Phenotype* and *Clone* breeding strategies. For the two-stage strategy, optimum number of test plants and optimum selection age (counted from establishment of the selection test) and genetic gain (%) are given for each stage of selection (for the first-stage of selection, number of plants refers to each full-sib family, while for the second stage of selection, the number of ramets per selected candidate (ortet) is given).

Parameter	Value	Two-stage Phenotype/*Clone strategy*								Single-stage strategies	
		Stage 1: Phenotype test			Stage 2: Clonal test			Overall 2 stages			
		Genet. gain	Select. age	Selection *n* out of *N*	Genet. gain	Sel. age	Ramet no. per ortet	Cycle time	GMG/Y	*Phenotype* GMG/Y	*Clone* GMG/Y
σ^2_D, % of σ^2_A	0	0.3	3	15/32	8.3	22	17	32	**0.255**	**0.150**	**0.259**
	25*	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.152**	**0.250**
	100	0.3	3	18/39	7.2	20	13	30	**0.230**	**0.157**	**0.229**
σ^2_E, % of total variance	94	0.2	3	12/26	6.8	23	23	33	**0.197**	**0.098**	**0.201**
	88*	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.152**	**0.250**
	38	4.9	10	17/169	8.3	14	6	31	**0.420**	**0.442**	**0.406**
	0	13.0	4	4/239	3.0	6	1	27	**0.568**	**0.681**	**0.529**
σ_{Am}, %	5	0.2	3	16/34	4.3	24	17	34	**0.116**	**0.065**	**0.117**
	10*	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.152**	**0.250**
	20	0.6	3	15/32	15.4	20	16	30	**0.511**	**0.328**	**0.517**
Diversity loss, %	0.25	0.3	3	15/32	7.7	20	16	30	**0.255**	**0.164**	**0.258**
	0.50*	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.152**	**0.250**
	1	0.3	3	15/36	8.1	22	17	32	**0.231**	**0.128**	**0.234**
	5	1.8	12	19/124	12.2	44	25	63	**0.144**	**0.033**	**0.141**
Rotation age, years	10	2.0	3	9/42	8.4	8	12	18	**0.546**	**0.365**	**0.504**
	20	1.5	3	10/44	8.0	11	14	21	**0.414**	**0.271**	**0.398**
	60*	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.152**	**0.250**
	120	0.1	3	19/33	7.4	31	18	41	**0.172**	**0.097**	**0.175**
Tb, years	1^{Ph}	–	–	–	–	–	–	–	–	**0.152**	–
	3	0.0	1	17/21	7.8	21	14	29	**0.253**	**0.141**	**0.263**
	$5^{Cl,\ Ph/Cl}$	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.132**	**0.250**
	7	0.7	5	14/51	7.6	20	17	32	**0.244**	–	**0.239**
Cg, $	0.1^{Cl}	0.3	3	15/34	7.7	21	16	31	**0.247**	–	**0.250**
	1.0^{Pr}	0.3	3	14/33	7.9	22	12	32	**0.244**	–	**0.246**
	5	0.3	3	12/29	7.9	23	18	33	**0.230**	–	**0.233**
Cp, $	0.5	0.4	3	21/61	8.8	18	21	28	**0.297**	**0.167**	**0.297**
	1*	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.152**	**0.250**
	3	0.1	8	8/30	6.0	24	11	39	**0.171**	**0.127**	**0.180**
Total budget, $	5	0.1	7	10/35	7.0	25	13	39	**0.191**	**0.132**	**0.198**
	10*	0.3	3	15/34	7.7	21	16	31	**0.247**	**0.152**	**0.250**
	20	0.4	3	22/64	8.6	18	21	28	**0.302**	**0.168**	**0.301**
	50	0.5	3	38/148	9.9	16	30	26	**0.372**	**0.187**	**0.366**

Superscripts of the parameter values indicate the main scenario values for: (*) all breeding strategies, (Ph) *Phenotype* strategy, (Cl) *Clone* strategy, (Pr) *Progeny* strategy, (Ph/Cl) two-stage *Phenotype/Clone* strategy and (Ph/Pr) two-stage *Phenotype/Progeny* strategy.

[1)] GMG/Y values for the single-stage strategies are taken from DANUSEVICIUS and LINDGREN (2002), which also gives more details on the optimisation of these strategies.

Table 3. Comparison of Group Merit Gain per year (GMG/Y in %) obtained in simulations based on the main and alternative scenarios of the two-stage *Phenotype/Progeny* and single-stage *Progeny* breeding strategies. For the two-stage strategy, optimum number of test plants and optimum selection age (counted from establishment of the selection test) and the genetic gain (%) are given for each stage of selection (for the first-stage of selection, number of plants refers to each full-sib family, while for the second-stage, the number of seedlings per candidate is given).

Parameter	Value	Two-stage *Phenotype/Progeny* strategy								Single-stage
		Stage1: Phenotype test			Stage 2: Progeny test			Overall 2 stages		
		Genetic gain	Sel. age	Selection *n* out of *N*	Genetic gain	Sel. age	Plant no. per candidate	Cycle time	GMG/Y	*Progeny* GMG/Y
σ^2_D , % of σ^2_A	0	2.7	15	5/101	5.5	20	59	43	**0.180**	**0.138**
	25*	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	100	2.9	15	5/103	5.6	20	58	43	**0.185**	**0.141**
σ^2_E, % of total variance	94	1.8	15	5/82	5.1	25	72	48	**0.134**	**0.107**
	88*	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	38	8.5	15	4/189	4.3	9	25	32	**0.383**	**0.236**
	0	14.2	15	2/202	0.3	1	4	24	**0.578**	**0.282**
σ_{Am}, %	5	1.3	15	6/102	3.1	24	55	47	**0.085**	**0.065**
	10*	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	20	5.5	15	5/101	10.7	19	57	42	**0.375**	**0.287**
Diversity loss, %	0.25	2.8	15	5/101	5.4	19	57	42	**0.187**	**0.144**
	0.50*	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	1	2.7	15	6/102	6.3	24	55	47	**0.170**	**0.130**
	5	3.5	23	8/168	10.2	52	78	83	**0.104**	**0.069**
Rotation age, years	10	4.8	15	4/94	6.8	9	48	32	**0.346**	**0.236**
	20	4.5	15	4/97	5.9	11	52	34	**0.291**	**0.188**
	60*	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	120	1.8	16	104	6.0	34	63	58	**0.124**	**0.104**
Tb, years	5^{Cl}	2.2	11	6/81	5.7	21	48	40	**0.183**	**0.164**
	7	2.2	11	6/81	5.7	21	48	40	**0.183**	**0.159**
	$17^{Pr,\ Ph/Pr}$	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
Cg, $	0.1^{Cl}	2.7	15	6/100	5.8	21	51	44	**0.182**	**0.139**
	1.0^{Pr}	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	5	2.8	15	5/98	5.6	21	57	44	**0.179**	**0.135**
Cp, $	0.5	3.0	15	7/188	6.9	20	84	43	**0.219**	**0.171**
	1*	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	3	2.5	17	4/45	3.8	22	25	47	**0.125**	**0.087**
Total budget, $	5	2.6	16	4/55	4.5	23	36	47	**0.141**	**0.106**
	10*	2.8	15	5/101	5.5	20	59	43	**0.181**	**0.139**
	20	2.9	15	8/191	7.1	20	78	43	**0.222**	**0.173**
	50	3.2	15	13/494	8.8	19	120	42	**0.274**	**0.220**

Superscripts of the parameter values indicate the main scenario values for: (*) all breeding strategies, (Ph) *Phenotype* strategy, (Cl) *Clone* strategy, (Pr) *Progeny* strategy, (Ph/Cl) two-stage *Phenotype/Clone* strategy and (Ph/Pr) two-stage *Phenotype/Progeny* strategy.

[1] GMG/Y values for the single-stage strategies are taken from DANUSEVICIUS and LINDGREN (2002), which also gives more details on the optimisation of these strategies.

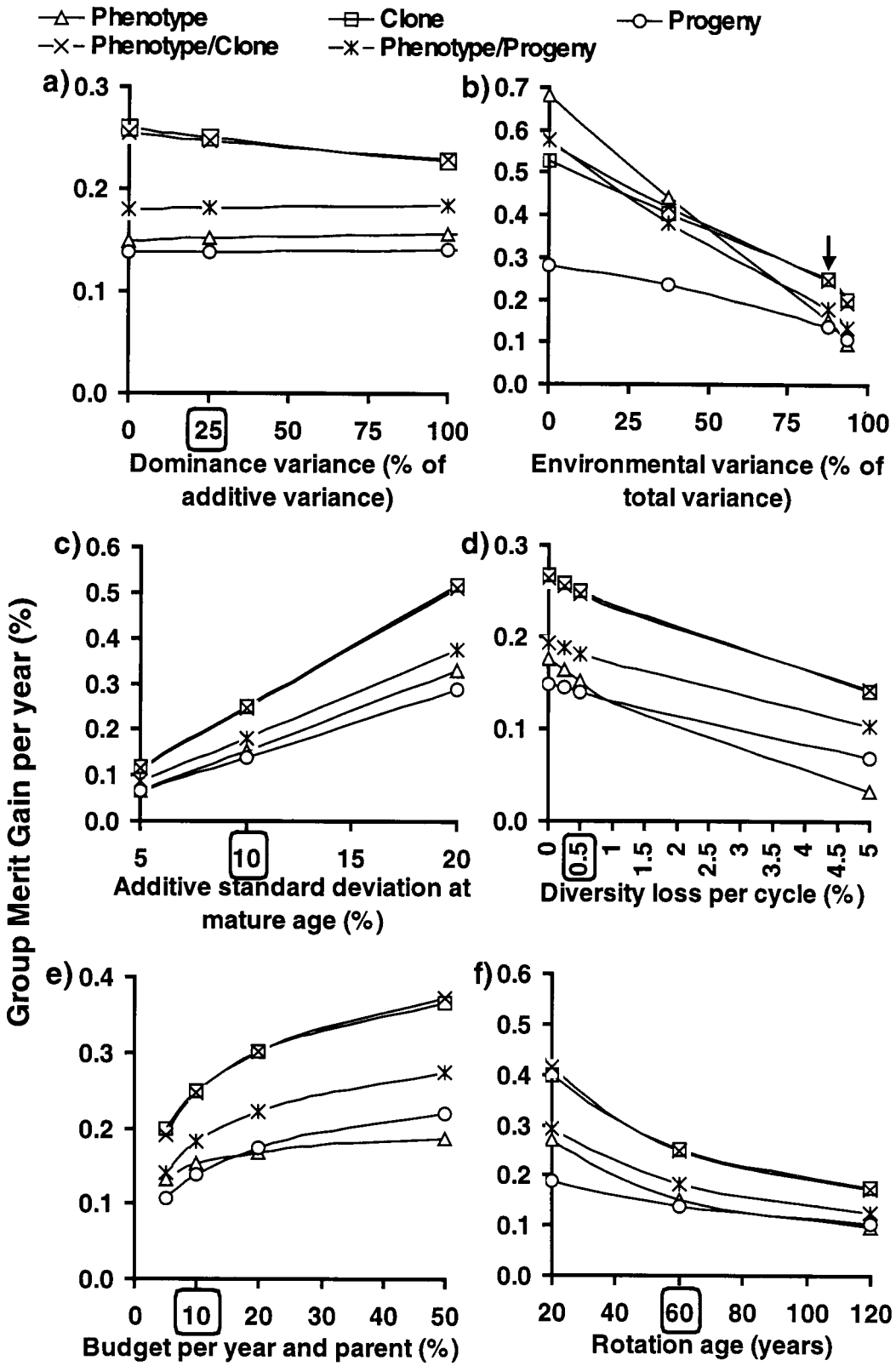

Figure 2. Ranking of the breeding strategies according to Group Merit Gain per year, with the main and alternative values for genetic parameters (plots a, b, c, d), total budget as the constraint (e) and rotation age (f). The boxed points on the X-axis and arrows above the curve indicate the values for the main scenario. Phenotype/Progeny and Phenotype/Clone are the two-stage strategies.

additional parameters concerning goals associated with time horizons. Some may consider it valuable to prolong generation time in our system to obtain the same *GMG* at a certain time with less loss of diversity, but again this requires more inputs to account for changes in the value of various factors at different times. It is possible that this could be done using interest functions to discount benefits and costs at the same time.

We have assumed, that for the single-stage *Phenotype* strategy the crossing of the selected individuals for next breeding cycle will be performed on the top-grafted selections, where an earlier pre-selection and top grafting of some percentage of the best candidates within each family were performed. However, this may be quite an optimistic assumption, and a more secure, but less beneficial, way would be to graft only the final selections and to wait for some 10 years until they will start flowering. In the later case, benefit from the single-stage *Phenotype* strategy reported here is overestimated and, thus, shall be interpreted with caution and assuming that the top grafting and the pre-selection are realistic options for Norway spruce. This also means that, if the pre-selection and top grafting are not feasible opinions for the single-stage *Phenotype* strategy, a strategy involving clonal testing would rank still more superior in all the scenarios investigated.

Optimisation of the two-stage *Phenotype/Clone* strategy

To achieve the highest overall gain from the two-stage *Phenotype/Clone* strategy, the resources were reallocated from the first-stage phenotypic selection to the second-stage selection based on clonal tests, as the latter provided a relatively greater gain per unit of time (Figure 5). Thus, in most of the scenarios, the optimum age for the phenotypic selection was as short as necessary to get the plants of a sufficient size to provide enough cuttings, i.e. four years old. Owing to maternal and establishment effects, it may also be risky to base the first-stage selection on plants younger than this. Even if it proved possible to find a method for early selection with a high juvenile-mature correlation, it would probably not be worth using it at an earlier stage as sufficient time must still be allowed to get cuttings.

The main advantage of the first-stage phenotypic selection is that the time before production of the hedges for the clonal test can be utilised to generate gain at a relatively low cost. However, except for the scenarios with high heritability and short rotation age, this gain per unit time made a minor contribution to the overall gain (Figure 5, Table 2). In cases of relatively high heritability, phenotype-based first-stage selection

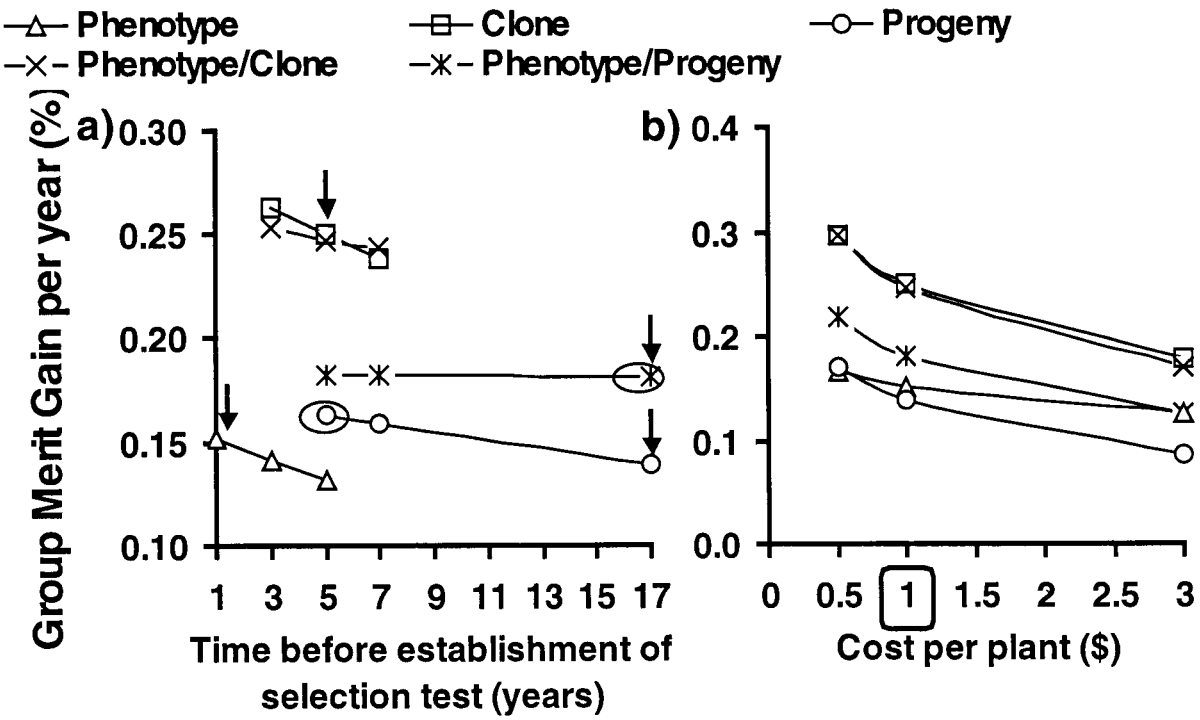

Figure 3. Ranking of the breeding strategies according to Group Merit Gain per year with the main and alternative values for time before establishment of the selection test (a) and cost per test plant (b). The boxed points on the X-axis and arrows above the curve indicate the values for the main scenario. The ringed points relate to the comparison between the two-stage Phenotype/Progeny strategy (with "Time before" set to 17 years) and the single-stage Progeny strategy (with "Time before" set to five years).

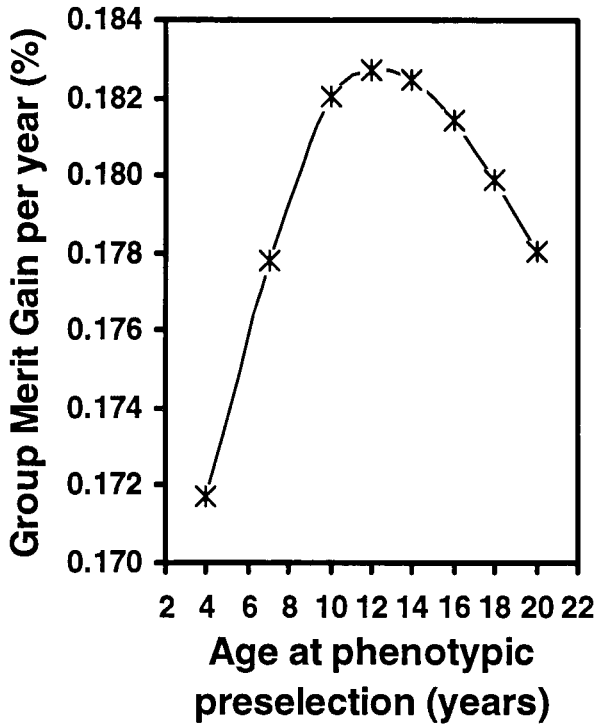

Figure 4. Group Merit Gain per year obtained from the two-stage *Phenotype/Progeny* strategy as a function of tree age at the first selection stage (X axis) based on the main scenario values of the genetic parameters and cost components. The optimum age for the first-stage selection is 12 years. It seems doubtful that it would be generally beneficial to shorten the reproductive maturity before age 12 to start progeny testing earlier.

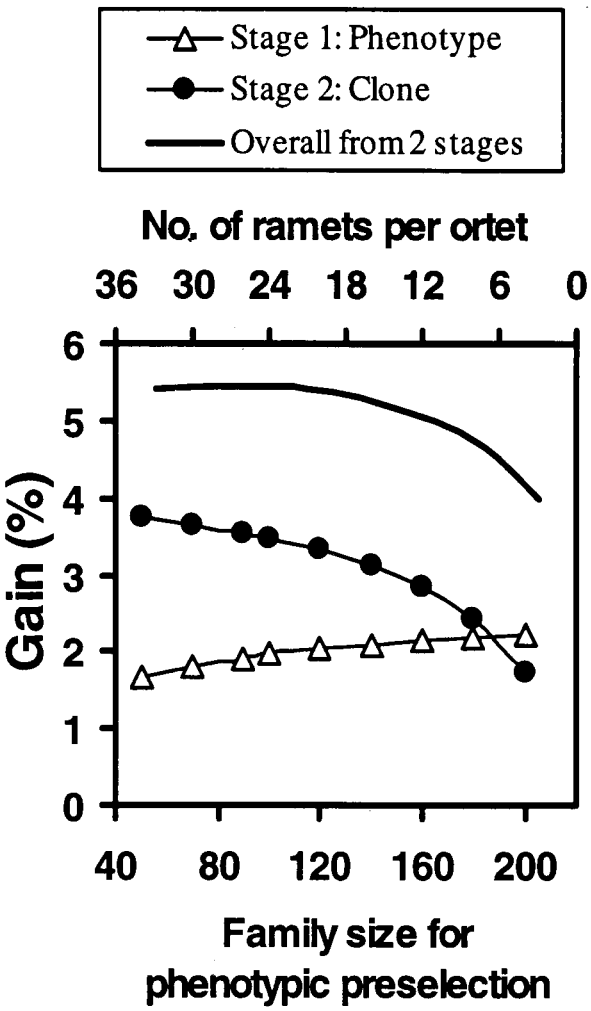

Figure 5. Comparison of simultaneous change in genetic gain from each of the interdependent selection stages of the *Phenotype/Clone* two-stage strategy in response to increasing family size for the first-stage phenotypic selection (lower X axis), which leads to a simultaneous reduction in the number of ramets per ortet in the clonal test (upper X axis). For this reason, gradually more resources were reallocated from the clonal test in stage 2 to the phenotype test in stage 1 (thereby increasing selection intensity for the phenotype test and reducing the precision of the clonal test), provided that the other key parameters remained constant. The selection age was 8.5 years for the phenotype test and nine years for the clonal test. Five candidates were phenotypically selected at the first stage, and main scenario values were used.

may be a cost-efficient approach as it could reduce the size of field test, without sacrificing genetic gain (WU 1998). However, if these resources were allocated to a single-stage clonal test, or a phenotype test when heritability was high, an adequate gain per unit time may be obtained (Table 2, COTTERILL 1986). Therefore, there was no marked difference in GMG/Y between the two-stage *Phenotype/Clone* and the single-stage *Clone* strategy. This suggests that the choice here may depend on factors other than those considered in this study. As there was almost no difference between these strategies, it would probably be advantageous when using the two-stage selection to select for different characters at the different stages (for instance, traits with high heritability and J-M correlation such as growth rhythm at the first-stage phenotypic selection and growth traits at the second-stage selection, based on a clonal test). Support for this hypothesis is given by COTTERILL & JAMES (1981) as well as by the results of the alternative scenarios with short rotation age (high J-M correlation) and high heritability, in which the two-stage *Phenotype/Clone* strategy was superior to the single-stage *Clone* strategy (Table 2). In contrast, if heritability of the measured trait is low, phenotypic selection should be used with caution, as it provides little gain and may introduce the risk of biased selection, which may markedly reduce the efficiency of the subsequent selection stage.

Optimisation of the two-stage *Phenotype/Progeny* strategy

Even if the *Clone* strategy seems superior, it is often impossible to put into practice, and even if technically possible, it may be suspected that it will not work as well in reality as assumed in this study. In all the scenarios, the first-stage phenotypic selection markedly raised the genetic gain per unit time from the two-stage *Phenotype/Progeny* strategy, what lead a greater GMG/Y than from the single-stage *Progeny* strategy (Table 3). COTTERILL (1986) obtained similar results. Furthermore, maturation will occur while waiting for the first-stage phenotypic selection, making it more likely that pollen or cones will be available at the time of the first-stage phenotypic selection. The two-stage *Phenotype/Progeny* strategy, with no investment to promote early flowering ("Time before" set to 17 years, which may be realistic for Norway spruce) was superior to the single-stage *Progeny* strategy, in which it was assumed that the seeds for testing can be obtained at three years of age ("Time before", five years) (Figure 3a). This suggests that use of two-stage selection with phenotypic selection, to benefit from the time preceding sexual maturity, followed by a progeny test of the selected candidates is more beneficial than investment to hasten flowering of the individuals in a single-stage progeny test.

Under the two-stage *Phenotype/Progeny* strategy, the optimum age for the first-stage phenotypic selection was 12 years (Figure 4). Thus, under this two-stage strategy, it seems doubtful that it would generally be beneficial to accelerate reproductive maturity to before 12 years of age in order to start the progeny test earlier. Estimates of the optimum age for the first-stage selection may be affected by Lambeth's formula to estimate J-M genetic correlation (LAMBETH 1980), for which there are several alternatives (e.g. WEI & LINDGREN 2001, GWAZE *et al.* 1997). It may be noted that the loss in efficiency is not very large if the selection age is anywhere between seven and 20 years. Thus, breeders who want to make a somewhat earlier selection (they may believe that Lambeth's J-M correlation seriously underestimates J-M correlation at young age or if they select for another character than at late age) may conclude that our results support selection at a young age. It seems also that it does not matter very much if selection is made some years later than the optimum.

Concluding remarks

For recurrent cycles of balanced within-family selection, the single-stage *Clone* strategy followed by the two-stage *Phenotype/Clone* strategy are the best choices to maximise GMG/Y, except for traits with high heritability, for which the single-stage *Phenotype* strategy is best.

There is no marked difference in GMG/Y between the single-stage *Clone* and the two-stage *Phenotype/Clone* strategies. Thus, the two-stage strategy would probably be advantageous if different characters, that are not strongly correlated, were selected at different stages: traits with high heritability and J-M correlation (growth rhythm) at the first, phenotypic selection stage and growth traits at the second selection stage, based on clonal tests.

For all reasonable values of genetic parameters, cost and time components, it is better to invest in the first-stage phenotypic selection, to utilise the time before the seeds for the progeny test are obtained, rather than in attempts to hasten the reproductive maturity of the candidates in the single-stage selection strategy based on progeny tests.

In the two-stage *Phenotype/Progeny* selection strategy, the optimum age for the first-stage phenotypic selection was 12 years. Therefore, to increase genetic gain per unit time, it would be beneficial to shorten the sexual maturity of the candidates to the age of circa 12 years.

In any second-stage selection method within a two-stage selection strategy, the first-stage phenotypic selection should always be more beneficial if it is based on traits with high heritability and high J-M correlation.

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