

Sustainability Robustness and Efficiency of a Multi-generation Breeding Strategy Based on Within-family Clonal Selection

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Abstract

Sustainability and robustness of genetic gain and diversity were analysed for breeding and production populations managed according to the Swedish breeding programme for Norway spruce. This strategy is based on double-pair mating and balanced, within-family selection with clonal testing within a number of reproductively isolated breeding populations. The importance of different characteristics was quantified by computer simulation, using a stochastic quantitative infinitesimal genetic model.

After ten generations, the linear increase in the additive genetic effect for the baseline scenario reached $11.0 \sigma_A$ and status effective number was reduced from 48 to 8.1 in a breeding population with 48 members. Six trees with status number 3.9 could be selected as seed orchard parents with a predicted inbreeding in the seed crop of less than 5% resulting in a total gain including inbreeding depression of $11.7 \sigma_A$. Meanwhile, available gain from a similar inbred clone mix of 6 genotypes was $13.1 \sigma_A$, mainly as a result of one more cycle of testing. Provided that populations had no fewer than 24 members, increases over ten generations in group coancestry, inbreeding and inbreeding depression were not substantial, and the drop in additive gain per generation was negligible. Reduction of additive variance and increased inbreeding had no apparent effect on accumulation of gain per generation, even after ten breeding cycles. Thus, it is concluded that the current programme is sustainable.

Clonal testing continued to be efficient throughout ten generations at population sizes down to 24, allowing a substructure of two sibilines within a population of 48 members. Non-additive variance reduced the rate of increase of the additive mean. A continuous accumulation of additive effects was more important than exploitation of non-additive variation, even when cloned planting stock is deployed. Clonal testing was highly effective and robust, even at low numbers of ramets and weak heritability. Low stochastic variation among replicate runs indicated high precision, thus predictions of the breeding programme outcome are reliable within the limits of the model.

Key words: additivity, dominance, epistasis, clonal testing, effective population size, inbreeding, genetic diversity, within-family selection.

FDC: 165.3; 165.441; 165.6; 232.13; 232.311.3; 174.7 *Picea abies*; (485).

Introduction

The purpose of long-term tree breeding is to achieve genetic gain on a sustainable basis in reforestation material through intensively selected production populations. Therefore, long-term breeding populations must be managed for both increased

genetic mean and sustained diversity. Initially, genetic diversity is determined by the sampling of alleles when the breeding population is founded and thereafter by the methods of breeding (mating and selection), natural processes within the population (genetic drift and mutation) and intentional or unintentional introduction of new genes (migration). A suitable diversity in the resulting forest stands (within and among stands) should be determined considering the influence of genotypic diversity on resource utilisation and ecological stability, and, in certain circumstances, the genetic diversity of the eventual natural regeneration.

Most long-term tree breeding programmes include strategies to balance gain and diversity by considering size, structure and management of breeding populations (COTTERILL, 1984; COTTERILL *et al.*, 1989; FOWLER, 1986; SHELBORNE *et al.*, 1986; KANG and NIENSTAEDT, 1987; MCKEAND and BRIDGWATER, 1992; WHITE, 1993; WHITE *et al.*, 1993; WILLIAMS and HAMRICK, 1996). Although recognising that diversity is a concern, most of them lack a clear long-term objective which could be operationally monitored as precisely as the accumulation of gain. The loss of gene diversity has been directly investigated by DNA markers in hierarchical and multiple population strategies as well as elite lines (WILLIAMS *et al.*, 1995), but these kinds of results have limited utility for detailed planning of breeding programmes. In simulation studies, KING and JOHNSON (1993) used inbreeding effective population size to describe diversity after five generations of breeding, and MAHALOVICH (1989) and BRIDGWATER *et al.* (1992) used inbreeding coefficient over 20 generations.

The loss of diversity by inbreeding and random genetic drift is reflected in both a loss of allelic variants in the population and a decline in heterozygosity of the genotypes. Decreased heterozygosity often results in reduced individual tree performance (lower average fitness due to inbreeding depression), whereas a lack of allelic variants inhibits long-term response to selection (FALCONER and MACKAY, 1996). Gene diversity is often measured as expected heterozygosity following random mating (NEI, 1973), i.e., the probability that two alleles sampled from the gene pool are different. It is the mean heterozygosity that would exist if the population were in HARDY-WEINBERG equilibrium. Gene diversity can also be measured by the numbers of different alleles (ALLENDORF, 1986). A common technique is to observe diversity by marker genes, but it is not possible to distinguish genes that are independently identical from those that are identical by descent. In this context, we consider only identity by recent descent as a criteria for gene identity; thus, in a population of non-inbred and non-related trees, all genes (all alleles) are seen as different.

The group coancestry of a population (Θ) (the probability that a pair of genes taken at random with replacement from the gene pool of the population are identical by descent) (COCKERHAM, 1967), describes the genetic similarity caused by relatedness, and can also be used as a measure of population diversity. The inbreeding coefficient (F) (the probability that the two homologous genes from an individual are identical by descent) is used as a corresponding measure on the genotype

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level. Θ and F can be calculated exactly from the pedigree and Θ in one generation becomes the expected F in its progeny if the population members mate at random and contribute uniformly to the gamete pool. For a population, the proportional loss of gene diversity (GD) from the founders may be expressed as $1-\Theta$ (NEI, 1973; WEIR, 1990, p. 127; LACY, 1995), where the gene diversity of the source population (or an infinite population of unrelated genotypes) from which the founders originate is 1.

Based on group coancestry, diversity can also be expressed as status effective number N_s (LINDGREN *et al.*, 1996; LINDGREN and MULLIN, 1998). A similar concept, founder genome equivalent (FGE), was developed by LACY (1995), who unfortunately defined FGE in two non-equivalent ways, only one of which is analogous to N_s . N_s and the analogous variant of FGE express the theoretically expected number of unrelated, non-inbred genotypes that would be required to provide the level of genetic diversity equivalent to that in the population under study. Both of these effective population sizes are relative to a reference population which is without coancestry between members and without inbreeding.

A strategy that minimises group coancestry will maximise the maintenance of gene (allele) diversity (BALLOU and LACY, 1995). In a population without substructure, diversity is maximised by equal founder contribution following balanced within-family selection which will minimise the increase in coancestry (BALLOU and LACY, 1995; LINDGREN *et al.*, 1996). Additive variance is the origin of long-term accumulated genetic improvement. Since half the initial additive variance is among families for initially non-inbred full-sib progeny, a balanced within-family selection procedure will accumulate gain more slowly, compared to a less restrictive strategy, although it will increase the long-term limit of selection (DEMPFLE, 1975). If individual family members are sexually propagated and breeding values estimated from their tested progeny, the genetic improvement from classical within-family selection in a long-term breeding population is much enhanced, compared to individual phenotypic selection (COTTERILL, 1984). Improvement can be even greater if candidates are vegetatively propagated and tested as clones (LIBBY, 1969; BURDON and SHELBOURNE, 1974; BURDON, 1986; SHELBOURNE, 1991; SHAW and HOOD, 1985; RUSSELL and LOO-DINKINS, 1993; MULLIN and PARK, 1992; FOSTER, 1986, 1992), and can be achieved faster, increasing the gain per unit time (SHELBOURNE, 1991; MATHESON and LINDGREN, 1985).

The objective for a long-term breeding programme can be to maximise the combined value of genetic improvement and diversity (expressed in the same units as gain) of the population (LINDGREN and MULLIN, 1997). A concern for sustainable long-term breeding can be emphasised by giving the diversity component a high value when the optimal balance between genetic improvement and diversity is sought.

Computer simulation is a technique suitable for comparing alternative breeding strategies and to analyse management procedures in complex breeding programmes over many generations. Stochastic simulations can be much closer to reality than predictions by exact formula, since no formulae are usually available for realistic complicated situations. A breeding scenario can be repeated a sufficient number of times to estimate the central tendency of parameters for a real-life breeding programme which is itself only carried out once, but which is subject to stochastic variation in actual outcome. Computer simulations are frequently used in theoretical and evolutionary genetics, and in applied animal breeding, and have recently also been used for evaluating forest tree breeding strategies (MAHALOVICH, 1989; BRIDGWATER *et al.*, 1993; KING and JOHNSON, 1993).

The objective of this study was to analyse sustainability, robustness and efficiency of the breeding strategy used in the Swedish Norway spruce programme, which relies on clonal testing and within-family selection. The focus was on sensitivity to population size and structure, test accuracy, and variation in genetic parameters that cannot be controlled by the breeder.

Material and Methods

The Swedish breeding strategy

The Swedish long-term tree breeding programmes are described in general by DANELL (1991a and b, 1993a and b). For adequate conservation of alleles, to achieve long-term adaptation, and to improve general-purpose goals for Norway spruce and Scots pine, a multiple breeding population strategy is applied (BURDON and NAMKOONG, 1983). A meta-population of more than 1000 founder trees is subdivided into 20 or more unrelated breeding populations each of size 50, and each adapted to a particular range of photoperiod and temperature. Scandinavia has a harsh climate with steep environmental gradients causing genotype-by-environment interactions, and preventing long distance transfer of adapted material. The population sizes were chosen to keep the probability of losing "rare" alleles (<1%) low in the metapopulation, and of losing "common" alleles (5% to 10%) low in each of the breeding populations, and their inbreeding effective population size should remain above 50 (DANELL, 1993a and b). Thus, for the main reforestation species, the Swedish breeding programme has the objectives of both economic improvement and dynamic gene conservation for an uncertain future, while considering evolutionary potential (NAMKOONG, 1980, 1984; ERIKSSON *et al.*, 1993). The breeding programme intends to promote a cost-benefit balance between high testing accuracy and high selection intensity. The current management plan for breeding of Norway spruce in Sweden is summarised in table 1 (KARLSSON and ROSVALL, 1993). Double-pair mating, clonal testing, and balanced within-family selection are characteristic features of the plan.

Simulation model

A stochastic simulation tool POPSIM (MULLIN and PARK, 1995) based on a quantitative infinitesimal model was modified to more closely simulate certain features of the operational breeding plan in Sweden. In the model, each genotype is considered to be the sum of independent genetic and environmental effects, and the total phenotypic variation in a population is described as the sum of independent variances for each of these effects. The additive effect for an individual is generated by:

$$A'_i = \frac{A_f + A_m}{2} + r_i \sqrt{\frac{1 - 0.5(F_f + F_m)}{2}} \sigma_A^2 \quad (1)$$

where A'_i is the additive effect for progeny i ; A_f and A_m are the additive effects of the female and male parents, respectively; F_f and F_m are the corresponding coefficients of inbreeding; σ_A^2 is the additive variance and r is a normal random deviate. The dominance effect for an individual is generated as:

$$D'_i = r_{fm} \sqrt{\frac{1}{4}} \sigma_D^2 + r_i \sqrt{\frac{3(1 - 0.5(F_f + F_m))}{4}} \sigma_D^2 + bF_{fm} \sigma_P \quad (2)$$

where σ_D^2 is the dominance variance; r_{fm} and r_i are random normal deviates for each family (fm) and individual (i), respectively. Inbreeding depression was simulated by reducing the

Table 1. – Schedule for the Swedish Norway spruce baseline breeding programme (modified from KARLSSON and ROSVALL, 1993).

Starting year	Management action	Size	State of breeding
			F0 founder population with tested plus trees and clones
0	Selection	50	F0 breeding population
1	Controlled crosses 2 families/parent	5 000	F1 progeny
4	Screening in nursery Cloning	2 000	F1 clones 40 clones/family, 14 ramets/clone
6	Establishment of clonal-family tests	28 000	stecklings on 4 test sites plus a breeding archive
15	Evaluation and selection	50	selected clones, F1 breeding population
15-20	Controlled crosses 2 families/parent clone	5000	F2 progeny
			and so on

dominance effect D_i by the regression of inbreeding depression on the inbreeding coefficient of the family members F_{fm} , where the regression coefficient b expresses the reduction in phenotypic value in units of phenotypic standard deviation, σ_p , for the trait in the unselected base population. As a simplification the epistatic variance σ^2_I and effects I_i and were considered to be within-family:

$$I'_i = r\sqrt{\sigma^2_I} \quad (3)$$

The environmental effect E'_{ik} for the k th ramet of the i th genotype assumes that the environmental variance σ^2_E is constant in all generations:

$$E'_{ik} = r\sqrt{\sigma^2_E} \quad (4)$$

For each of the genetic and environmental effects, estimates of mean and variance are calculated from the generated data for the N trees in the breeding population. Group coancestry and inbreeding are calculated from the pedigree. Status number N_s is an effective number calculated for each generation t from group coancestry Θ_t (LINDGREN *et al.*, 1996):

$$N_{s_t} = \frac{1}{2\Theta_t} \quad (5)$$

Inbreeding effective population size, N_e , was calculated from the rate at which inbreeding accumulates ΔF (FALCONER and MACKAY, 1996):

$$N_e = \frac{1}{2\Delta F} \quad (6)$$

By repeating the simulation of a given scenario 100 times, both the stochastic variation in expected genetic effects and variances of a single scenario and the average outcome of repeated scenarios was analysed by the standard deviation (sd) and the standard error ($sd/n^{1/2}$) respectively.

Simulations of the Swedish Norway spruce breeding programme

The Swedish Norway spruce breeding programme was first simulated according to the original management plan (KARLSSON and ROSVALL, 1993) (Table 1), although both the initial selection from the founder population and the nursery screening at each generation shift was omitted from the simulations. Clonal testing of candidate trees was applied for both the advanced-generation breeding population and for selection of clones for the production population. In the baseline strategy, 40 clones per family were each tested using 14 ramets corresponding to a test size of 560 planted trees per breeding population member, totalling 26 880 test plants per generation (Table 1). Testing was assumed to use single-tree plots (no environmental covariances) in a single constant environment (no $G \times E$ interaction), and no variances associated with cloning (no C -effects) were considered. The genetic parameters were chosen to represent Swedish conditions based on experience (DANELLE, 1991b). The breeding objective was regarded as an index of economic merit assuming no changes in genetic correlations among the components (infinitesimal model). The measurement unit is the initial standard deviation of additive genetic effects, $\sigma_A = V_{A-initial}^{1/2}$, which is also applied to non-additive genetic effects.

A new generation of trees was created and selected in exactly the same way in all scenarios by non-assortative, double-pair mating among the breeding population and by balanced selection within families, i.e., the best clone was selected from each and every test family. A combined index (CI) where family and individual clone values are weighted by their respective heritabilities was calculated according to MULLIN and PARK (1995) for each progeny and used as a selection criterion for selecting seed orchard populations as a 6-tree subset of the breeding population. Selection of a 6-clone mix was based on clone means for progeny of the breeding population. The selected clones thus belong to the same generation as the progeny of the seed orchard. Selection of the six genotypes for the seed

orchard or clone mix was restricted to be as unrelated as possible (i.e., no parent in common) to determine what maximum level of diversity could be obtained.

Regression coefficients for inbreeding depression on inbreeding coefficient (F) were chosen to generate 0.5%, 1.0% and 2.0% inbreeding depression for each percentage point increase in inbreeding at additive variance coefficient 0.1, irrespective of the phenotypic variance or heritability chosen. A 20% to 50% reduction in early height growth plus increased mortality due to inbreeding depression after selfing is typical for Norway spruce (ANDERSSON *et al.*, 1974; ERIKSSON *et al.*, 1973; SKRÖPPA, 1996) and related species (WILLIAMS and SAVOLAINEN, 1996). Considering the economic merit including fitness, an intermediate depression rate (1%) was chosen to be most appropriate as a standard rate of long-term inbreeding depression in most simulation runs.

The influence of (1) population size and structure, (2) inbreeding depression, (3) genetic variance components and heritability, and (4) test accuracy on genetic mean and diversity in both the breeding and production populations were analysed by changing the input variables one at a time. The trade-offs between the number of clones and ramets were analysed at different total test sizes and heritabilities for a fixed number of families. The main settings are given in table 2.

Table 2. – Parameters used for simulation of the Swedish Norway spruce breeding programme according to the baseline strategy and alternative scenarios.

Parameters	Baseline strategy	Alternative scenarios
<i>Structure of base population at generation 0</i>		
Number of trees, N	48	(8–144)
Number of sublines	1	(1–6)
Standard rate of inbreeding depression at CVA 10%	0%	(0–2%)
Additive variance, σ_A^2	100	
Dominance variance, σ_D^2	25	(0–100)
Epistatic variance, σ_i^2	0	(0–100)
Environmental variance, σ_E^2	375	(0–9875)
Narrow-sense heritability, h^2	0.2	(0.01–0.8)
Broad-sense heritability, H^2	0.25	(0.0125–1.0)
<i>Clone test structure</i>		
Total test size per full sib family	560	(70–2000)
Number of clone x number of ramets per full sib family	40x14	(1x2000–2000x1)

Results

Number of replicate simulations

Replicating the simulation 100 times gave stable repeatable mean values and standard deviations expressing the expected variation for a single breeding scenario. It was also sufficient to identify significant differences, since the standard errors of the mean were small compared to differences among alternatives of possible practical importance.

Development of the baseline breeding population

Genetic parameters after ten generations for a breeding population with 48 founders according to the baseline Norway spruce breeding plan and corresponding seed orchard and clone mix of size 6 are given in table 3, under an assumption of no inbreeding depression. The development over generations is illustrated in figure 1 and table 5. The increase in additive effects (A_{eff}) in the breeding population was essentially linear, and approximately $1.1 \sigma_A$ per generation ($\sigma_A = V_{A-initial}^{1/2}$) over ten generations of breeding (Figure 1a). Most of the almost

50% loss of additive variance (V_A) among the trees in the breeding population occurred during the first three generations (Figure 1a). The dominance effect (D_{eff}) remained fairly constant at about $0.4 \sigma_A$ and the dominance variance (V_D) did not change over generations (Table 3, Figure 6).

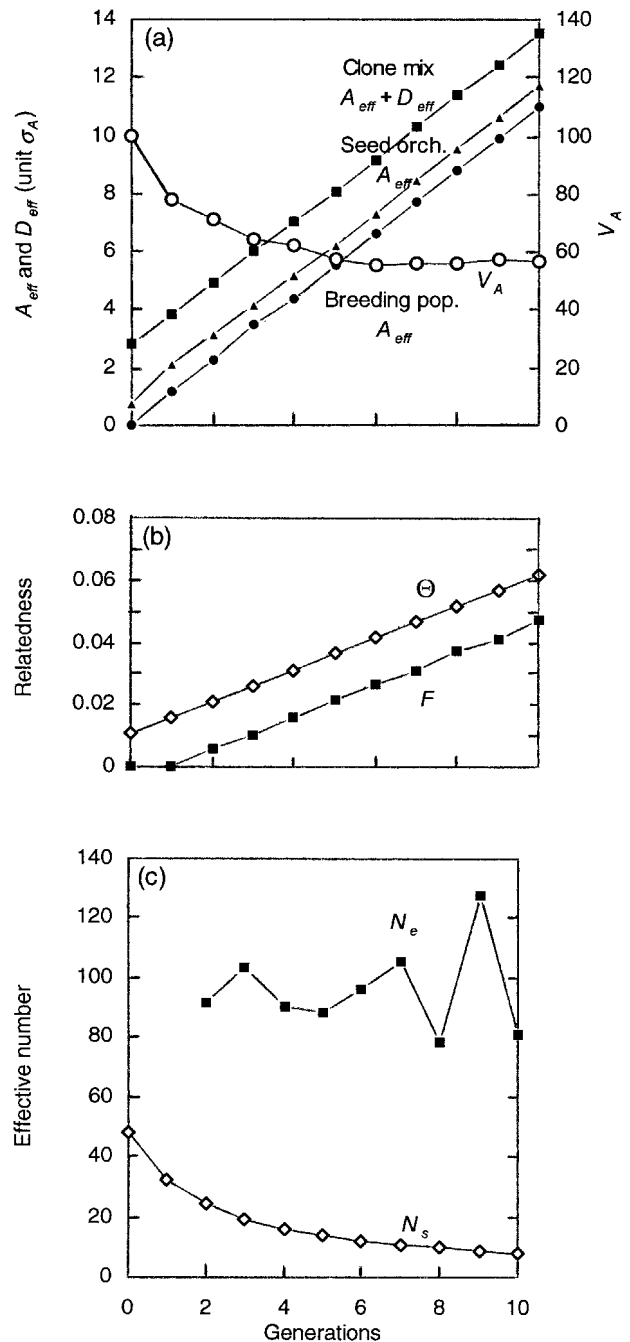


Figure 1. – Development over ten generations of (a) additive effect (A_{eff}) and variance (V_A) in the breeding population and genetic gain from a seed orchard (A_{eff}) and clone mix ($A_{eff} + D_{eff}$) of six clones respectively, (b) group coancestry (Θ) and inbreeding coefficient (F) and (c) corresponding status number (N_s) and effective population size (N_e) according to the baseline Norway spruce breeding strategy with population size $N = 48$ and no inbreeding depression.

Group coancestry (Θ) increased from 0.010 to 0.062 after ten generations and the inbreeding coefficient (F) reached 0.047 (Table 5, Figure 1b). By excluding selfing from the mating design, and with restrictions on the relatedness of selections, F reached Θ after approximately three generations instead of

Table 3. – Genetic effects (A_{eff} , D_{eff}) in units of σ_A ; variances (V_A , V_D); group coancestry (Θ); status number (N_s) and inbreeding coefficient (F), after ten generations in the breeding and production populations according to the baseline Norway spruce breeding strategy, assuming no inbreeding depression. Inbreeding coefficient in the seed orchard progeny (F_{prog}) with self pollination excluded. Standard deviations are for 100 iterations.

	Breeding population (48 genotypes)		Seed orchard (6 clones)		Clone mix (6 clones)	
	Mean	sd/mean (%)	Mean	sd/mean (%)	Mean	sd/mean (%)
Additive effect, A_{eff}	11.0	2.5	11.7	3.0	12.9	3.0
Additive variance, V_A	57	21.8	38	71.2	34	72.4
Dominance effect, D_{eff}	0.4	14.8	0.6	31.6	0.6	32.2
Dominance variance, V_D	22	19.6	20	55.3	20	65.4
Additive + Dominance effect, $A_{eff} + D_{eff}$					13.5	
Average coancestry, Θ	0.062		0.127		0.139	
Status number, N_s	8.1	0.09	3.9	2.1	3.6	4.7
Inbreeding coefficient, F	0.047	7.7	0.050	26.5	0.053	25.0
Inbreeding coefficient in progeny, F_{prog}			0.048			

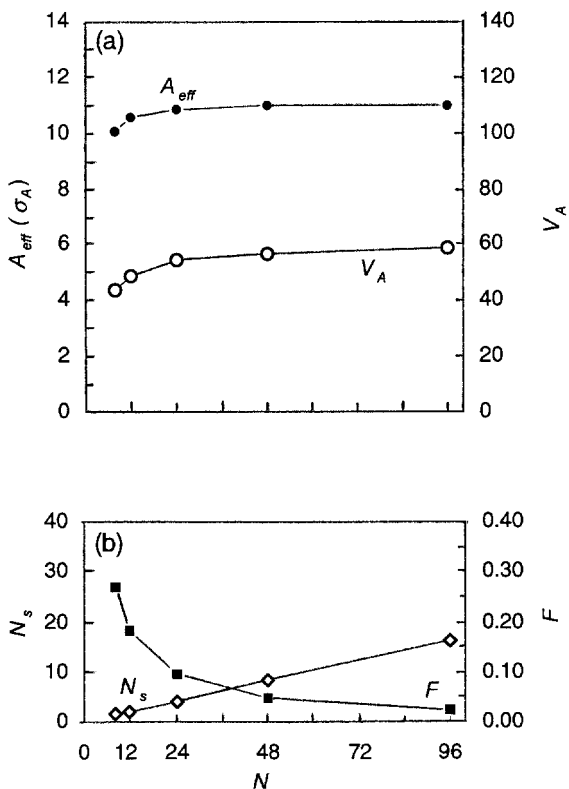


Figure 2. – Effect of population size (N) on (a) additive effect (A_{eff}) and variance (V_A) and (b) status number (N_s) and inbreeding coefficient (F) in the breeding population.

one, which is expected after random mating (Figure 1b). The Θ of 0.062 after ten generations resulted in a reduction of status number (N_s) from 48 to 8.1 (Table 3, Figure 2c) and a corresponding expected 6.2 % loss of initial gene diversity (GD) (Figure 3). Inbreeding effective population size (N_e) is not defined until inbreeding first occurred at generation two (Figure 1c). N_e then varied according to the overall increase in F , around an average value of 95.7 (Figure 1c, Table 5). Theoretically, the value of N_e should approach $96 - 1$ over a large number of generations (FALCONER and MACKAY, 1996).

Baseline production populations

The extra additive gain from the six seed orchard genotypes compared to the average of all 48 trees in the breeding popula-

tion was $0.96\sigma_A$ in the first generation, and decreased to 0.74 in the tenth generation due to the reduced V_A of the breeding population and the restrictions on relatedness applied (Table 3, Figure 1a). The extra additive gain in a mixture of six clones was $2.1\sigma_A$ initially and decreased to 1.9 after ten generations. The clone mixture also captured a dominance effect varying between 0.59 and $0.64\sigma_A$ over generations (assuming no inbreeding depression) (Figure 1a).

After ten generations, the status number (N_s) of the six orchard trees was 3.9, compared to 8.1 for the 48 trees in the breeding population (Table 3). The average F for the six orchard trees and the six-tree clone mix was 0.050 and 0.053, respectively, compared to 0.047 in the breeding population. If random mating, including selfing, with equal gamete contributions is assumed, the average inbreeding coefficient of the orchard progeny (F_{prog}) will be 0.127 at generation ten. However, if it is assumed that no selfing occurs in the seed orchard (as for controlled pollination), F_{prog} will be 0.048, which is close to the inbreeding of the breeding population (Table 3).

Population size and structure

Considering alternative numbers of trees in the breeding population (N) from 8 to 96, there was a significant increase up

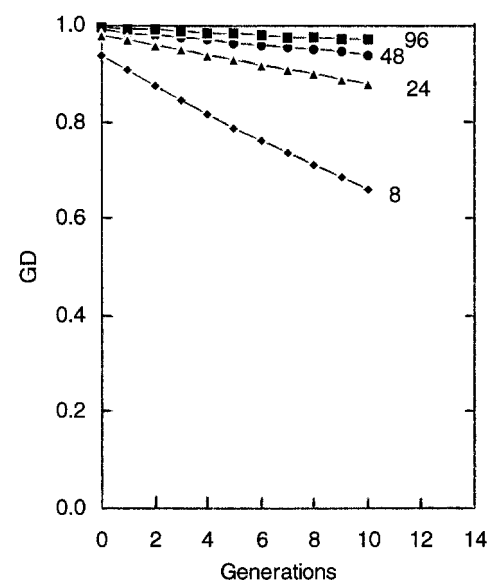


Figure 3. – Proportional gene diversity ($GD = 1 - \Theta$) over ten generations of breeding at population sizes (N) kept at 8, 24, 48 and 96 (the same number of founders).

Table 4. – Influence of population size and structure (number of sublimes and founders) on additive and dominance effect (A_{eff} , D_{eff}) in units of σ_A ; variances (V_A , V_D); inbreeding coefficient (F); group coancestry (Θ) and status number (N_s) after ten generations in the breeding and corresponding production populations. At the 1x48 + 3x48 scenario, 6 + 2x3 clones were selected. Inbreeding coefficient in the seed orchard progeny (F_{prog}) is with self pollination excluded. Standard deviations are for 100 iterations.

Population structure and size	Mean value in different populations															
	Breeding population (48 genotypes)								Seed orchard (6 clones)				Clone mix (6 clones)			
	A_{eff}	sd (%)	V_A	D_{eff}	V_D	F	Θ	N_s	A_{eff}	F	F_{prog}	N_s	A_{eff}	D_{eff}	F	N_s
1x8	10.1	7.0	44	0.3	19	0.271	0.339	1.5	10.2	0.269	0.298	1.4	11.1	0.4	0.296	1.3
1x24	10.8	3.7	55	0.4	22	0.094	0.121	4.1	11.3	0.095	0.094	2.9	12.5	0.6	0.105	2.6
1x48	11.0	2.5	57	0.4	22	0.047	0.062	8.1	11.7	0.050	0.048	3.9	12.9	0.6	0.053	3.6
1x96	11.0	1.8	59	0.4	22	0.023	0.031	16.1	11.9	0.024	0.024	4.7	13.1	0.7	0.027	4.5
6x8	10.2	2.6	77	0.3	19	0.272	0.056	8.9	10.8	0.275	0.000	4.7	11.7	0.5	0.297	4.6
2x24	10.8	2.5	60	0.4	21	0.093	0.061	8.3	11.5	0.094	0.038	4.1	12.6	0.6	0.104	3.8
3x48	10.9	1.5	61	0.4	21	0.047	0.021	24.4	11.9	0.047	0.010	5.2	13.1	0.7	0.051	5.1
1x48 + 3x48									11.8		0.013	9.9				

Table 5. – Development of inbreeding coefficient (F); effective population size (N_e) (not defined for generation 0 and 1); group coancestry (Θ) and status number (N_s) during ten generations at different breeding population sizes (N).

Generation	Breeding populations size (N)															
	8				24				48				96			
	F	N_e	Θ	N_s	F	N_e	Θ	N_s	F	N_e	Θ	N_s	F	N_e	Θ	N_s
0	0.000		0.063	8.0	0.000		0.021	24.0	0.000		0.010	48.0	0.000		0.005	96.0
1	0.000		0.094	5.3	0.000		0.031	16.0	0.000		0.016	32.0	0.000		0.008	64.0
2	0.035	14.2	0.125	4.0	0.011	46.4	0.042	12.0	0.005	91.4	0.021	24.0	0.003	185.5	0.010	48.0
3	0.069	14.3	0.155	3.2	0.021	48.8	0.052	9.6	0.010	103.5	0.026	19.2	0.006	167.6	0.013	38.4
4	0.096	17.5	0.184	2.7	0.032	43.4	0.062	8.0	0.016	90.2	0.031	16.0	0.008	226.0	0.016	32.0
5	0.133	12.1	0.213	2.4	0.043	45.7	0.072	6.9	0.021	88.4	0.036	13.8	0.011	180.4	0.018	27.5
6	0.164	14.2	0.240	2.1	0.053	46.3	0.082	6.1	0.026	95.8	0.041	12.1	0.013	216.5	0.021	24.1
7	0.192	14.7	0.266	1.9	0.063	46.9	0.092	5.4	0.031	105.3	0.046	10.8	0.016	168.0	0.023	21.4
8	0.219	15.1	0.291	1.7	0.072	50.7	0.102	4.9	0.037	78.2	0.052	9.7	0.018	192.1	0.026	19.3
9	0.246	14.5	0.315	1.6	0.082	46.2	0.112	4.5	0.041	127.7	0.057	8.8	0.021	193.3	0.028	17.6
10	0.271	15.1	0.339	1.5	0.094	40.7	0.121	4.1	0.047	80.9	0.062	8.1	0.023	207.3	0.031	16.1
Average		14.6				46.1				95.7				193.0		

to $N = 24$ for A_{eff} and D_{eff} , V_A and V_D after ten generations (assuming no inbreeding depression) but for N larger than 24 the increase was small (Table 4, Figure 2). N_s increased linearly with N from 1.5 to 16.1 (Table 4, Figure 2), while Θ was roughly proportional to $1/N$, corresponding to an expected loss of GD of 33.9% and 3.1% when N is 8 and 96, respectively (Table 4, Figure 3). For N equal to 8 and 24, it took 1 and 8 generations, respectively, to lose about 10 % of the initial GD (Figure 3). The average F was much larger at smaller N , decreasing from 0.271 to 0.023 (Table 4, Figure 2) for a change in N from 8 to 96 (almost proportional to $1/N$, with a time delay due to selection restrictions on relatedness). For N equal to 24 and 48, the balanced within-family selection procedure accumulated inbreeding per generation by approximately 0.01 and 0.005 respectively (Table 5). For N is 8, 24 and 48, $F = 0.050$ was reached after 2, 5 and 10 generations, respectively, and for N is 8 and 24, $F = 0.10$ was reached after 4 and 10 generations.

Each individual subline in a subdivided breeding population (BURDON and NAMKOONG, 1983) will be equivalent to a single breeding population of similar size (Table 4). For subline sizes 8 to 48, a linear relationship was found between the number of sublimes and the performance of the aggregated population.

With an increase in the number of sublimes, genetic effects decreased and V_A increased considerably (Table 4, Figure 4a); the average F increased markedly while Θ decreased and N_s increased slightly (Table 4, Figure 4c). N_s is expected to remain larger in a subdivided population (LINDGREN *et al.*, 1996) but the effect is not substantial unless the sublimes are very small. In contrast, when 3 adjacent breeding populations were considered together as an aggregate population of 144 trees (run as a single scenario, i.e., 3 sublimes of size 48), there was less increase in V_A and change in mean effects. Θ was 0.021, indicating three times the N_s of one population and a 2 % loss of GD (Table 4).

The larger A_{eff} in the production populations with increased size of the breeding population (Table 4) was explained primarily by increased selection intensity when a constant number of 6 trees are selected. The greater V_A maintained by larger population size acted in the same direction, but had little influence on the gain. The production populations derived from structured breeding populations followed the same linear pattern as the breeding populations, with increased inbreeding and reduced gain as the number of sublimes increased (Table 4, Figure 4b and d). Although inbred ($F = 0.275$), the 6 seed orchard trees

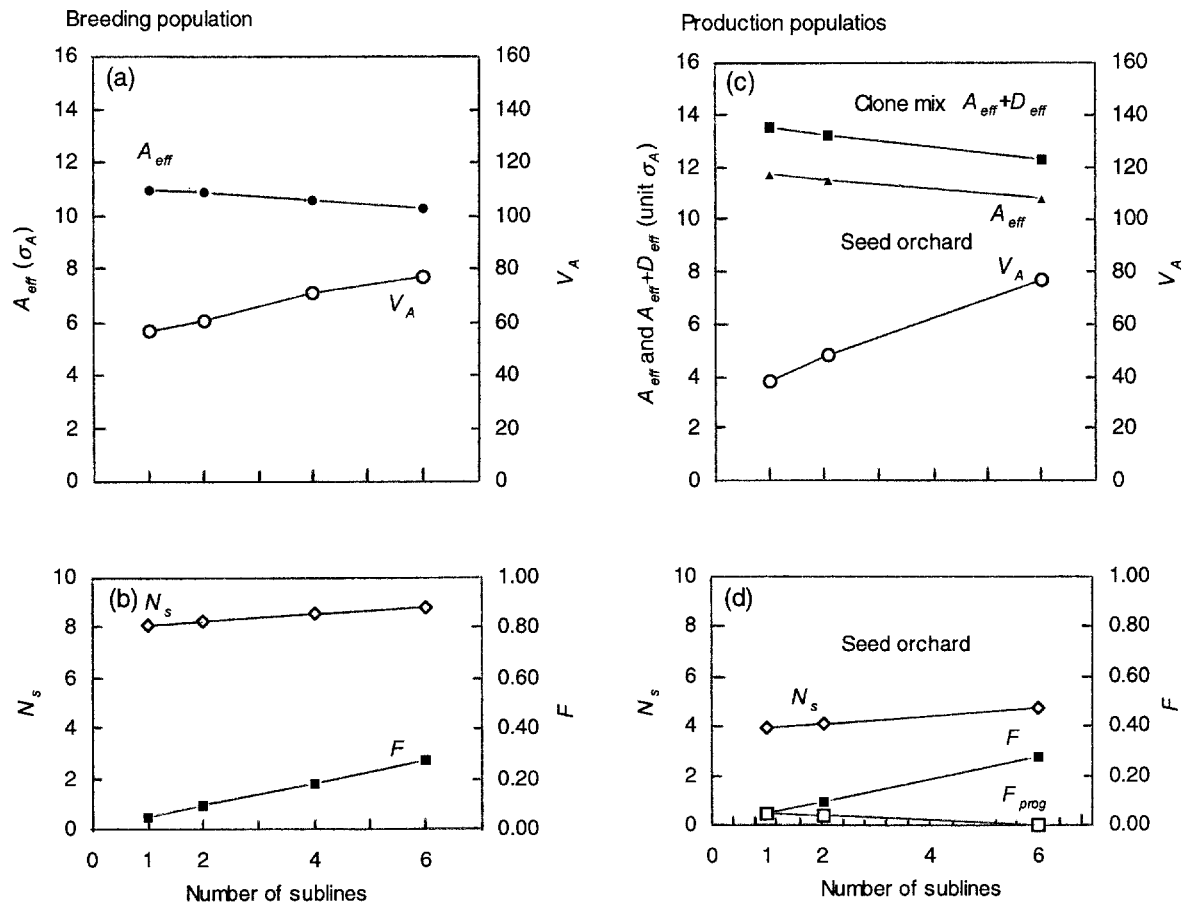


Figure 4. – Effect of population structure (number of sublimes) at total population size $N = 48$ on (a) additive effect (A_{eff}) and variance (V_A) and (b) status number (N_s) and inbreeding coefficient (F) in the breeding population and (c) additive gain (A_{eff}) and variance (V_A) in the seed orchard and total genetic gain ($D_{eff} + A_{eff}$), in the clone mix and (d) status number (N_s) and inbreeding coefficient (F) in the seed orchard and inbreeding coefficient (F_{prog}) in the seed orchard progeny if self-pollination is avoided.

from 6 sublimes of 8 trees were unrelated with $N_s = 4.7$, instead of 3.9. With no self-pollination in the orchard, the F_{prog} will be zero as compared to 0.048 for the seed orchard progeny of 6 related seed orchard parents from an unstructured breeding population of 48 trees. For the six-sublime alternative, F of the 6 clones in the clone mix had risen from 0.053 to 0.297, which would likely cause too much inbreeding depression for clonal testing or deployment (Table 4). However, in a structured population with 2 sublimes of size 24, F was kept below 0.10 for 10 generations, which should be low enough to retain clonal options.

Inbreeding depression

After ten generations, inbreeding depression (expressed in the model as a negative component of dominance) remained small for population sizes 48 and 96, even at the higher depression rate (2% per 0.01 F), but increased exponentially at smaller population sizes (Figure 5). The putative realistic depression rate of 1% per 0.01 F reduced the mean significantly only for breeding populations of size 8 and 24 trees (Figure 5). At this depression rate in the baseline scenario ($N = 48$) and the ratio of initial additive to dominance variance of 100/25, the resulting D_{eff} was positive, despite the effects of inbreeding depression, until generation 9 ($F = 0.041$, Table 5). Thereafter the total D_{eff} became negative in generation ten ($D_{eff} = -0.1 \sigma_A$, Table 6 and $F = 0.047$, Table 5). For the baseline alternative V_D increased from 25 in the initial population to 29 at generation

ten at the 1% rate of inbreeding depression (Table 6, Figure 6). Higher depression rates increased V_D more.

In this simulation, all individuals within a family were regarded as being inbred at all loci to a degree identical to the family average by pedigree analysis. Ranking within families

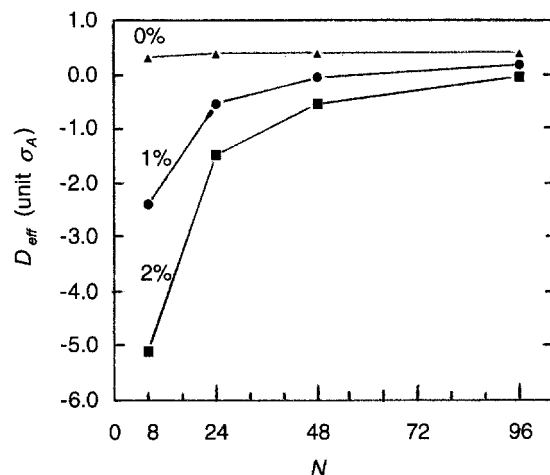


Figure 5. – Inbreeding depression after ten generations at various population sizes (N) and inbreeding depression rates 0.0%, 1.0%, and 2.0% per 0.01 F (different regression coefficients of F on inbreeding depression).

Table 6 – Influence of environmental (V_E), dominance (V_D) and epistatic (V_I) variance components on additive, dominance and epistatic effects (A_{eff} , D_{eff} , I_{eff}) in units of σ_A ; variances (V_A , V_D , V_I); inbreeding coefficient (F) and status number (N_s) after ten generations. Initial V_A was kept at 100 and inbreeding depression rate corresponded to 1 % per 0.01 F at CV 10 %. F and N_s in the breeding populations reached 0.047 and 8.1 respectively for all scenarios.

Individual variance components					Mean value in different populations														
V_D	V_I	V_E	h^2	H^2	Breeding population (48 genotypes)						Seed orchard (6 clones)			Clone mix (6 clones)					
					A_{eff}	V_A	D_{eff}	V_D	I_{eff}	V_I	A_{eff}	F	N_s	A_{eff}	D_{eff}	I_{eff}	F	N_s	
25	0	9875	0.01	0.0125	3.8	91	-0.3	32				4.6	0.039	3.9	4.7	-0.2	0.047	3.7	
25	0	1875	0.05	0.0625	7.5	78	-0.2	31				8.3	0.038	3.9	9.0	0.0	0.045	3.6	
25	0	875	0.10	0.1250	9.3	67	-0.1	30				10.0	0.039	4.0	11.1	0.1	0.044	3.6	
25 ¹⁾	0	375	0.20	0.25	11.0	57	-0.1	29				11.7	0.038	3.9	12.9	0.2	0.044	3.6	
25	0	125	0.40	0.50	12.1	48	0.0	29				12.8	0.037	3.9	14.1	0.3	0.044	3.6	
25	0	0	0.80	1.00	12.9	42	0.0	29				13.6	0.037	4.0	14.9	0.3	0.043	3.6	
0	0	0	1.00	1.00	15.2	22	-0.5	8				15.8	0.036	3.9	17.4	-0.4	0.043	3.6	
0	0	400	0.20	0.20	12.1	50	-0.5	8				12.8	0.036	3.9	14.1	-0.4	0.044	3.6	
50	0	350	0.20	0.30	10.1	61	0.3	47				10.8	0.039	4.0	11.9	0.7	0.044	3.6	
100	0	300	0.20	0.40	8.8	67	0.8	77				9.5	0.039	4.0	10.4	1.5	0.044	3.6	
0	25	375	0.20	0.25	10.6	59	-0.5	8	0.5	20		11.4	0.036	3.9	12.5	-0.4	0.7	0.044	3.6
0	50	350	0.20	0.30	9.6	66	-0.5	8	0.9	34		10.4	0.037	3.9	11.4	-0.4	1.2	0.044	3.6
0	100	300	0.20	0.40	8.2	74	-0.5	8	1.6	52		9.0	0.037	3.9	9.8	-0.4	2.1	0.044	3.6

¹⁾ Baseline scenario

was thus not affected by inbreeding depression. Therefore, in the breeding population, inbreeding depression only affected the dominance mean effect and variance, while all other parameters remained unchanged. In the production populations, where selection was permitted among families, inbreeding depression encouraged selection of trees with less inbreeding, resulting in reduced F and slightly reduced Θ , and increased N_s . For the baseline programme, the reduced F from 0.050 to 0.038 for the seed orchard and from 0.053 to 0.044 for the clone mix can be seen by comparing table 6 with table 3.

Environmental variance and heritability

Increased narrow-sense heritability, h^2 , (in this case, constant additive variance and decreased environmental variance) improved genetic effects and reduced genetic variances in the breeding population after ten generations (Table 6 and Figure 7). With clonal testing, selection response was close to maximum at $h^2 = 0.2$. At higher h^2 , a slightly lower F in the production populations and slightly greater D_{eff} in the clone mix also reflected more effective selection against inbreeding depression (Table 6).

Dominance and epistasis variances

Non-additive variances will bias the prediction of additive effects (breeding values) from clonal testing since some of the total genotype will be caused by these non-additive effects, including C-effects due to the propagation method (LIBBY and JUND, 1962; BURDON and SHELBORNE, 1974). When the initial ratio of additive to dominance variance was changed from 100/25 to 100/100, the A_{eff} after ten generations of breeding decreased from 12.1 to 8.8 σ_A (Table 6) and, in parallel, V_A increased somewhat. The influence of epistatic variance (V_I) on the additive components was similar to that of V_D but slightly stronger, since V_I was assumed to be totally within families, reducing A_{eff} more and V_A less (Table 6).

The reduced A_{eff} of the breeding population when non-additive variances were increased was also reflected in reduced

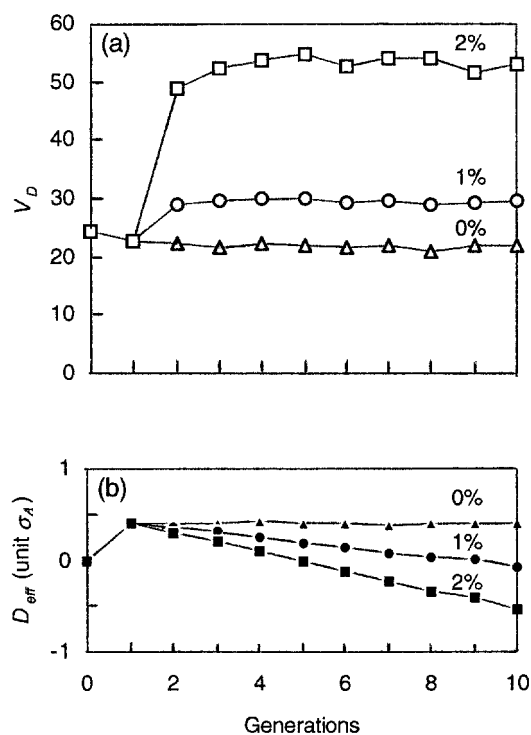


Figure 6. – Development over ten generations of (a) dominance variance (V_D) and (b) dominance effect including inbreeding depression (D_{eff}) in the breeding population at inbreeding depression rates 0.0%, 1.0%, and 2.0% per 0.01 F (different regression coefficients of F on inbreeding depression) according to the baseline strategy ($N = 48$).

additive gain from the seed orchard and clone mix (Table 6). In the clone mix, when initial V_D increased from 0 to 100, D_{eff} increased from $-0.4 \sigma_A$ (due solely to inbreeding depression) to 1.5, but the total gain ($A_{eff} + D_{eff}$) decreased from 13.7 to 12.0 σ_A . If gain from clone mixes at additive to dominance ratios

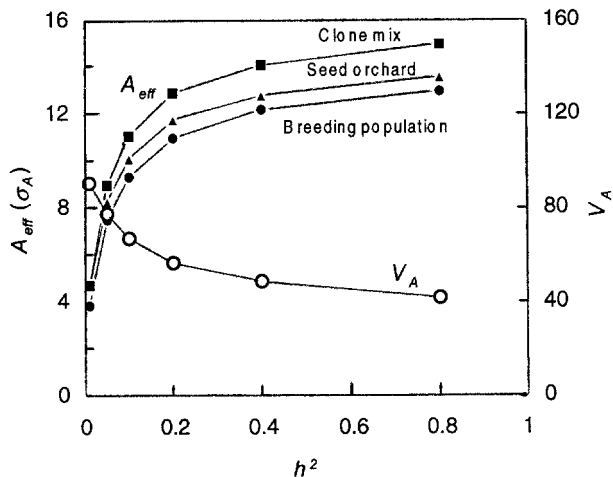


Figure 7. – Influence of initial heritability (h^2) on additive effect (A_{eff}) and variance (V_A) in the breeding population and on additive gain (A_{eff}) in the production populations according to the baseline breeding strategy ($N = 48$) and inbreeding depression rate 1.0%.

100/25 and 100/100 were compared over generations, the break-even point for total genetic effect occurred after 5 generations. After 7 generations, the additive gain of the 100/25 scenario alone reached the total gain of the 100/100 scenario (Figure 8). There were very small differences in N_s and F with altered genetic variance components in the production populations (Table 6), as expected when selection in the breeding populations is restricted to exactly 2 per family.

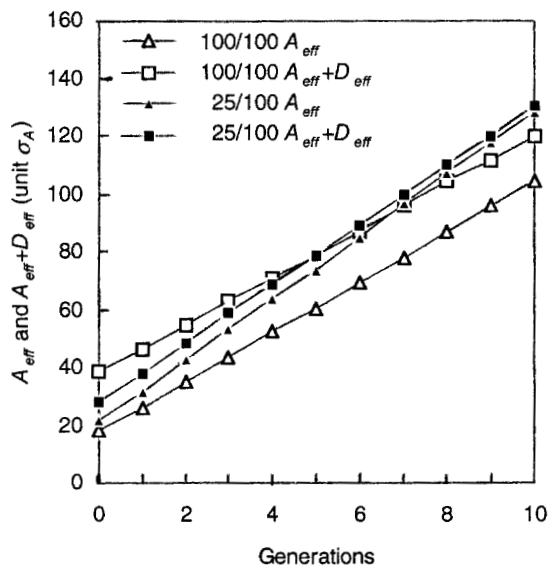


Figure 8. – Development over generations of additive (A_{eff}) and total genetic effect ($A_{eff} + D_{eff}$) in a clone mixture at dominance to additive variance ratios (V_D/V_A) 25/100 and 100/100 according to the baseline breeding strategy ($N = 48$) and inbreeding depression rate 1.0%.

Testing and evaluation method

The trade-off between precision (number of ramets per clone) and selection intensity (number of clones) was examined at different levels of resources (total number of trees tested) (Figure 9). When total test size was increased from 140 to 2240 stecklings per family at $h^2 = 0.2$, the optimal number of ramets was fairly stable in the interval 7 to 16 ramets per clone. However, the optimal number of clones tested per family which

maximises the A_{eff} after ten generations increased considerably, from 20 to 140 (Figure 9). Doubling the total test size suggested by the baseline strategy (560 to 1120 stecklings per family) increased gain by 9.7%, but this will increase the total cost considerably. For the baseline test size, the optimal number of tested clones per family increased with heritability from 20 clones at $h^2 = 0.05$ to 140 at $h^2 = 0.40$, with corresponding ramet numbers decreasing from 28 to 4 (Figure 10). This gave 6% to 7% more gain than with the baseline alternative of 40 clones and 14 ramets, and thus the ramet number is rather insensitive to differences in heritability. In general, the test composition gave similar results for both additive and dominance effects so the same combination of clone and ramet numbers are suitable also for seed orchard and clone mix production populations (data not shown). From figures 9 and 10 it can also be seen that, at the baseline test size of 560 test plants per family, clonal testing accumulated $3.8 \sigma_A$ (+ 53 %) more A_{eff} after ten generations compared to individual phenotypic selection (number of ramets = 1) and that the difference in A_{eff} increased with larger total test size and smaller h^2 .

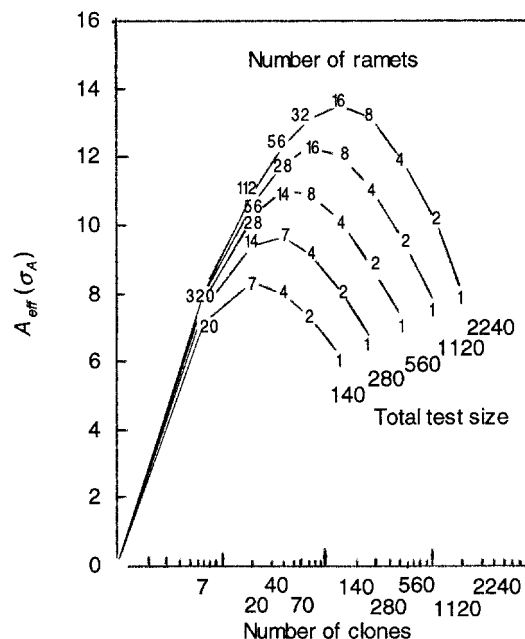


Figure 9. – Influence of distribution of clone and ramet numbers at total test sizes 140–2240 on additive effect (A_{eff}) in the breeding population after ten generations of breeding according to the baseline breeding strategy ($N = 48$) and inbreeding depression rate 1.0%.

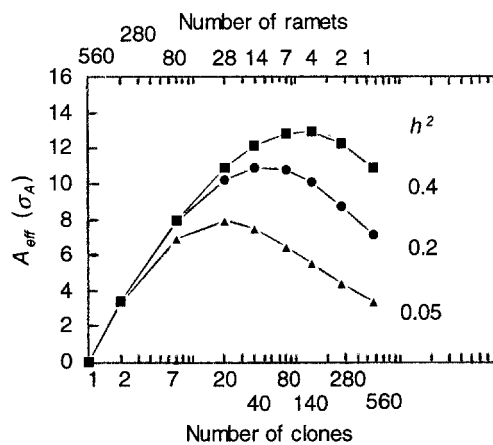


Figure 10. – Effect of re-partitioning of clone and ramet numbers on additive effect (A_{eff}) after ten generations of breeding at different heritabilities (h^2) according to the baseline breeding strategy ($N = 48$ and total test size = 560) and inbreeding depression rate 1.0%.

Stochastic variation in programme outcome

For one baseline breeding population, the standard deviation for A_{eff} and V_A after ten generations was estimated to be 0.28 σ_A (2.5 %) and 1.23 (21.8 %), respectively (Table 3). This illustrates the stochastic variation of a single breeding scenario. Due to sampling effects, the standard deviation for the A_{eff} after ten generations increased greatly with decreasing population size to 7.0 % for N is 8 (Table 4). In all other scenarios, the variation among replicate runs for A_{eff} was in the interval 0.17 to 0.37 σ_A (data not shown). In general, the stochastic variation in outcome for a single scenario was greater for parameter settings that reduced testing accuracy and precision, such as increased non-additive and environmental variance components, few ramets tested per clone (less than 7 ramets) as in the extreme case of phenotypic selection. Reducing the selected proportion (less than 7 clones per family tested per generation) also increased the stochastic variability. A large sd was associated with a small A_{eff} , thus the relative uncertainty of predicted outcome was even greater.

The coefficient of variation of V_A followed the same pattern as for the A_{eff} , increasing markedly if the breeding population was small or subdivided. Non-additive effects and variances also varied greatly. N_s remained relatively constant, due to the balanced selection regime, while the average F varied due to the non-assortative mating procedure. Stochastic variation for gain in the production population followed that of the mean in the breeding population.

Discussion

Extending the simulations for ten generations was done, not so much to predict the future outcome, but rather to study the sensitivity of the breeding strategy to combinations of management options and circumstances out of the breeder's control. From an evolutionary perspective, ten generations is a short time, but for tree breeding it is a long time, representing perhaps 200 years for Norway spruce. Ten generations might also be longer than a specific breeding goal is maintained.

It is probable that forces outside the model (migration, mutation, release of coupling, selection against inbreeding and inbreeding depression, selection advantages for heterozygotes (overdominance), environmental change, mistaken identities and pedigrees, changes in breeding strategy, changes in the mode of inheritance, etc.) make the model used by POPSIM increasingly unrealistic in subsequent generations. However, these limitations are also shared by mathematical approaches. Provided comparisons of major alternatives are considered, the simplifications may be acceptable, especially since there are no more exact alternative methods. It can be seen as an ethical requirement of a forest tree breeding operation to investigate the consequences as far in the future as can be meaningfully done.

Interpretation of variances

The development over generations of mean effects and variances in the simulation depends on how the breeding population is selected and mated, and how the new variances are modelled (VERRIER *et al.*, 1990, 1991). The infinitesimal model used in POPSIM assumes an infinite number of unlinked loci, each with a small effect, which results in a negligible change in allele frequencies due to selection (BULMER, 1980). The additive variance reported is among the selected genotypes and its decline indicates that these diploid genotypes have more similar additive values, the *Bulmer effect*, but the decrease is expressed much less in the gene pool from which the next generation is drawn. The family variance component is not

systematically and only little affected by selection in this case, since selection only acts on within-family variance. The family variance, however, is reduced when transmitted to progeny by averaging of parental effects. Under random mating, this reduction is substantial. Assuming no changes in gene frequencies, the proportion of initial additive variance, representing MENDELIAN sampling of gametes from the female and male parent, is normally distributed and independent of female or male genetic values even after selection (VERRIER *et al.*, 1989, 1990). Therefore, after mating, the within-family variance will be re-established except for the reduction due to inbreeding. The initial sharp reduction of additive variance during the first few generations corresponds to an increase in gametic phase linkage disequilibrium following truncation selection (BULMER, 1971; FALCONER and MACKAY, 1996). The additive variance stabilises when a balance is reached between the increase in disequilibrium by selection and loss due to recombination. The reduction is temporary and disappears if selection is relaxed.

Thus, the long-term decrease in additive variance under this model results only from accumulated inbreeding. However, the low levels of inbreeding reached after ten generations in most scenarios did not reduce additive effects and variances to the degree that a deviation from a linear accumulation of gain was visible. Only for $N = 8$ was there an observed decline in additive effect and variance, caused by increased relatedness and sampling effects. If, as an alternative, genetic variance is modelled for a limited number of alleles, there will generally be a decline in variance due to changes in gene frequencies as well as loss and fixation of alleles due to genetic drift and selection, even if individual rare alleles may contribute more to the variance if their frequencies increase.

DE BOER and VAN ARENDONK (1992) have outlined the difficulties in simulating dominance effects and variances correctly in presence of inbreeding under an infinitesimal model. Including the average effect of inbreeding depression on the mean, while ignoring the effect of inbreeding on dominance variance and genetic covariances associated with dominance, and changes in gene frequencies, as is done in our simulation study, gave similar genetic effects as unbiased predictions by an allelic model with few loci that did not impose these simplifications. The resulting dominance variances, however, differed between the infinitesimal and allelic simulation models. Dominance variance is expected to decrease as a result of inbreeding, but increase due to variation in the inbreeding coefficient (DE BOER and VAN ARENDONK, 1992). Due to the balanced selection and mating pattern used here, there was no decrease in the dominance variance except at the smallest population size with the highest level of inbreeding. The increase in dominance variance reported for the breeding population, when accounting for inbreeding depression, was not transmitted to the progeny in the model used here. In practise, varying degrees of dominance at different loci and varying gene frequencies would result in covariances between additive and dominance effects tending to cancel each other out, as discussed by JOHANSSON *et al.* (1993). The amount of epistatic variation among families depend on details of the nature of the gene interactions (FALCONER and MACKAY, 1996). The simplification to allow only for within family epistatic variation is used since the higher-order gene interactions controlling how much variance is between family is regarded relatively less important. Thus, for these different reasons the simplified model used for simulating non-additive effects seems to be appropriate for our study.

Genetic effects and gain

The sustained linear increase in additive mean over generations at the population sizes studied is in agreement with the

infinitesimal theory and with other studies based on that model (VERRIER *et al.*, 1990, 1993). Also, mathematical approaches considering gene frequency distributions (HILL and RASBASH, 1986) and simulation studies with allelic models and various kinds of selection regimes give similar results for 50 to 200 loci and population sizes in the order of 20 to 60 (KANG, 1979; BAKER and CURNOW, 1969; MAHALOVICH, 1989). Almost linear long-term response is also found in empirical selection experiments with many different species (DUDLEY and LAMBERT, 1992; FALCONER and MACKAY, 1996). The results should be interpreted in the context of truly quantitative characters, under the influence of a fairly large number of genes. A character controlled by only a few genes would lose variance more quickly, resulting in a slower rate of progress.

Within-family selection has its greatest potential at high heritabilities and large family sizes, where the frequency of the desired allele is low (DEMPFFLE, 1975). In the long-term perspective, non-additive effects do not add to the genetic improvement of the breeding population, but rather slow down progress by reducing narrow-sense heritability and thus selection efficiency for additive effects. However being largely within families, selection for non-additive effects has an impact on short term gain realised in the production population by vegetative propagation without reducing diversity. In the clone mixes studied, a high diversity was achieved by restricting selection to one clone per parent. It is interesting to note that a long-term breeding programme intended for a clonal mix deployment becomes less attractive as the proportion of non-additive variance increases, in contrast to the additional gains that are possible in any single generation by clonal selection and deployment. This supports the use of clones as a method to effectively exploit the additive variance.

The degree of desirable diversity in the production populations is not discussed in this study. The specification of six clones in seed orchards and clone mixes number was done to study the variation in the additional gain and diversity that can be achieved by intense selection among the trees within the breeding population. Therefore it is rational to disregard self pollination in the simulated seed orchard when comparing the coefficient of inbreeding after 10 generations, since the degree of self pollination is not strongly dependent on the advances in breeding, but very much depends on the number of seed orchard parents chosen. Any inbreeding depression from selfing will also have a low impact on final forest production due to low yield of filled seed, low seedling survival and growth compensation from neighbouring trees in the stand (KOSKI, 1973; LESICA and ALLENDORF, 1992). In the future, it is also possible that seed will be produced from controlled pollination of intensively selected, unrelated trees, to better exploit additive genetic gain and the family part of the non-additive gain from selected mate combinations and to avoid pollen contamination.

To improve selection intensity, to reduce inbreeding depression by outcrossing and to enhance diversity, clones established in seed orchards and those in clone mixes can be selected from more than one population. However, in the current Swedish situation, there are limitations due to the different climatic adaptation of the breeding populations. If, for example after ten generations of breeding, six trees were to be selected from the target population and two additional trees from each of three adjacent populations, the resulting status number of the 12-clone seed orchard would be 9.9. This is even higher than the status number of a single breeding population. The average F in the progeny, excluding self pollination, would be as low as 0.013. The increased selection intensity when 12 trees are

chosen from 192 raises the additive gain to $11.8 \sigma_A [(6j_{(6,48)} + 2 \times 3i_{(2,48)})/12]$, compared to 10.8 if the 12 trees are selected in one population of 48. To keep this option available, the individual populations must remain unrelated.

The higher gain in the clone mix is an effect of one more generation of breeding and testing, a greater exploitation of additive variance, and the exploitation of non-additive genetic variance, as discussed by MATHESON and LINDGREN (1985). In practise, this advantage can be further enhanced, since more related clones can be accepted in a clone mix for vegetative propagation than is possible for a seed orchard, where related clones would result in inbreeding, reduced seed production and reduced performance due to inbreeding depression. The waiting period associated with commercial multiplication is much shorter for tested clones in clonal mixes than in seed orchards. For Norway spruce, the time lag of 20 years from selection to the start of commercial seed production in a conventional seed orchard is as long or longer than the generation interval possible for the breeding programme, and the orchard may be used for an additional 20 to 30 years.

If controlled pollination after flower stimulation is used and combined with vegetative bulk propagation (i.e., family forestry), the effective time lag can be much reduced. The genetic gain from this alternative may also be greater than from conventional seed orchards since a higher selection intensity can be allowed and related crosses avoided. By selecting mates, inbreeding can be controlled and among family dominance variance can be captured. Less pollen contamination also increases genetic efficiency. In addition, both the clone mix propagation and controlled pollination bulking-up alternatives are flexible and can be kept up-to-date with new test result and market requirements by changes made to the clone mix, as and when desired (SWEET and KRUGMAN, 1977).

Inbreeding and inbreeding depression

The build up of inbreeding and the presence of inbreeding depression was counteracted by balanced within-family selection and exclusion of self-pollination, and had no significant effect other than at breeding population size 8. For an ideal population of size $N = 48$, F would have been 0.10 in generation 10. For the baseline alternative where $N_s = 8.1$ after ten generations and random pairing of mates, F would have been 0.057 (= Θ in generation 9) but is only 0.047, which demonstrates a strong effect of the balanced regime compared to the mating system in the long run. Inbreeding can be further delayed if relatedness between mates is also considered. However, balanced recruitment and minimum coancestry mating is able to delay the first crossing of related individuals 5 generations in a breeding population of size above 31 (= $2^5 - 1$).

If no selfing is assumed, inbreeding in the seed orchard trees has no impact on inbreeding depression in the seed crop; rather, inbreeding in the progeny will depend only on the average of the coancestry among non-identical genotypes in the orchard. In a clone mix, however, inbreeding depression will be present in advanced generations and will decrease gain. For most scenarios after ten generations, the total gain from the clone mix was still higher than the additive gain in the seed orchards except for population size 8 when the depression rate was at least 1 % per 0.01 F , and for population size 24 at depression rate 2 % per 0.01 F (data not shown).

Variation in inbreeding depression changed the pattern of selection of the production populations, favouring clones from families with less inbreeding and inbreeding depression. This effect included, after ten generations for the baseline programme with $N = 48$ and $N_s = 8.1$, it will still be possible to se-

lect 6 trees for a seed-orchard with N_s as high as 3.9. The inbreeding depression in the progeny will reduce the 11.7 σ_A gain from the seed orchard by a maximum of only 0.4 σ_A . Similarly, in a clone mix, the total genetic effect of 13.5 σ_A will be reduced by not more than 0.4 σ_A .

Our limited understanding of how inbreeding depression develops in a population under selection (CHARLESWORTH and CHARLESWORTH, 1987; HEDRICK, 1994; WILLIAMS and SAVOLAINEN, 1996) motivates the simplified approach to its simulation. One consequence of the model used here is that all trees with the same pedigree have exactly the same inbreeding, and therefore inbreeding depression is equal at all loci. In reality, the dynamics of sampling and linkage will cause inbreeding and inbreeding depression to vary among different individuals with the same F (SCHULTS and WILLEY, 1995; SKRØPPA, 1996). Selection against and eventual purging of deleterious genes will slow down the increase in inbreeding depression even more (LESICA and ALLENDORF, 1992; HEDRICK, 1994; WILLIAMS and SAVOLAINEN, 1996). Although the expected inbreeding F is the same for all full sibs, the chance variation at meiosis causes identical chromosome segments in the maternal and paternal gametes to vary. Trees with rather little inbreeding can be expected to be relatively more variable around the expected inbreeding than trees with a higher coefficient of inbreeding. This effect strengthens a trend for reducing inbreeding by selection. Furthermore, the selected individuals in a full sib family will tend to be those who suffer least from inbreeding depression at a certain level of inbreeding. Thus, the slow accumulation of inbreeding in trees under the baseline breeding strategy ($\Delta F = 0.005$ to 0.010 per generation) will have smaller effect than predicted for both future seed orchards and control-pollinated propagation. On the other hand, orchards or clone mixes derived from unrelated sublines keeps the option open to avoid inbreeding completely in the production population.

Genetic diversity

Concepts concerning inbreeding and genetic diversity are often derived from figures where alleles are identical or not. Genes can be identical independently or by descent (FALCONER and MACKAY, 1996). To make identity by descent operative, a relative concept is used by defining a reference population where all genomes are assumed to carry different alleles. Here, the reference population is the wild forest from which plus trees are selected, assuming that all trees are non-inbred and unrelated. Thus, Θ , F and N_s are relative to the values in that reference population. There is certainly some inbreeding and coancestry in the natural forest, but to speculate about amounts needs some other reference to be defined.

Inbreeding effective population size, N_e , is a dynamic measure based on how fast F accumulates over time. It gives the size of an ideal population which would accumulate inbreeding at the same rate. For managed populations, as was shown here and elsewhere, the measure is neither fit for describing the development over time nor as a snap-shot at a particular moment (BALLOU and LACY, 1995; LINDGREN *et al.*, 1996, 1997). Also the variance effective population size N_{ev} , which is the size of an ideal population having the same variance of change in allele frequencies between generations as the studied population (CROW and KIMURA, 1970) is a rate measure. Since Θ measures the relatedness among the members of a population, it is directly connected to a certain situation and time. Thus, when Θ is converted to an effective number, either status number N_s (LINDGREN *et al.*, 1996), or the equivalent founder genome equivalent FGE (LACY, 1995), the actual status of a population is

described. N_s and FGE measure the cumulative genetic drift or loss of gene diversity, while variance effective size represents the per-generation loss (LACY, 1995). The longer the time perspective; the more relevant is the classical effective population size as an operational tool. However, status number has advantages when describing what happens over a limited number of generations for essentially neutral genes.

N_s and FGE give information about the allele-carrying capacity of the population (BALLOU and LACY, 1995; LINDGREN *et al.*, 1997) in terms of expected number of founders that would be required to provide the level of allelic diversity existing in that population if the founders were equally represented and if no genetic drift had occurred (no alleles had been lost) (LACY, 1995). Numerically small changes in Θ give initially large changes in the numeric value of N_s , but at a diminishing rate as coancestry increases. If low levels of relatedness and inbreeding are assumed among the founders, the observed declines of N_s over generations would be much less drastic numerically. This reflects the fast loss of low-frequency neutral alleles, expected initially due to sampling from one generation to the next. This pattern of allelic loss is confirmed by simulation studies (LACY, 1987; VERRIER *et al.*, 1993). Although important for genetic diversity, the number of alleles at a locus (allelic diversity) has low impact on gene diversity (heterozygosity) (ALLENDORF, 1986), and genetic variance. Increases in Θ and F result in a proportional decrease in additive variance (VERRIER *et al.*, 1989, 1991) and thus also a proportional decrease in the short-term response to selection. However, long-term response to selection may be more dependent on the number of alleles present in the population than on heterozygosity (ALLENDORF, 1986).

Applying the results from a single breeding population of 48 trees to the total Norway spruce meta-population of 24 such breeding populations, the overall status number will drop from 1152 to 194 over 10 generations. Since N_s is a measure of allele retention capacity, the meta-population retains alleles with a frequency of 1% with probability 0.98, compared to 0.86 for a scenario with 24 populations, each with $N = 24$ and $N_s = 4.1$ (probabilities for a binominal distribution). The effect of population sub-structure is to increase the allele-carrying capacity, compared to that possible if all trees were bred in a single large, unstructured meta-population. After the initial sampling, rare alleles will be present in much higher frequencies in those smaller populations where they exist, thus decreasing the risk of their random loss. However, this has a value only for the long-term conservation, over longer time scales than considered here. The probability of retaining common alleles with a frequency of 10% in an individual population will be 0.8 and 0.56 for $N_s = 8.1$ and 4.1, corresponding to population sizes 48 and 24, respectively. This latter probability was also predicted by simulation (ALLENDORF, 1986).

At any specified size for an unstructured population, when the founders are unrelated, balanced within-family selection, as used for the baseline Norway spruce breeding programme, conserves maximum genetic diversity, as measured by N_s . However, sublining of the breeding populations and intentional inbreeding can increase N_s and conserve allelic diversity even more if inbred lines are not lost (LINDGREN *et al.*, 1996).

An optimal combination of gain and diversity in a closed breeding population can only be found if gain and diversity are valued on a comparable scale. The inclusion of inbreeding depression in these simulations translates reduced gene diversity by inbreeding to units of genetic gain by the regression of F on gain, giving a precise value to that kind of reduced diversity. However, the additional future loss of gain due to loss of

allelic diversity by random genetic drift can be considered better by including group coancestry and giving it a suitable weight depending on the objectives and time horizon of the breeding programme (BRISBANE and GIBSON, 1995; LINDGREN and MULLIN, 1997).

Clonal testing as an integrated strategic part of long-term breeding

Improved accuracy by genetic testing will ultimately increase diversity-use efficiency according to the infinitesimal model, since there is no cost in diversity from increased selection intensity within families (VERRIER *et al.*, 1989, 1991). However the improved gain is at the price of more resources and time.

The trade-off between number of clones and ramets at a given resource level to maximise genetic gain has been examined previously for different heritability and ratios of additive to non-additive genetic variance (SHAW and HOOD, 1985; RUSSELL and LOO-DINKINS, 1993; SHELBOURNE, 1991) and different family numbers (RUSSELL and LOO-DINKINS, 1993). For a single test site situation and a range of conditions, SHAW and HOOD (1985) found that the optimum number of ramets ranged from 1 to 6 at total test size of 144 trees per family. When applying combined-index selection with no restrictions on family selection, 1 ramet was best, but when family selection was restricted to various degrees more ramets were optimal (SHAW and HOOD, 1985; SHELBOURNE, 1991). RUSSELL and LOO-DINKINS (1993) included the influence of genotype-by-environment interaction and found an optimum allocation by decreasing the number of ramets per clone per site and increasing the number of sites. Generally 1 to 2 ramets tested on each of 2 to 6 sites (total of 4 to 10 ramets per clone) optimised genetic gain at test sizes of 97 and 156 trees per family. They also found similar distributions of effort among sites, clones and ramets for maximising both production and breeding population gains, and for most accurately estimating additive genetic variance.

The current results are in general agreement with those cited above. When larger optimal ramet numbers were found, it was at larger total test sizes than considered in the other studies. In addition, our study demonstrates that the advantages of clonal testing can be robust over many generations and under more complicated circumstances considering non-additive variance components, inbreeding and inbreeding depression. The different relationships between gain and test resource distribution were flat above a threshold level of 10 ramets. Thus, for the Norway spruce breeding programme with the total test size of 560 trees per family, the baseline alternative of 14 ramets and 40 clones per family seems to be an appropriate and robust compromise. This number refers to out-planted stecklings on four or more sites before any mortality has taken place. The optimal allocation might, however, be improved by also considering the family number (RUSSELL and LOO-DINKINS, 1993) and by including economic parameters in the analysis (LINDGREN and WERNER, 1989; LINDGREN *et al.*, 1997).

Although clonal testing as it is applied here may give biased breeding value estimates due to confounding of additive and non-additive effects (a property shared with phenotypic selection), this may be compensated for by high precision per unit test size even at high levels of non-additive variance (BURDON and SHELBOURNE, 1974; MATHESON and LINDGREN, 1985). Polycross progeny testing of selected trees is more accurate but substantially less precise (BURDON and SHELBOURNE, 1974; MATHESON and LINDGREN, 1985; DANELL, 1993b). At a given test size, the disadvantages of low accuracy (but high precision) with clonal testing as compared to progeny testing is compen-

sated for by the higher selection intensity made possible through testing more genotypes by clonal testing. Variation in inbreeding depression and some types of C-effects add more to the confounded error variance when predicting breeding values with clone tests (LIBBY and JUND, 1962; BURDON and SHELBOURNE, 1974; FRAMPTON and FOSTER, 1993; BORRALHO and KANOWSKI, 1995). However, C-effects are not considered to be large for adult characters when juvenile ortets are cloned (CANNELL *et al.*, 1988) and may have even less influence when selection is kept within families.

Clonal testing, as compared to progeny testing, also offers the possibility of faster production of test material of the new generation before sexual maturity. The clonal tests in target field areas and corresponding breeding archives can be managed in parallel under conditions suitable for genetic discrimination and early flowering respectively. Selected trees can be crossed and the new generation propagated for testing without any time delay. The generation turnover time will be the shortest possible and used for testing to a maximum.

Clonal testing, sublining and elite lines

If inbreeding depression reaches levels where outcrossing is needed to produce test material to predict breeding values, clonal testing will be of no value for testing the breeding population. In that case, if tested clones are to be deployed for forest production by vegetative propagation, tests of outcrossed clones must be added to the programme at extra cost. In addition, to keep the superior genetic quality of the clones, at least two sublines are required to produce pairs of unrelated mates. At a given level of investment, doubling the number of populations by sublining will half the individual population size and increase the rate of inbreeding even more. Thus, in a strategy where clonal tests have the dual purpose of advancing the breeding population and supplying clones for vegetative mass propagation, inbreeding should be minimised. In the long run, new technology such as somatic embryogenesis (VON ARNOLD *et al.*, 1995) and cryopreservation, which can serve clonal testing for selection to the breeding population and to mass propagation equally well, strengthens the argument to postpone inbreeding.

This study indicates that, with balanced within-family selection, inbreeding and inbreeding depression will be kept at such a low level that a breeding programme based on clonal testing as well as deployment of these clones is sustainable at population sizes half as large as those in current use ($N = 24$ instead of 48). For the current Norway spruce breeding strategy, this opens the opportunity either to apply a structure with two sublines per population or to successively reduce the effective population size by 50% corresponding to 24 founders and still rely on clonal testing with the option of deploying the tested clones. If and when problems arise from inbreeding depression in the sublines, it can be postponed by fusion of two sublines. Alternatively, that the breeding programme is more sustainable than needed could give some room for an element of family selection, which probably would boost the genetic improvement (LINDGREN *et al.*, 1993). Considering the original minimum effective population size target of $N_e = 50$ (DANELL, 1993a), this is acceptable as this corresponds to an average increase in inbreeding of $\Delta F = 0.01$ per generation.

The Swedish breeding strategy intends to take advantage of relaxed selection restrictions to exploit the best part of one or more long-term breeding populations, by use of intensively selected elite lines (DANELL, 1993a). They are flexible in breeding goal and management and need not be sustainable for a long period. An elite line with 8, top-ranked unrelated trees

from one or a few breeding populations can be bred for 2 to 3 generations to a status number of 3 to 4, keeping 85% of the initial gene diversity and the inbreeding below $F = 0.05$, without any drop in additive gain per generation. Thus, the initial gain from selecting 8 trees of 48 would be sustained and improved during these generations at a similar rate as for the larger population sizes, but at a lower cost. Within elite lines, coancestry and inbreeding are allowed to increase even more. They can be enriched, as in open nucleus breeding systems (COTTERILL *et al.*, 1989), or be abandoned and new lines started from the long-term populations after some generations. The ultimate constraint for diversity in the elite populations comes from the desired genotypic diversity in deployed reforestation material and acceptable loss of gain from inbreeding depression.

Conclusion

The longer a breeding programme progresses, the more it has to rely on balanced within-family selection, which is the most conserving strategy considering gene diversity. This study has demonstrated that balanced recruitment of the breeding population based on genetic testing of individual family candidates, can combine the highest genetic diversity possible with a considerable improvement of the population genetic mean. In addition, the diversity preserved in the breeding population can partly be utilised to boost the gain in the production population. However, the most suitable mating design to keep the family variance high in the breeding population has still to be found. Clonal testing of the family members, although not accurate, is highly effective and robust under a wide range of sub-optimal conditions. Clonal testing offers flexible solutions to many problems associated with breeding and deployment of forest trees. Advances in biotechnology may further enhance the use of clones, favouring similar breeding strategies that avoid inbreeding. As currently planned, the Norway spruce breeding programme in Sweden is sustainable considering both gain and diversity, and allows continuous breeding far into the future to respond to shifting environments and new objectives, and will be able to function as a source for production populations in the long term.

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Genetic Interpretation of Malate Dehydrogenase (MDH) Isozyme Gene Loci Using a New Staining Approach and the Genetic Control of Ten Other Isozymes in *Pinus roxburghii* SARG.

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Abstract

Genetic control of malate dehydrogenase and ten other enzymes have been investigated in *Pinus roxburghii*, the most extensively distributed pine of India. Seeds were collected from

eight natural populations covering about one third of the species' distribution range. In total, 2560 samples consisting of equal number of embryos and endosperms were analysed for ACO, AAT, GDH, IDH, LAP, MDH, MNR, PGI, PGM, 6PGDH and SKDH using starch gel electrophoresis. Eighteen polymorphic loci were found to code for these enzymes. Two to three alleles per locus were identified. Mostly the isozyme loci followed the expected 1:1 segregation ratio. MDH was stained

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