Theor Appl Genet (2001) 103:1099–1107

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Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies

Received: 16 December 2000 / Accepted: 13 March 2001

Abstract Genetic gain and the gene diversity of seed crops from clonal seed orchards were formulated considering genetic selection, fertility variation and pollen contamination, and compared for five different management strategies. Genetic response was studied as a function of orchard management tactics. Management variables included the proportion of clones left after genetic thinning and/or selective seed harvesting. Formulae were derived to calculate gene diversity (expressed as group coancestry or status number) based on the sex ratio in an orchard population. The influence of having different sets of clones serving as seed parents, or pollen parents, or as both, was analysed. In addition, the impact on genetic gain and the gene diversity of seed crops was studied quantitatively as a function of the quantity and quality of gene flow from outside the orchard. The negative impact of fertility variation among orchard genotypes on the gene diversity of the seed crop was quantified. Numerical examples were given to illustrate the impact of orchard management alternatives on genetic gain and gene diversity. The formulae and results of this study can be used for identifying favourable alternatives for the management of seed orchards.

Keywords Genetic gain · Gene diversity · Fertility variation · Roguing · Selective cone harvest

Communicated by H.C. Becker

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Introduction

Seed orchards are established with the aim of producing seed with high genetic worth, combined with an adequate amount of gene diversity. The genetic worth of seeds is a function of the breeding values of the parent genotypes, the distribution of maternal and paternal gametes produced by the orchard trees, and the amount of pollen contamination from outside the orchard (Stoehr et al. 1998). Maximum gene diversity for a given clone number occurs when mating among the seed orchard parents approaches panmixis, and all orchard genotypes contribute equally to the seed crop. In general, however, this ideal situation is violated to some degree in most seed orchards.

Genetic improvement can be defined as a process whereby genetic value is improved while joint consideration is given to the gene diversity of deployed material (Rosvall 1999). There are different orchard-management techniques to increase genetic gain while retaining diversity, including: (1) selective seed harvesting, (2) genetic thinning, and (3) combinations of these. In selective seed harvest, selection is applied only to seed parents, as seeds are collected from clones with high breeding value. In many seed orchard programs, clones are evaluated in genetic tests and those that prove genetically inferior are removed (Jett 1986). This procedure is referred to as "genetic thinning" or "roguing". In genetic thinning, selection is applied simultaneously to both seed and pollen parents. While the genetic value of the remaining clones is important, the effective number of parents in the orchard must also be considered (Lindgren and El-Kassaby 1989; Kang and Lindgren 1999). The relatedness among the orchard progeny can be monitored and managed in terms of group coancestry, also expressed as an effective population size by status number (N_s) (Lindgren et al. 1996; Lindgren and Mullin 1998). The variation in the fertility of orchard clones is also important. Seed demand, variation in numbers of ramets, selfing rate and the need for flexibility may be additional factors in planning the genetic thinning of seed orchards.

The calculations of genetic gain and gene diversity in seed orchard populations are of theoretical as well as of great practical importance. Knowledge of these values makes it possible to decide the composition and design of seed orchards, to evaluate the genetic composition of seed crops, and to assess the factors influencing seed quality. However, genetic value, relatedness and fertility among parental genotypes, and gene flow from surrounding stands, which strongly determine the genetic gain and diversity of the seed crop, should be considered for these calculations in seed orchards (Kang and Lindgren 1998; Lindgren and Mullin 1998).

Association with management variables has never been derived in a way for making quantitative assessments possible in the management strategy of seed orchards. So, the objective of the present study was to formulate predictions of genetic gain and gene diversity (status number) of the seed crop from a clonal seed orchard considering pollen contamination, fertility variation, and different numbers of clones functioning as seed and pollen parents. Variance-effective population sizes were also calculated under the various scenarios.

Materials and methods

Theory

Designations; the following symbols are used:

- M, gene migration by pollen contamination (half of background pollination);
- C, breeding value of contaminating pollen (measured with the same units as gain);
- Θ, group coancestry (average coancestry, average kinship) of the seed crop;
- CV, coefficient of variation (%) for fertility;
- A, sibling coefficient (the probability that two genes will originate from the same parent);
- G, genetic gain (breeding value compared to average plus trees, genetic gain is the predicted breeding value of the seed orchard crop);
- N, initial census number of genotypes in a seed orchard;
- N_s, status effective number;
- N_r, relative status number (= N_s/N);
- $N_e^{(v)}$, variance effective population size;
- i, selection intensity;
- σ_A, additive variance (standard deviation of breeding value for orchard clones);
- N_m, number of clones producing seeds (i.e. functioning as the mother);
- N_f, number of clones producing pollen (i.e. functioning as the father); and
- N_{mf}, number of clones that are functioning as both mother and father at the same time.

Theoretical background

The situation and prerequisites

The situation we have in mind is that a number of genotypes are taken from a wild forest where all trees are unrelated and non-inbred. It can be formulated that the wild forest is the reference point compared to which inbreeding and relatedness are measured. The wild forest is assumed to be without internal structure (such as a small number of half-sibs or provenances, in which case genotypes would be more or less related). The breeding values are known, and this information is used for decisions on how genotypes are used as parents represented in the seed orchard crop. There is no connection between breeding value and the relatedness of the trees (all are anyway unrelated). It is assumed that fertility is not correlated with breeding value. The effect of selfing is considered negligible.

The calculations concern a "large" seed orchard crop, considering fertility variation and pollen contamination, and do not describe the population of seed orchard genotypes themselves. The fertility of individual genotypes is unknown, while the variation in fertility is known. The fertility of a genotype can be regarded as the sum of its fertility as mother and as father. The relative fertility of the orchard genotypes is assumed to be equal as mother and as father. Thus, the correlation between the fertilities as mother and as father for the orchard clones is 1. Gamete contributions can differ between genders due to pollen contamination, but the relationships among genotypes are the same within each sex. It is assumed that contaminating pollen is contributing equally to all seed parents. The derived gene diversity is also valid for a seedling seed orchard or a stand, although we primarily have clonal seed orchards in mind. For seedling seed orchards, it is more relevant if their half-sib family structure is considered.

Gene diversity, group coancestry and status number

Group coancestry (Cockerham 1967) is the average of all coancestries between population members in a coancestry matrix, including self coancestry. It is also the probability that two genes taken at random (with replacement) from a population are identical by descent (Lindgren and Mullin 1998). Gene diversity is most naturally defined as the probability that genes are different by descent, thus the loss of gene diversity (compared to an infinite wild forest) is the group coancestry. Here, the considered population is the predicted seed crop of the orchard. The group coancestry of the seed crop becomes the coefficient of inbreeding in the offspring following random mating of the seed-crop genotypes. Group coancestry can be expressed as an effective number, the status number, which describes the census number of unrelated trees corresponding to the gene diversity of the seed orchard crop.

Status number (N_s) is defined as half the inverse of group coancestry (Θ) (Lindgren et al. 1996):

$$N_s = \frac{0.5}{\Theta}. \quad (1)$$

The status number of orchard genotypes can be considered at the stage when these genotypes are selected. The status number could help in deciding which, and how many, genotypes are to be used in the seed orchard. Status number will be more useful in advanced generations as candidate genotypes become inbred and related to greater and varying degrees (Lindgren and Mullin 1998; Kang et al. 2001).

Group coancestry for the gamete gene pool of the seed crop is calculated as (cf. Lindgren and Mullin 1998):

$$\Theta = \sum_{i=1}^{N} \left(m_i + (1-2M) f_i\right) \sum_{j=1}^{N} \left(m_j + (1-2M) f_j\right) \theta_{ij}, \quad (2)$$

where m_i and f_i are the maternal and paternal contributions of genotype i (sums up to 0.5, respectively), and m_j and f_j are those of genotype j. θ_{ij} is the coancestry between genotypes i and j. Here, f_i and f_j refer to paternal contributions in the absence of pollen contamination. Thus, the contribution of orchard fathers cannot sum to 0.5 if there is pollen contamination.

Clones are not equally fertile in seed orchards; thus, variation in fertility should be included in calculating relatedness in the seed crop. Fertility variation can be described by the sibling coefficient A (Kang and Lindgren 1998, 1999), and calculated from the census number and the sum of squared individual fertility values. The sibling coefficient (A) can also be related to the coefficient of variation [CV(%)] as follows:

$$A = N\sum_{i=1}^{N} p_i^2 = \left(\frac{CV(\%)}{100}\right)^2 + 1, \quad (3)$$

where p_i is the fertility of genotype *i*. If all orchard genotypes have equal fertility, there is no variation in fertility, and thus *CV* has a value of 0 and *A* equals 1. But, we prefer to use the probabilistic definition of *A*. *A*=1 means that relatedness and inbreeding of seed crops will build up over generations at the same speed and amount as the wild forest which is the reference population. The reference population is defined as having an infinite number of unrelated individuals and thus a group coancestry and inbreeding of 0 (Kang and Lindgren 1999).

For an orchard composed of unrelated and non-inbred clones, N_s can also be calculated based only on the fertility variation among parents, which was introduced by Kang and Lindgren (1999) as a concept of the effective number of parents. Here, N_s is the status number of the seed orchard crop, and *A* associates to the offspring but *CV* refers to the parental population:

$$N_s = \frac{N}{A} = \frac{1}{\sum_{i=1}^{N} p_i^2}. \quad (4)$$

Theoretical development with respect to prediction of gain and gene diversity

The breeding values of orchard clones are assumed to be known. The breeding values of the initial set of plus trees established in the orchard are expressed in standardized units with a mean of 0 and a standard deviation of σ_A (it is often convenient to use σ_A as a scale unit, thus to set σ_A=1). If the breeding values are not known, heritability and phenotypic standard deviation can be used. The contaminating pollen has a breeding value which is usually less than zero. In calculations of genetic gain, the breeding value of contaminating pollen (*C*) can be expressed as the difference in units of additive standard deviation. It is important to note that gene migration into the orchard is half the pollen contamination rate, as the father contributes only half of the genes to the crop, and that there is no contamination on the maternal side. Note also that if gene migration (*M*) is 0.5 (i.e. all pollen in the orchard is coming from outside the orchard), all orchard clones will produce only half-sib families. The influence of pollen contamination on the genetic value of the crop can then be predicted as *MC*. It is assumed that the alien pollen gametes are unrelated to all seed orchard clones as well as to each other, and this enhances the diversity considerably.

By truncation selection, the expected breeding value of the orchard crop (*G*) following selection can be predicted as

$$G = i_e\sigma_A + MC = 0.5\left(i_{(N_m,N)} + (1-2M)i_{(N_f,N)}\right)\sigma_A + MC, \quad (5)$$

where i_e is the effective selection intensity; that is, the average selection intensity applied to maternal and paternal parents. The expected genetic gain is unaffected by fertility variation because of the assumption of non-correlation between breeding value and fertility.

In the seed orchard composed of unrelated and non-inbred clones, the probability that two genes are identical by descent for genes in clone *i* is the probability that both come from clone *i* times the probability that both are identical. The group coancestry of the seed crop (Θ) is the chance that any two genes are identical by descent, which is obtained from summing over all clones with self-coancestry (0.5) and pollen contamination as:

$$\Theta = \sum_{i=1}^{N} (m_i + (1-2M) f_i)^2 0.5. \quad (6)$$

Note that as contaminating pollen gametes are unrelated, their group coancestry is 0, which simplifies the formulation. Now we study the case where the fertility is different when the genotypes function as fathers and as mothers. Although we can easily count the number of mothers by collecting seeds, the number of fathers is regarded as infinite. First, let us look at the case where all father genotypes and all mother genotypes have the same fertility. Since m_i=0.5/N_m and f_i=0.5/N_f, some genotypes may be acting as both fathers and mothers. The fertility of parents functioning as both genders is implicitly included in m_i and f_i. The census number of parents (*N*) can thus be partitioned into N_m, N_f and N_{fm} [formula (7)]. Note that N_{fm} can be the same as N_m or N_f, but cannot be larger than the smaller of them. Note also that there is an unbalanced contribution between genders because of the gene migration. From formula (6), group coancestry is re-formulated, with the pollen contamination and the number of mother and father parents as follows:

$$\begin{aligned}\Theta &= 0.5\sum_{i=1}^{N} m_i^2 + 0.5(1-2M)^2\sum_{i=1}^{N} f_i^2 + (1-2M)\sum_{i=1}^{N} m_i f_i \\ &= 0.5\sum_{i=1}^{N_m}\left(\frac{0.5}{N_m}\right)^2 + 0.5(1-2M)^2\sum_{i=1}^{N_f}\left(\frac{0.5}{N_f}\right)^2 + (1-2M)\sum_{i=1}^{N_{fm}}\left(\frac{0.25}{N_f N_m}\right) \\ &= \frac{N_f + (1-2M)^2 N_m + 2(1-2M)N_{fm}}{8N_f N_m}. \end{aligned} \quad (7)$$

The status number (N_s) for the seed crop is then described with group coancestry and fertility variation from formulae (1) and (4) as:

$$N_s = \frac{4N_f N_m}{A\left(N_f + (1-2M)^2 N_m + 2(1-2M)N_{fm}\right)}. \quad (8)$$

This is equivalent to the effective population size given by Frankel and Soulé (p. 38, 1981) and by Falconer and Mackay (p. 67, 1996) when considering the sex ratio: if there is equal fertility and no gene migration, formula (8) becomes N_s=4*N_f*N_m/(N_f+N_m) in dioecious species. If the sexes are not equal, N_s is less than *N*. On the other hand, N_s is the same as *N* when the sexes are all equal (N_f=N_m=N_{fm}), under an idealised situation. The amount of genetic drift in a seed crop where the sex ratio of the parents is skewed is higher than that for a crop where the parental sex ratio is balanced. From the sex ratio standpoints, therefore, N_s is the size of an ideal population having a 1:1 sex ratio, which is subject to the same degree of genetic drift as a real orchard population. In other words, the amount of random genetic drift in the orchard population size of *N* is equal to that in an ideal population of size N_s, where genders are represented equally and mated randomly. It should be noted that inbreeding or genetic drift depends mainly on the numbers of the less-numerous sex. For instance, if an orchard were maintained with an infinitely large number of pollen parents but only one seed parent, the status effective number would be only 4.

Based on the theory developed in this study, we now look at the impact of five alternative management scenarios on the genetic gain and status number of a seed orchard crop:

Alternative 1. Entire crop from an unthinned orchard

Selection intensity in an unthinned seed orchard, hereafter referred to as *Alternative 1*, is zero. The scale for breeding value is defined so that the average of considered clones is zero. The genetic gain (*G*) of the seed crop will only be affected by gene migration (*M*) by pollen contamination, so that:

$$G = MC. \quad (9)$$

Note that *C* usually has a negative value, thus the contaminating pollen has a negative breeding value. It may, for example, represent average trees in the surrounding forest that have lower breeding values than the orchard clones.

Status number can be expressed as a function of census number, gene migration, and fertility variation (i.e., N=N_f=N_m=N_{fm}) as:

$$N_s = \frac{N}{(1-M)^2 A}. \quad (10)$$

Alternative 2. Harvest from best clones, no thinning

In *Alternative 2*, seeds are collected only from clones with high breeding values, thus only the seed parents are improved. The

number of maternal clones used for seed harvest is N_m, while the number of clones contributing as pollen parents (father clones, N_f) is the census size of the orchard (N_f=N). Genetic gain and status effective number can then be obtained as:

$$G = 0.5 i_{(N_m,N)} \sigma_A + MC \quad (11)$$

$$N_s = \frac{4NN_m}{A(N + (1-2M)(3-2M)N_m)}. \quad (12)$$

Selection is only for the maternal parent, and there is no selection against fathers [i.e. $i_{(N_f, N)}$=0] in this option. The inferiority of con tamination is also inserted for calculating genetic gain.

Alternative 3. Genetic thinning (roguing)

In *Alternative 3*, seeds are collected from all of the clones remaining after genetic thinning. Pollen parents (N_f) are thus improved, as well as seed parents (N_m), by the irreversible removal of clones with low breeding values. The clones selected to remain, contribute as both mothers and fathers (i.e. N_f=N_m). Selection occurs for both parents at the same time and with the same intensity in this option. Gain and status number can be calculated as:

$$G = (1-M) i_{(N_m,N)} \sigma_A + MC \quad (13)$$

$$N_s = \frac{N_m}{(1-M)^2 A}. \quad (14)$$

Alternative 4. Genetic thinning, followed by harvest from best clones

After genetic thinning, all remaining clones will serve as the pollen source, while seeds are harvested only from a portion of these clones with higher breeding values. Thus, selection occurs twice in *Alternative 4*. The first is simultaneous selection against pollen and seed parents at the time of thinning, so that the selection intensity is derived from the initial number of clones. The second is the selection against seed parents only, due to the selective cone harvest. The combined genetic effect and status number become:

$$G = \frac{\left(i_{(N_m,N)} + (1-2M) i_{(N_f,N)}\right)\sigma_A}{2} + MC \quad (15)$$

$$N_s = \frac{4N_m N_f}{A\left(N_f + (3-8M+4M^2)N_m\right)}. \quad (16)$$

Alternative 5. Different seed and pollen parents

In some orchards, some clones may serve as seed or pollen parents, but not as both. This can occur when young or fresh materials, which have not yet started to produce pollen, are added to the orchard (e.g. orchard refreshment or rejuvenation). In *Alternative 5*, one set of clones is identified that serves as pollen parents (N_f), while a second set produces seed (N_m). Some number of clones may be common between these two sets, producing both pollen and seed (N_{mf}). The genetic gain and status number of the seed crop are given as:

$$G = \frac{\left(i_{(N_m,N)} + (1-2M) i_{(N_f,N)}\right)\sigma_A}{2} + MC \quad (17)$$

$$N_s = \frac{4N_m N_f}{A\left(N_f + (1-2M)^2 N_m + 2(1-2M)N_{fm}\right)}. \quad (18)$$

Clones, which do not serve as both pollen and seed parents, are still chosen with the same selection intensity of those that do. Thus, there is no consideration of selection intensity against clones serving as both seed and pollen source in *Alternative 5*.

Variance effective population size

Variance-effective population size predicts the drift in gene frequencies between generations, in this case between the gene frequencies of the orchard genotypes and those of the offspring, where the gene frequency will be different because of differences among genotypes in fertility (Kjær 1999).

The variance effective population size [$N_e^{(v)}$] is a function of fertility variation and the number of orchard genotypes, so that $N_e^{(v)}$=N/(A–1) (Kang and Lindgren 1998). Thus, $N_e^{(v)}$ will approach infinity under ideal situations with no migration and equal fertility. Under those conditions, the gene frequency in the seed orchard crop and the gene frequency of the seed orchard genotypes will be the same, as if the genotype number is infinite (Kjær 1999). It can also be described with group coancestry including gene migration [i.e. with formula (7)] as:

$$N_e^{(v)} = \frac{A}{2\Theta(A-1)}. \quad (19)$$

For all management alternatives, $N_e^{(v)}$ was calculated on the basis of orchard clones and a large gamete pool of the seed crop. Thus, variance effective population size considers the change of gene contributions between the orchard clones and the seed crop. It describes the size of sample that would give the same drift in gene frequencies as found in the seed crop (Lindgren and Mullin 1998): the variance of gene frequency between generations. If there is gene migration, $N_e^{(v)}$ will be biased. So, we did not calculate it under pollen contamination. When the sibling coefficient (A) is 2 (i.e. CV for fertility=100%), $N_e^{(v)}$ is twice as large as N_s in the seed orchard.

Results

Numerical example

We now present a numerical comparison of these orchard management situations. Starting with an initial seed orchard of N=100 clones with a mean breeding value of zero and additive genetic variance (σ^2_A) of unity (*Alternative 1*), we collect seeds from 50% of clones with the highest breeding values (*Alternative 2*, N_m=50), or from all clones remaining after 50% thinning of clones with lowest breeding values (*Alternative 3*). Also, seeds will be harvested from the best 20 clones after the thinning (*Alternative 4*). In *Alternative 5*, different numbers of seed and pollen parents are considered after genetic thinning (i.e. N_f=40, N_m=20 and N_{fm}=10, and thus the census number of clones is N=50).

Rates of pollen contamination were assumed to be 0% and 40%, equivalent to gene migration rates, 0% (M=0.0) and 20% (0.2), respectively. Orchard pollen is expected to be genetically better than that from the wild forest, so here we assume that the quality of alien pollen is inferior to that of the orchard so that the contamination inferiority, C=–1. That it is –1 in additive standard deviation units means that the inferiority is the same as the superiority of the selected clones if selection intensity is 1. Comparisons were also made over a range of values for selection intensity (i), gene migration (M), and fertility variation (A).

The results of our calculations are presented in Tables 1 and 2. Selective cone harvest, genetic thinning, and the combination of the two increased gain compared to the

Table 1 Genetic gain (G), status number (N_s) and variance effective number [$N_e^{(v)}$] for the case when sibling coefficients (A) were 1 and 1.75, and gene migrations (M) were 0.0 and 0.2, respectively. The breeding value of the contaminating pollen (C) was −1. Note that N_s is dependent only on the number of clones in different categories, not their ranks

Management options[a]	M=0.0			M=0.2	
	G	N_s	$N_e^{(v)}$	G	N_s
A=1					
Alternative 1	0.000	100.0	Infinite	–0.200	156.3
Alternative 2	0.396	80.0	Infinite	0.196	112.4
Alternative 3	0.792	50.0	Infinite	0.433	78.1
Alternative 4	1.089	36.4	Infinite	0.730	49.3
Alternative 5	1.172	40.0	Infinite	0.780	54.1
A=1.75					
Alternative 1	0.000	57.1	133.3	–0.200	89.3
Alternative 2	0.396	45.7	106.7	0.196	64.2
Alternative 3	0.792	28.6	66.7	0.433	44.6
Alternative 4	1.089	20.8	48.5	0.730	28.1
Alternative 5	1.172	22.9	53.3	0.780	30.9

[a] *Alternative 1*: initial seed orchard consisted of 100 clones
Alternative 2: selective cone harvest from the best 50%
Alternative 3: genetic thinning; the best 50% remains
Alternative 4: selective cone harvest from the best 20% after removing the worst 50% according to the previous alternative
Alternative 5: different seed and pollen parents (N_f=40, N_m=20 and N_{fm}=10), note that the gains obtained are based on the certain assumptions given in the text

Table 2 Comparison of genetic gain (G) and status number (N_s) in the *Alternatives 2* and *3* where N=100, M=0.2, C=–1 and A=1.75 (CV=86.6%)

Portion selected to contribute gametes	Selective cone harvest (*Alternative 2*)		Genetic thinning (*Alternative 3*)	
	G	N_s	G	N_s
0.1	0.665	19.8	1.184	8.9
0.2	0.493	34.8	0.909	17.9
0.3	0.375	46.7	0.719	26.8
0.4	0.279	56.3	0.567	35.7
0.5	0.196	64.2	0.433	44.6
0.6	0.119	70.8	0.311	53.6
0.7	0.046	76.5	0.194	62.5
0.8	–0.027	81.3	0.077	71.4
0.9	–0.104	85.6	–0.046	80.4
1.0	–0.200	89.3	–0.200	89.3

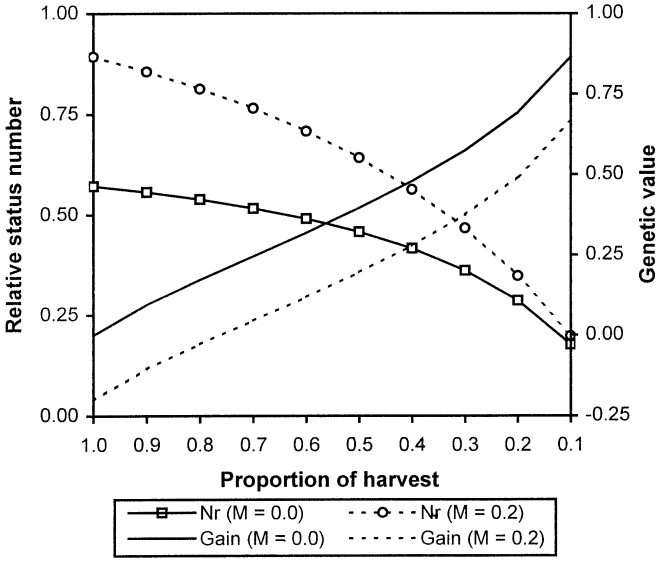

Fig. 1 Relationship between the genetic value and the relative status number (N_r) for selective harvesting, with different levels of foreign gene migration (M). It was assumed that the coefficient of variation (CV) of fertility was 86.6% (A=1.75)

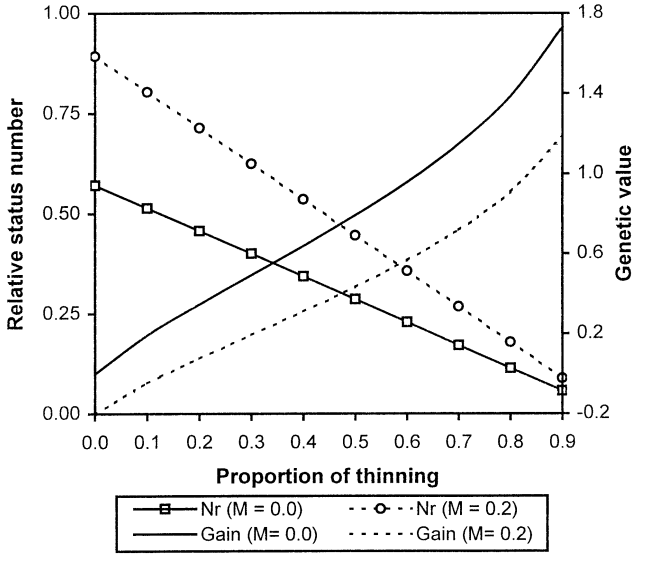

Fig. 2 The genetic value and the relative status number (N_r) for genetic thinning, with different levels of foreign gene migration (M). It was assumed that the coefficient of variation of fertility (CV) was 86.6%

initial seed orchard. The increase was, however, coupled with a decrease in status number and variance effective number. Pollen contamination increased status number and variance effective number, but decreased genetic gain. Fertility variation decreased N_s and $N_e^{(v)}$, but did not change the gain.

Genetic value (G) and relative status number (N_r) under the option of selective cone harvest (*Alternative 2*) are presented in Fig. 1 at different levels of gene migration. If we want to achieve N_r=0.49, and if there is no contaminating pollen, we need to harvest from about 60% of the clones and will obtain a gain of 0.319. On the other hand, if the migration is 0.2, we can obtain the same N_r when we harvest from 32% of clones, and this will yield a genetic gain of 0.354. The results also illustrate that the proportion of harvested clones necessary for any given balance between gain and diversity decreases as the level of gene migration from outside of the seed orchard increases.

The effect of genetic thinning alone (*Alternative 3*) on genetic gain and N_r is presented in Fig. 2, under the assumption of pollen contamination. Truncation selection

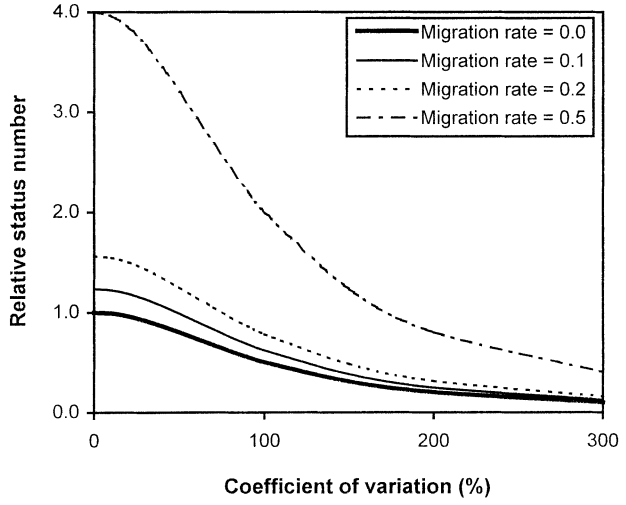

Fig. 3 Relationship between the relative status number (N_r) and the coefficient of variation (*CV*) in fertility, while considering different levels of gene migration (*M*)

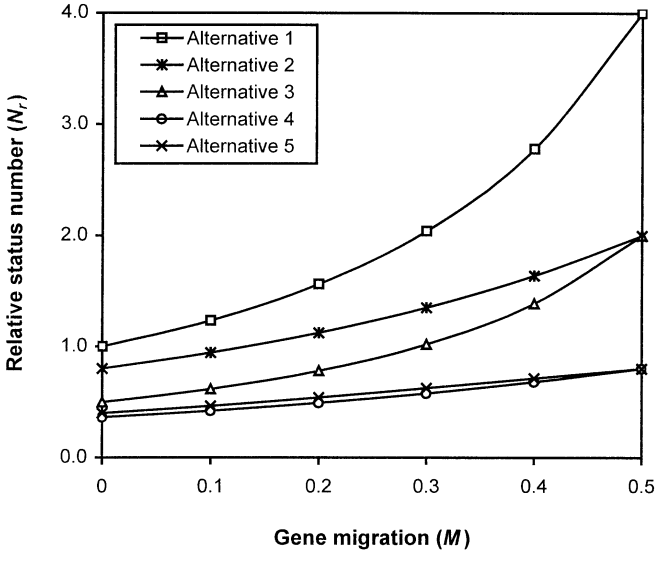

Fig. 4 Relative status number (N_r) at different levels of gene migration (*M*) for the different orchard management strategies described in Table 1, and applied to a first-generation seed orchard beginning with 100 clones

resulted in a large decrease in N_r, which was accompanied by a larger increase in genetic gain than was possible by selective harvesting alone in *Alternative 2* (see also Table 2). The figure also suggests that an orchard manager could determine the level of genetic thinning necessary to maintain a balance between gain and gene diversity; as pollen contamination increases, the selection intensity should be higher. To maintain a given N_r, one can thin more heavily if there is more gene flow into the orchard, and, at the same time, achieve greater genetic gain. The only negative effect is that the potential quantity of seed collected can be decreased, as the orchard is thinned more heavily.

Relative status number decreases and group coancestry increases when fertility is more variable (Fig. 3). It was confirmed that the status number of the seed crop could be increased up to four-times the census number of orchard clones, if all fertilization is by contaminating pollen (Lindgren and Mullin 1998).

Relative status numbers at different levels of gene migration are presented for the five management alternatives in Fig. 4. Pollen contamination from outside the orchard had a large impact on relative status number, which increases at higher rates of gene migration in all management alternatives. For complete pollen contamination (M=0.5), N_r of *Alternative 2* was the same as that of *Alternative 3*, indicating that only mother parents in the orchard population affect the gene diversity of the seed crop when fertilization is solely by alien pollen.

EXCEL spreadsheets that apply the math developed in this paper are available on the web site managed by Dag Lindgren, http://www.genfys.slu.se/staff/dagl/. Some additional programs and examples that may be helpful in understanding and applying the techniques described in this study are also available at the site.

Discussion

Fertility variation and gene migration

Parents in seed orchards are never equally fertile. Variation in reproductive output can be described by the sibling coefficient *A* (i.e. *CV*). A consequence of fertility variation is the reduction of effective population size, which contributes to higher levels of inbreeding (Kimura and Crow 1963; Kang and Namkoong 1988; Harju 1995). Gene diversity (i.e. N_s and $N_e^{(v)}$) at any given level of gene migration decreases as fertility variation increases, but the effect of fertility variation on diversity is not linear (Fig. 3). The decrease in status number resulting from fertility variation could be diluted by the pollen contamination. The fertility variation can be partitioned into maternal and paternal components in formula (3). When the difference in fertility between genders is large, it may significantly affect the gene diversity of the seed crop.

The coefficient of variation in the fertility of orchard clones was considered to be *CV*=86.6% (equivalent to *A*=1.75), a typical value of those found in a survey of first-generation seed orchards in Korea and from other references (Harju 1995; Bila and Lindgren 1998; Kang and Lindgren 1998). As shown in Table 1, a higher N_s is obtained when the fertility is equal among clones (*A*=1). Keeping female fertility constant may increase the effective number of parents (Kang and Lindgren 1999).

Pollen contamination is detrimental because it reduces the genetic gain from the selection of orchard genotypes, but it is nevertheless helpful in terms of gene diversity (Figs. 3 and 4). In this study, gene migration from outside the orchard decreased genetic gain, but increased status number and effective population size for all alter-

natives (Table 1 and Fig. 4). Thus, it is important that pollen contamination should be considered for the optimal balancing between genetic gain and gene diversity in the management of seed orchards.

The impact of gene flow depends on the magnitude of migration and the degree of genetic differentiation between the donor and recipient populations (El-Kassaby et al. 1989; Hartl and Clark 1989). Large differences in the genetic composition of orchard pollen and contaminating pollen could profoundly influence the genetic composition and adaptation potential of orchard seeds, even when the amount of gene flow into the seed orchard is small if contaminating pollen is related to itself or to orchard pollen (Lindgren and Mullin 1998).

It seems likely that contaminating pollen will generally have a lower breeding value than that of the orchard (inferiority of contaminating pollen, *C*). However, it is difficult to assess the genetic quality and quantity of incoming pollen from surrounding stands. Only in some cases can the pollen sources be defined, e.g. if there is a rare allele in the surrounding gene pool or if surrounding populations are known to have clear differences in gene frequencies (Pulkkinen 1994). If there are only a few clones or if genetic thinning of the orchard has been very heavy, the genetic quality and magnitude of alien pollen is subsequently more important.

Among the factors that influence the magnitude of pollen contamination in seed orchards are: (1) the degree of isolation from background stands, (2) the orchard size, (3) pollen production within the orchard, and (4) synchrony of flowering of the orchard with that in background stands (Adams and Burczyk 1998). It is still difficult to make a good determination of either the exact rate of pollen contamination in seed orchards (Savolainen 1991) or the relationship between the level of contamination and production of within-orchard pollen. A direct assay of the effective pollen contribution is possible using biochemical markers such as isozymes (Friedman and Adams 1985); but isozyme markers show little genetic variation and are thus limited in their ability to discriminate different pollen sources (El-Kassaby et al. 1989; Stoehr et al. 1998). On the other hand, Stoehr et al. (1998) reported that it was possible to use a chloroplast DNA marker for the estimation of background pollination in seed orchards.

Genetic gain and gene diversity

Relative gain from the management strategies changes with the proportion of selected and thinned clones. Table 2 indicates that *Alternative 3* gives more gain than *Alternative 2* at the same selection intensity, but this is accompanied by a greater loss of status number and variance-effective population size. Genetic thinning by truncation selection is a form of family selection (Libby 1964); thus the rapid loss of diversity is unavoidable in *Alternative 3* compared to *Alternative 2*. In general, however, *Alternative 3* would be better than *Alternative 2* because the main purpose of a seed orchard in most breeding programs is as a mechanism to get higher gain into the field, albeit with an appropriate amount of gene diversity.

The gain from orchard roguing will depend on the heritability, the genetic value and proportion of culled clones (Cotterill and Jackson 1989). When heritability is low, roguing of a clonal seed orchard (backward selection) is favoured (Toda 1964; Falconer and Mackay 1996); but if it is sufficiently high, selecting from the offspring of crosses among plus trees (Ruotsalainen and Lindgren 1998) and/or replacement with better phenotypes will be better.

Increased selection intensity will yield a greater gain but will also reduce the effective population size, thus reducing genetic variability and increasing inbreeding (Kerr et al. 1998). Selection intensity will be lower than expected because the arrangement of the ramets in the orchard is often carefully balanced and this discourages the removal of many clones. It is obvious that strong selection intensity will increase the genetic gain in the seed orchards, but if the juvenile-adult correlation is weak, the first-generation seed orchard cannot be rogued heavily while young (Lindgren 1994).

In all alternatives with parental selection based on the performance of the progeny or clonal replications, status numbers are exactly equal to selected numbers (census number) for seed orchards where the parents are unrelated and non-inbred. Nevertheless, fertility variation and pollen contamination can each have a strong effect on the status number of orchard crops, as shown Table 1, Fig. 3 and Fig. 4. In *Alternative 2*, for example, if seeds are harvested from only a few clones with high breeding value, the other clones functioning as pollen parents, including contamination, have a considerable impact on diversity (Lindgren and Mullin 1998). Pollen contamination in the seed orchard will normally lead to lower genetic gain. The loss could be as much as one-half if all the seeds are fertilized by contaminating pollen; i.e. as much as the influence of contamination on the genetic value (*MC*).

Loss of gene diversity can be calculated, relative to the group coancestry of the reference population, where gene diversity is considered to be 1 (Kang and Lindgren 1998). Relative gene diversity (*GD*) is calculated as $GD=1-\Theta$ (Lacy 1995) or equivalently $1-1/(2N_s)$. In the initial seed orchard consisting of 100 clones (*Alternative 1*), the gene diversity of seed crop equals 0.995 in the case of no pollen contamination and no fertility variation. Fertility variation will decrease the gene diversity, but pollen contamination will increase it (Harju 1995).

In this study, it was assumed that genotypes were unrelated and non-inbred. If there was, however, relatedness among parents, there would be a strong effect on the genetic diversity of the seed crop (Lindgren and Mullin 1998). Ramet variations could also contribute to increased fertility variation and thus to lower diversity (Kang et al. 2001). Controlling fertility and gain by adjusting ramet number is possible when breeding values are known.

We also assumed that there was no correlation between breeding value and fertility. In practice, however, they could be correlated with each other. It may be advantageous to maximize genetic gain if they are positively correlated, but if there is strongly negative correlation, the increased fertility variation may reduce greatly the genetic value of orchard seeds.

Orchard management tactics

The gain calculation in *Alternative 5* is theoretically the same as in *Alternative 4*, but group coancestry and N_s may be different. For increasing diversity, it may be beneficial to include some portion of new plus trees into an existing seed orchard, although genetic gain may decrease.

As discussed earlier, if low-ranking clones are thinned from orchards when progeny test results become available, improvements in genetic gain will be made, but it is expensive to have extra grafts in seed orchards during the test period (Lindgren 1994). For unbiased estimates of gain, one must weigh the costs and time against orchard benefits for various selection strategies (Namkoong et al. 1966; Lindgren 1994). Genetic thinning can be done at different ages; the older are the progeny tests at evaluation, the higher will be the genetic gain, although it will be realized later.

A seed orchard has a finite lifespan after which it is no longer used (Matheson and Lindgren 1985). The length of time between the establishment of the orchard and the average time of usage in plantations depends on the species and the breeding program. It might therefore be advantageous to use an 'advancing front' in order to increase the average lifespan of the seed orchard by replacing obsolete clones with better, more recently selected ones.

First-generation seed orchards are not the only source of genetic material for future seed orchards, and they are not established primarily as a base for future selections (Zobel and McElwee 1964). They are intended to provide the maximum possible improvement in the shortest possible time. Many orchard programs are now at a stage where they must consider rejuvenation and/or a generation shift. In some cases, it is desirable to replace some genotypes in existing orchards with better clones, rather than replacing the entire orchard.

This paper presents different options of orchard management to increase genetic gain while retaining gene diversity, and indicates that these may vary considerably depending on the sex ratio, pollen contamination and fertility variation of orchard genotypes. Orchard managers should consider these options to be used for balancing genetic gain and gene diversity. The different options can also be expanded into seedling seed orchards with the calculation of group coancestry.

Acknowledgements This study was funded by Korea Forest Research Institute, Kempe Stiftelsen and Föreningen Skogsträdsförädling. The authors thank Erik Kjær at the Danida Forest Seed Centre, Denmark and Seppo Ruotsalainen at the Finnish Forest Research Institute for their valuable comments on the manuscript.

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