

would be confounded with σ_f^2 . An increase in σ_f^2 would have the effect of increasing h_1^2 and h_f^2 to an unknown degree.

The advantage of obtaining variance components from an unselected population means they will not be biased as a result of selection for one or a number of traits. Unbiased estimates of heritabilities and genetic correlations allows the construction of more accurate mathematic models. Such models can then be used to investigate the implications of various selection scenarios in terms of direct and indirect selection which are not possible when variance components are inherently biased.

Conclusions

Based on the analysis of height and diameter data collected from a large population of half-sib progeny of known single origin growing at just one site over a 23-year period, certain conclusions can be drawn regarding heritability, age:age correlations and optimum selection ages in Sitka spruce.

Individual tree heritabilities are higher for early height traits than for later diameter traits. Family heritabilities are little changed across years regardless of whether the trait is height or diameter. Genetic correlations for early height traits with the selection goal of DM23 were all greater than 0.60 beyond HT03 and then 0.80 beyond HT08. All diameter traits (DM10 to DM19) had a genetic correlation with DM23 in excess of 0.90.

Generation efficiency (Q_{gen}) for individual tree selection reached a peak at HT09 due to rising genetic correlations and high individual tree heritabilities compared to the breeding goal of DM23. Since family heritability varied only slightly across ages, Q_{gen} for family selection did not exceed that achievable at DM23.

Q_{year} for individual tree selection was maximised by selecting for HT09 assuming a selection goal of DM23, although there was little loss in efficiency through indirect selection for HT07. Q_{year} for family selection peaked at HT05. If the delay (d) taken to establish the next generation of genetic tests could be reduced to 5 years, then Q_{year} for individual tree selection would be reduced to HT06.

Estimated Q_{year} based on various LAMBETH regression equations assuming a selection goal of 40-year height or diameter

tended to reflect the results from DM23 selection goal. The most optimistic LAMBETH regression equation involved genetic correlations of juvenile height with mature diameter (r_{AHD}) against LAR. This model predicted an optimum individual tree selection age of 7 to 9 years height when d = variable and family-mean selection age of 3 to 4 year height when d = 5 years.

Based on these conclusions it would appear that for practical purposes indirect selections for family-mean mid-rotation diameter can be safely made based on 5-year height, whereas individual tree mid-rotation diameter should be delayed a further 2 to 4 years.

References

- FALCONER, D. S.: Introduction to quantitative genetics. Second Edition. Longman Group Limited, Essex, UK, 340 pp. (1981). — FLETCHER, A. M. and FAULKNER, R.: A plan for the improvement of Sitka spruce by breeding and selection. Forestry Commission Research and Development Paper, No. 85, HMSO, London (1972). — Forestry Industry Council of Great Britain: Handbook 1998. FICGB, Stirling, Scotland, 72 pp. (1998). — GILL, J. G. S.: Juvenile-Mature correlations and trends in genetic variances in Sitka spruce in Britain. *Silvae Genetica* **36**(5–6): 189–194 (1987). — GILMOUR, A. R.: ASREML, a spatial REML program. Agriculture Research and Veterinary Centre, Orange, New South Wales 2800, Australia, 42 pp. (1996). — GILMOUR, A. R., THOMPSON, R. and CULLIS, B. R.: Average information REML: An efficient algorithm for variance parameter estimation in linear mixed models. *Biometrics* **51**: 1440–1450 (1995). — GORDON, A. G. and FAULKNER, R.: Identification and assessment of cone and seed crops. In: Seed Manual for forest Trees. Ed. A. G. GORDON. Forestry Commission Bulletin **83**: 71–85. HMSO, London (1992). — LAMBETH, C. C.: Juvenile-mature correlations in Pinaceae and implications for early selection. *Forest Science* **26**(4): 571–580 (1980). — LEE, S. J.: Breeding strategy for Sitka spruce in Britain. In: Proceedings of the Nordic Group for Tree Breeding, Ed. LEE, S. J. pp. 95–109. Forestry Commission, Edinburgh, Scotland (1993). — LEE, S. J.: Improving the timber quality of Sitka spruce through selection and breeding. *Forestry* **72**(2): 123–133 (1999). — LEE, S. J.: Selection of parents for the Sitka spruce breeding population in Britain and the strategy for the next breeding cycle. *Forestry* **74**(2): 129–143 (2001). — PATTERSON, H. D. and THOMPSON, R.: Recovery of interblock information when block sizes are unequal. *Biometrika* **58**: 545–554 (1971). — SAMUEL, C. J. A. and JOHNSTONE, R. C. B.: A Study of population variation and inheritance in Sitka spruce. I. Results of glasshouse, nursery and early forest progeny tests. *Silvae Genetica* **28**(1): 26–32 (1979). — SAS Institute Inc.: SAS User's Guide: Statistics. SAS Institute Inc., Cary, North Carolina, USA (1982). — WILLIAMSON, D. R. and LANE, P. B.: The use of herbicides in the forest. In: Forestry Commission Field Book 8, HMSO, London. 151 pp. (1989). — WRIGHT, J. W.: Introduction to forest genetics. Academic Press, London. 463 pp. (1976).

Using Single Family in Reforestation: Gene Diversity Concerns

By K. S. KANG¹), H.-L. LAI²) and D. LINDGREN³)

(Received 28th March 2001)

Abstract

Formulae for gene diversity measured by status number (group coancestry) for seeds collected from a single clone in a clonal seed orchard were derived. The formulae considered: number of seeds collected, ratio of seeds from selfing, fertility variation of pollen parents, relatedness among pollen parents and amount of pollination by alien fathers from outside the seed orchard (pollen contamination). The results showed that

¹) Tree Breeding Department, Korea Forest Research Institute, 44-3 Omokchun, Kwonsun, Suwon, Kyonggi, 441-350, Republic of Korea

²) Author to whom all correspondence should be addresses: KYU-SUK KANG (e-mail: kangks@foa.go.kr, fax: +82 31 292 8468)

³) College of Forest Resources and Environment, Nanjing Forestry University, 210037 Nanjing, People's Republic of China

³) Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S 901 83, Umeå, Sweden

the status number of seeds collected from a single clone generally varied between 2 and 4 in most cases. Typically it was close to 4, thus close to that of an “ideal” half-sib family. Relatedness among pollen parents and selfing decreased status number and gene diversity while pollen contamination increased it. Effect of the level of pollen contamination and fertility variation among pollen parents on gene diversity of crops from the same seed parent was usually small. High degree of relatedness and selfing can be important. If there were few pollen parents, the gene diversity depended greatly on relatedness, selfing and fertility variation. However, if there were many pollen parents (about 10 or more), these factors were not important. The influence of the number of pollen parents was discussed.

Key words: gene diversity, status number, single-family, fertility variation, pollen contamination, clonal seed orchard

Introduction

In first generation seed orchards, a large number of parents have been used to retain high diversity and flexibility at the later stages and to counteract possible negative effects of fertility variations among parents. If there is equal fertility, there will also be an equal size of family in the orchard crops. However, it has been proved that there is no equal fertility among orchard parents, and unequal contribution leads to faster accumulation of relatedness and inbreeding in their offspring (ERIKSSON et al., 1973; HARJU, 1995; KJÆR, 1996; KANG and LINDGREN, 1998; BILA, 2000).

Genetically improved stock shows not only outstanding growth but may sometimes also be linked to lower infection from diseases (LI et al., 1999). The quality of the seed crop from seed orchards depends on many factors of interest from a genetic point of view, such as genetic value and relatedness of parents, pollination processes within the orchard, pollination by alien pollen, amount of selfing and unequal contribution among parents (PAULE et al., 1993).

In the southeast USA and elsewhere, foresters use seeds collected from a single clone in clonal seed orchards for the establishment of individual stands for many years at a large scale (DUZAN and WILLIAMS, 1988; MCKEAND et al., 1997). Many companies are exclusively using single clone harvests for their plantations: the family block plantation system. In the family block plantation system, the identity of each maternal parent is retained for the half-sib family seed from the orchard through plantation establishment. Genotype x environmental (family x site) interaction is considered for the maximizing of the use of family block plantation. The best families should thus be planted on the best sites (MCKEAND et al., 1997; LI et al., 1999).

The advantages of the single block plantation system over conventional orchard seed bulking are the following: easier management of seeds, seedlings and forests (in particular easier to manage nursery crops); getting more homogenous product; better controlling and monitoring; and better utilization of the best material. Harvesting by maternal parents in the seed orchard also increases genetic gain over the orchard mix because it permits seeds of the highest genetic value to be used first (LINDGREN and EL-KASSABY, 1989). In many European countries and some other countries, however, such kind of forestation is not encouraged or not allowed (MUHS, 1993). The main reason seems to be concerns on the reduced gene diversity.

OECD scheme, for example, gives the minimum requirement for the approval of reforestation materials (OECD, 1974). The purpose of the requirement is to ensure the consistency and

high genetic diversity of forest reproductive materials (KANG et al., 2001b). To be certified as a qualified category, seed orchards should be managed and seed harvested in such a way that the objective of the orchard is attained, as to maximize genetic gain and keep gene diversity high. BONDESSON and LINDGREN (1993) proposed the use of optimal balancing between gain and diversity for optimizing the objective of seed orchard. In practice, however, orchard managers may try to increase gains by utilizing the best clones and produce homogeneous seedlings at a reduction in diversity.

The aims of the current study are to derive formulae on gene diversity (group coancestry and status number) of single-family seeds considering relatedness, fertility variation, selfing and pollen contamination, and to discuss the effect of such factors on gene diversity of the seeds from a single mother.

Theory

Formulae are derived for the calculation of gene diversity, measured by status number and group coancestry, of orchard crop from the same mother. The formula is for the case under which relatedness and fertility variation among trees, selfing and pollen contamination are assumed in seed production. For the case under which no relatedness is assumed to exist between trees, the formula is simplified from the former case. Orchard clones and alien fathers are assumed to be non-inbred.

Seeds are collected from one clone in a clonal seed orchard, which may be pollinated by orchard fathers (including selfing) or by contaminating pollen (alien pollen donators). The strobilus of pine receives pollen continuously during its receptive period. Of several pollen grains reaching ovule, however, only one succeeds in fertilizing the seed. A mature, pollinated cone will contain seeds of various paternal origins. Therefore, the relationship among the seeds can include selfing, relatives and sib-ships such as half-sib and full sib. Based on those cases, we define;

N	Total number of seeds collected from a seed parent,
S	Ratio of seeds resulted from selfing,
M	Ratio of seeds pollinated by alien pollen (pollen contamination),
n_i	Number of seeds produced by out-crossing between orchard clone i and the seed parent,
a_m	Number of seeds pollinated by an alien father m from outside the orchard,
f_w	Relatedness among clones within the seed orchard,
f_a	Relatedness among alien fathers outside the seed orchard,
N_w	Number of fathers within the orchard (excluding the seed parent),
N_a	Number of alien fathers outside the orchard,
ψ_w	Sibling coefficient (KANG and LINDGREN, 1999), it refers to the fertility variation among orchard fathers (the seed parent is excluded),
ψ_a	Sibling coefficient correspondent to the fertility variation among alien fathers

For convenience, we will use “selfed seeds” to refer to seeds from selfing, “non-selfed orchard seeds” to refer to out-crossing seeds produced by orchard pollen, and “contaminated seeds” to refer to seeds produced by alien pollen. Coancestry is the probability that two genes taken at random from two individuals are identical by descent (FALCONER and MACKAY, 1996). The coancestry consists of two parts; one is self-coancestry and the other is cross-coancestry.

Sum of self-coancestry

$$\sum \theta_{self} = 0.5SN + 0.5\sum n_i + 0.5NM$$

The three terms in this formula refer to the sum of self-coancestry from selfed seeds, non-selfed orchard seeds, and contaminated seeds, respectively. Note that the number of non-selfed orchard seeds, $\sum n_i = N^*(1-M-S)$. So, the sum of self-coancestry equals to be $0.5*N$ in the seeds from non-inbred parents.

Sum of cross-coancestry

$$\begin{aligned} \sum \theta_{cross} = & 0.5SN(SN-1)^2 + SN^2(0.5 + f_w)(1-M-S) + 0.5SMN^2 + \\ & 0.5(0.5 + f_w)\sum n_i(n_i-1) + 0.25(3f_w + 0.5)\sum \sum (n_i \cdot n_j) + \\ & 0.5MN^2(f_w + 0.25)(1-M-S) + \\ & 0.25\sum a_m(a_m-1) + 0.25(f_a + 0.25)(N^2M^2 - \sum a_m^2) \end{aligned}$$

The sum of cross-coancestry (group cross-coancestry) includes all pair-wise coancestry (including reciprocal) except only self-coancestry from possible combinations of sib-ships from a single mother (Table 1). Under the idealized situation, group cross-coancestry will be the inbreeding of offspring from a plantation established by orchard seeds.

Group coancestry

Group coancestry (Θ) is the sum of the above two parts (i.e., $\sum \theta_{self}$ and $\sum \theta_{cross}$) divided by N^2 as follows,

$$\begin{aligned} \Theta = & \frac{0.25(1-S)}{N} + \frac{0.25(0.5-f_a)\sum a_m^2}{N^2} + \frac{0.25(0.5-f_w)\sum n_i^2}{N^2} + \\ & 0.25\left[M^2(f_a + f_w) - 4Mf_w + 3f_w + 0.5 + S(1-2f_w - f_wS + 0.5S)\right] \end{aligned}$$

With replacement sibling coefficients (ψ_a and ψ_w) derived from KANG and LINDGREN (1999) into the formula (see Appendix); $\sum a_m^2 = \psi_a N^2 M^2 / N_a$ and $\sum n_i^2 = \psi_w N^2 [(1-M-S)]_2 / N_w$, we will get,

$$\begin{aligned} \Theta = & \frac{0.25(1-S)}{N} + \frac{0.25(0.5-f_a)\psi_a M^2}{N_a} + \frac{0.25(0.5-f_w)\psi_w(1-M-S)^2}{N_w} + \\ & 0.25M^2(f_a + f_w) - 4Mf_w + 0.75f_w + 0.125 + 0.25S(1-2f_w - f_wS + 0.5S) \end{aligned}$$

In general, almost infinite numbers of seeds are collected from the seed orchard. So, group coancestry can be simplified when assuming a large number of seed collected ($N \rightarrow \infty$) as follows,

$$\begin{aligned} \Theta = & 0.25\left(\frac{(0.5-f_a)\psi_a M^2}{N_a} + \frac{(0.5-f_w)\psi_w(1-M-S)^2}{N_w}\right) + \\ & 0.25\left(M^2(f_a + f_w) - 4Mf_w + 3f_w + 0.5 + S(1-2f_w - f_wS + 0.5S)\right) \end{aligned} \quad [1]$$

If there is no relatedness among parents (i.e., $f_w = f_a = 0$), the formula will then be simplified as,

$$\Theta = 0.125\left(\frac{\psi_a M^2}{N_a} + \frac{\psi_w(1-M-S)^2}{N_w} + (1+S)^2\right) \quad [2]$$

Status number

LINDGREN et al. (1996) defined status number (N_s) as the half the inverse of group coancestry as follows,

$$N_s = \frac{0.5}{\Theta}$$

We will use their approach to calculate the status number of seeds from the same mother based on the group coancestry calculations under two different situations. One is for the case under which relatedness, fertility variation, selfing and pollen contamination are assumed to exist among parents in the seed production (formula [1]), the other is for the simple case under which no relatedness among trees is assumed (formula [2]).

Results and discussion

If there is no relatedness among parents

The results under the situation of where trees were unrelated and non-inbred ($f_w = f_a = 0$) are presented in Table 2 and Figure 1. For comparison to an ideal situation (i.e., equal ferti-

Table 1. – Coancestry among seeds harvested from the same mother

Relatedness among seeds	Frequency	Coancestry ^{a)}
Self-coancestry sum for all seeds	$0.5N$	$\theta_{self} = 0.5(1+F) = 0.5$ (self-coancestry)
Cross-coancestry sum (CCS) among selfed seeds	$0.5SN(SN-1)^2$	$\theta_{ss} = 0.5$ (between selfed seeds)
CCS between selfed and non-selfed orchard seeds	$SN^2(0.5+f_w)(1-M-S)$	$\theta_{sn} = 0.25$ (between self-halves)
CCS between selfed and contaminated seeds	$0.5SMN^2$	$\theta_{sc} = 0.25$ (between self-halves)
CCS among non-selfed orchard seeds	$0.5(0.5+f_w)\sum(n_i(n_i-1)) + 0.25(3f_w+0.5)\sum \sum(n_i \cdot n_j)$	$\theta_{nn} = 0.125$ or 0.25 (half or full sib)
CCS between non-selfed and contaminated seeds	$0.5MN^2(f_w+0.5)(1-M-S)$	$\theta_{nc} = 0.125$ or 0.25 (half or full sib)
CCS among contaminated seeds	$0.25\sum(a_m^2 - a_m) + 0.25(f_a+0.25)(N^2M^2 - \sum a_m^2)$	$\theta_{cc} = 0.125$ or 0.25 (half or full sib)

a) Following LINDGREN et al. (1996) based on unrelated and non-inbred parents In this column, subscript letters (s, n and c) represent selfed seeds, non-selfed orchard seeds, and contaminated seeds, respectively.

Table 2. – Status number (N_s) in the situation with non-relatedness ($f_w = f_a = 0$), different number of fathers (N_w, N_a), pollen contamination ($M = 0.4$) and fertility variation ($\psi_w = 2, \psi_a = 3$). Amount of selfed seeds was assumed to be 10% ($S = 0.1$).

$N_w =$	1	2	3	4	5	10	15	20	30	40	50	100
$N_a = 1$	2.139	2.469	2.603	2.676	2.721	2.817	2.850	2.867	2.885	2.893	2.899	2.909
5	2.215	2.571	2.716	2.795	2.845	2.950	2.987	3.005	3.024	3.034	3.040	3.051
10	2.275	2.653	2.808	2.892	2.946	3.058	3.098	3.118	3.138	3.148	3.155	3.167
50	2.326	2.722	2.885	2.975	3.031	3.151	3.193	3.214	3.236	3.246	3.253	3.266
100	2.333	2.731	2.895	2.986	3.042	3.163	3.205	3.226	3.248	3.259	3.266	3.279
300	2.337	2.737	2.902	2.993	3.050	3.171	3.213	3.235	3.257	3.268	3.274	3.288
500	2.338	2.738	2.904	2.994	3.051	3.172	3.215	3.236	3.258	3.269	3.276	3.290

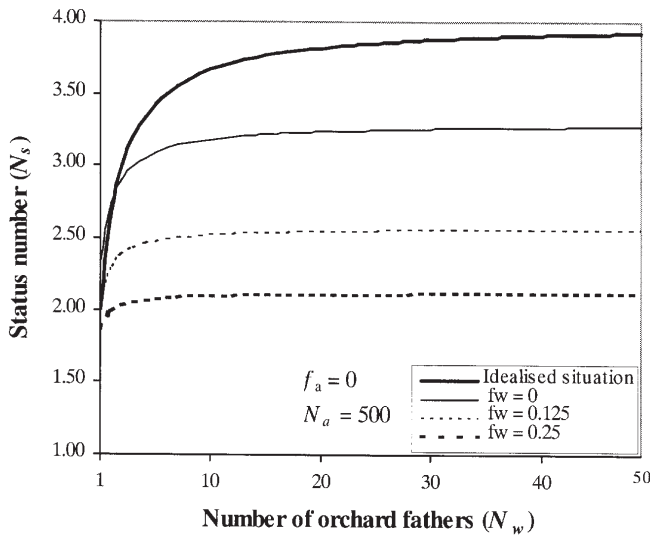


Fig. 1. – Status number (N_s) of seeds from a single clone. In the ideal situation ($f_w = f_a = 0, M = 0, S = 0, \psi_w = 1$), N_s approached four as the number of orchard fathers (N_w) increased. Relatedness among orchard fathers (f_w) decreased N_s . In the numerical scenarios, pollen contamination was 40% ($M = 0.4$), selfing was 10% ($S = 0.1$) and fertility variations $\psi_w = 2$ & $\psi_a = 3$. The number of alien fathers (N_a) was set to be 500 that was reasonably large enough.

lity, no pollen contamination and no selfing), it was set that the ratio of selfed seeds (S) was 10%, and the level of pollen contamination (M) was 40%, respectively, which both values could be acceptable in most seed orchards (EL-KASSABY et al., 1989; SAVOLAINEN, 1991). The typical values of ψ_w and ψ_a were taken from BILA (2000): $\psi_w = 2$ and $\psi_a = 3$. The numbers of alien fathers (N_a) and orchard fathers (N_w) varied.

In the ideal situation where all fathers are equally fertile, and no pollen contamination and no selfing are assumed, the status number of seeds (N_s) from a single clone ranged between the minimum of two and the maximum of four as the number of orchard fathers (N_w) increased (Fig. 1). The maximum in status number is 4 if all seeds are half-sibs, without selfing (LINDGREN et al., 1996). However, under the example situations with non-relatedness, selfing, pollen contamination and fertility variation, N_s ranged from 2.14 to 3.29. When the number of

orchard fathers (N_w) was higher than 10, the status number was steadily increased. The number of alien fathers (N_a) had a little effect on the gene diversity of seeds compared with the number of orchard parents.

Based on literature review (see BILA, 2000), sibling coefficients (Ψ) are most likely to be around 2 in seed orchards and 3 in forest stands. Thus, we used $\psi_w = 2$ in the orchard and $\psi_a = 3$ in the surround population for calculation status number. Amount of selfed seeds produced was assumed to be 10% in this study. For the level of selfed sound seeds, the selfing rates of seed orchards estimated by isozyme analysis are mostly less than 10% (MÜLLER-STARCK, 1982; RUDIN et al., 1986) but may be higher in particular cases (RUDIN and EKBERG, 1982; LAI and WANG, 1997). When the number of alien fathers (N_a) was higher than 500, its impact on status number became relatively small, further increase of the number of alien fathers had little influence on the status number. Therefore, we chose $N_a = 500$ to calculate status number when there was relatedness among father parents (see below).

Relatedness among seeds from a single mother is illustrated in Figure 2. In figure 2-a), it looks like self-sibs or double full sibs. If the seed parent A were inbred (e.g., $F_A = 0.5$), the coancestry between selfed seeds would be 0.75. But, we assumed in the present study that the parents were not inbred ($F_A = 0$), and thus the coancestry between selfed seeds X1 and X2 equals $f_{(X1,X2)} = 0.5$.

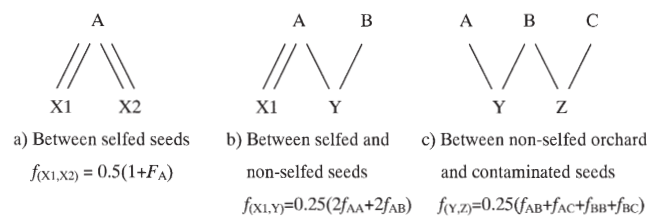


Fig. 2. – Relatedness (coancestry, f) among seeds from a single mother in a clonal seed orchard: a) self-sibs, b) double paring of a parent, and c) half-sibs. A and B are orchard parents and C represents an alien father.

For the coancestry between selfed and non-selfed seeds (Fig. 2-b); self-halves by ASKEW and EL-KASSABY (1994), f_{AA} equals to $0.5 \times (1 + F_A)$ and $f_{AB} = 0$ and thus $f_{(X1,Y)} = 0.25[2 \times (0.5(1+0) + 2(0))] = 0.25$ (Table 1). When the father

parents are equally fertile, there will be half sibs with the same family size (Fig. 2-c). However, as we deal with and discuss in this study, there may be half sibs, full sibs and relatives together if relatedness and fertility variation are assumed among the parents.

Relatedness between orchard pollen and contaminating pollen will not be considered through this study. Selected plus trees are transferred to the south or at low elevation for the establishment of seed orchards. Seed orchards have been established at the favourable place for better flowering and seed production. So, relatedness between orchard parents and trees near to the orchard can be ignored.

When there is some relatedness among parents

The results under the situation when there was some relatedness among trees are shown in Table 3 and Figure 1. Under this situation, various values of relatedness (f_w and f_a) among trees were assumed while other factors were the same as in the situation where there was no relatedness.

Under the situation where the relatedness among pollen parents existed, relatedness decreased the status number (Table 3 and Fig. 1). N_s decreased as relatedness among pollen parents (f_w and f_a) increased. On the other hand, N_s increased as the number of orchard fathers (N_w) increased. However, relatedness affected more strongly the decrease of status number than the number of orchard fathers. The effect of relatedness among alien fathers was small compared to that among orchard pollen parents.

If seeds were collected from a half-sib family parent as in seedling seed orchards ($f_w = 0.125$), N_s ranged between 2.08 and 2.45 depending on the relatedness among alien fathers and the number of orchard fathers. For all full-sib parents ($f_w = 0.25$), N_s was in general expected to be around 2.0 (Table 3).

Relatedness among individuals have built up through the aeons, the genes are copies of original ancestor's genes that are probably limited. The degree of relatedness depends on how many generations one goes back. On the other hand, there are mutations that make copies of the same gene different. Thus, relatedness and inbreeding are relative measures and related to the reference population where individuals are unrelated and non-inbred (KANG and LINDGREN, 1998). The formula [1] includes relatedness among seeds and fertility variation among

parents. Thus, the status number from the formula can be interpreted as the number of unrelated and non-inbred individuals with equal fertility.

Inbreeding means the mating among individuals that are related to each other by ancestry. The degree of relationship between the individuals in a population depends on the size of the population (FALCONER and MACKAY, 1996). The essential consequence of two individuals having a common ancestor is that they may carry replicates of one of the genes present in the ancestor, causing inbreeding and inbreeding depression in offspring.

The interesting matter is recent relatedness. For forest tree breeding, it is common to consider the plus trees as the starting point and reference. If they are considered as unrelated and non-inbred, we get the accumulated relatedness and inbreeding since the selection of plus trees. The more tree breeding is advanced, the more interest will be in the loss of gene diversity because of the consideration of alternatives where the orchard parents are relatives. It could also be the case for seedling seed orchards.

There might be no or very little relatedness among clones in first generation seed orchards because they are genotypes from different stands where plus trees were selected. In general, it is not allowed to select plus trees more than one within the same stand in order to avoid relatedness. This kind of effort might have the expected effect; there was no relatedness among plus trees, and thus no relatedness among clones. For alien pollen parents, however, they are likely to come from the same population with generation overlap or with related individuals who are growing closely in space, which all makes the higher probability of relatedness than that among orchard pollen parents.

Selfing effects

Similarly with relatedness, selfing decreased the status number of seeds from a single clone (Fig. 3). Under the idealized situation, N_s was ranged from 2 to maximum 4 (see also Fig. 1). Also, LINDGREN and MULLIN (1998) demonstrated that the maximum value of N_s was four under idealized situation. But, as selfing increased as 0%, 10%, 25% and 50%, N_s decreased as 3.94, 3.28, 2.55 and 1.78 under the same conditions as in Table 2 and $N_a = 500$.

Table 3. – Status number (N_s) in the case with that there was various relatedness among pollen parents (f_w, f_a) and different numbers of orchard fathers (N_w). The other factors were the same as in Table 2 ($M = 0.4, S = 0.1, \psi_w = 2$ and $\psi_a = 3$). The number of alien fathers (N_a) was set to be 500.

f_a	f_w	N_w											
		1	2	3	4	5	10	15	20	30	40	50	100
0	0	2.338	2.738	2.904	2.994	3.051	3.172	3.215	3.236	3.258	3.269	3.276	3.290
	0.125	2.080	2.304	2.390	2.436	2.464	2.522	2.542	2.552	2.563	2.568	2.571	2.577
	0.25	1.873	1.989	2.031	2.053	2.066	2.093	2.102	2.107	2.112	2.114	2.115	2.118
0.125	0	2.285	2.665	2.822	2.908	2.961	3.075	3.115	3.135	3.156	3.167	3.173	3.185
	0.125	2.037	2.253	2.335	2.378	2.405	2.460	2.480	2.489	2.499	2.504	2.507	2.513
	0.25	1.838	1.951	1.991	2.012	2.025	2.051	2.059	2.064	2.068	2.070	2.072	2.074
0.25	0	2.234	2.597	2.745	2.826	2.877	2.984	3.022	3.041	3.060	3.070	3.076	3.088
	0.125	1.997	2.203	2.282	2.323	2.349	2.402	2.420	2.429	2.438	2.443	2.446	2.451
	0.25	1.805	1.913	1.952	1.972	1.985	2.010	2.018	2.022	2.027	2.029	2.030	2.033

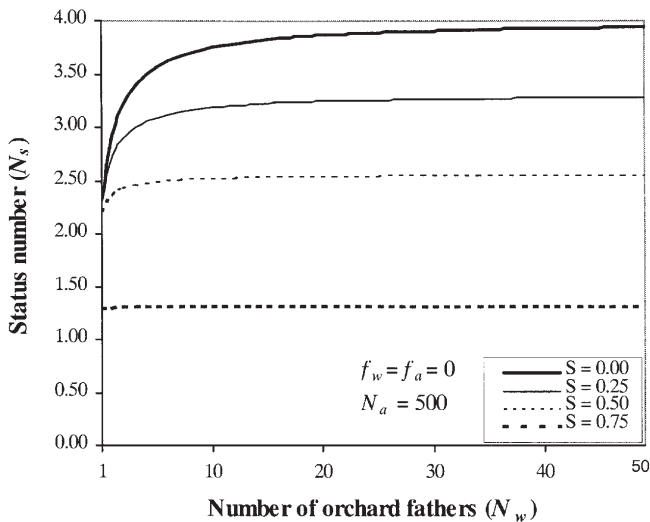


Fig. 3. – Status number (N_s) at the different levels of selfing (S) and orchard pollen parents (N_w) excluding the mother clone. Pollen contamination was 40% and fertility variations were $\psi_w = 2$ and $\psi_a = 3$. Non-relatedness was assumed.

In the numerical example, we did not consider the case when the number of orchard fathers equals zero ($N_w = 0$), except the mother tree. That doesn't seem to be realistic. But, if there is only one parent in the seed orchard, selfing equals 1-M. For this situation, the status number (N_s) increased from 1 to about 3.30 in the numerical example as the number of alien fathers (N_a) increased (results not shown).

If it is assumed that there is 100% selfing of a non-inbred individual, meaning that there is only one seed parent in the seed orchard and no pollen contamination, the status number of orchard crop is one because self- and cross-coancestries are all 0.5 and thus group coancestry becomes 0.5. On the other hand, if the seed parent is inbred and selfed completely ($F_A = 1$; a homogeneous line, $S = 100\%$), N_s of seeds for this case will be 0.5 that is the minimum value for the status number (LINDGREN et al., 1996).

With mating between related individuals, selfing further accelerates the reduction in gene diversity (heterozygosity) over generations (YEH, 2000). This is because inbreeding reduces the status effective number. If a species has, however, low rate of selfing as in most economic conifer species, the effect of inbreeding due to selfing on the decay of gene diversity is small relative to that of relatedness and fertility variation (YEH, 2000).

Pollen contamination

Pollen contamination increased N_s (LINDGREN and MULLIN, 1998). The large effect of pollen contamination appeared immediately when the number of orchard pollen parents increased only a few up to 10 (Fig. 4). After that, N_s was kept at almost the highest value of 3.29. The increase of status number caused by pollen contamination was relatively small when compared with the decrease caused by relatedness and selfing.

If there is 100% pollen contamination (i.e., $S = 0\%$, $N_w = 0$), N_s is a function of f_a , N_a and ψ_a . Contamination of seed orchard by background pollen depends on the isolation caused by distance and phenology as well as the ability of pollen dispersal and transport (PAULE et al., 1993). PRAT (1995) reported that effective pollen dispersal might be responsible for genetic variation in a Douglas-fir seed orchard.

Numerical results showed that the status number of seeds collected from a single clone generally ranged from 2 to 4 (Tables 2 and 3). Gene diversity (GD) of seeds can be calculated relative to the reference population as $GD = 1 - 1/2N_s$ (KANG and LINDGREN, 1998, 1999). Thus, the relative gene diversity of seeds collected from the same mother will range from 0.75 to 0.83. It means that loss of gene diversity will be about 15 to 27% if the parents were mating randomly.

From the results of relatedness, selfing, fertility variation and pollen contamination, it can be concluded that status number for a single clone harvest will seldom be expected to be much lower than that of an ideal half-sib family ($N_s = 4$). It may be lower if there is much selfing or high relatedness among clones. If there are more than 10 orchard pollen parents, it seems that the status number comes close to the maximum for any factors. When the number of pollen parents is only a few, the decrease of status number is very fast due to relatedness, selfing and fertility variation.

Number of fathers

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 (KANG et al., 2001a). From the sex ratio's point of view, 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 the population under consideration. KANG et al. (2001a) emphasized that inbreeding or genetic drift depends mainly on the numbers of the less-numerous sex. If there were a large number of pollen parents but only one seed parent, the status number of seeds would be only 4 as in the present study.

For both formulae derived in the present study, there is a common problem when estimating the status number of seeds from seed orchards. That is, we don't know exactly how many orchard fathers are contributing to produce orchard seeds from a single clone. It may be known when the number of male flowers or pollen production is accessed. But, that is still not the true contribution of fathers because of pollen dispersal, pollen competition and incompatibility.

It is hard to know the number of genotypes outside the orchard, which also contributes for seed production. However, it is obvious that the number of alien fathers is not equal to the number of the genotypes within the orchard but probably more.

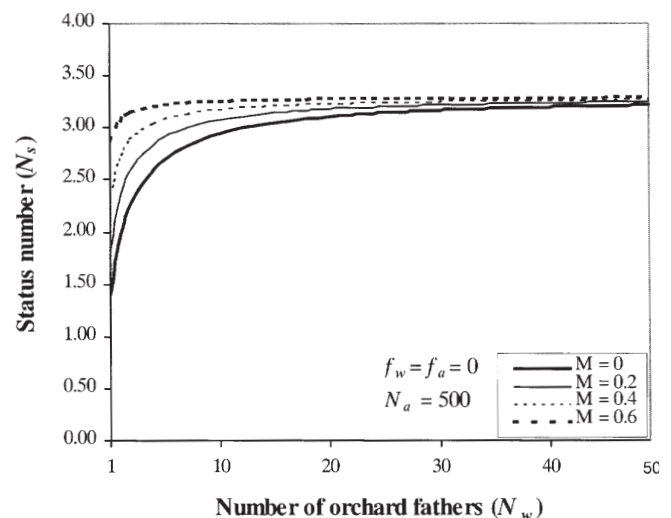


Fig. 4. – Status number (N_s) at the different levels of pollen contamination (M) and orchard pollen parents (N_w). Ratio of selfed seeds (S) was 10% and fertility variations were $\psi_w = 2$ and $\psi_a = 3$.

Because of many reasons such as asynchrony, fertility difference and spatial isolation, only a part of clones in the orchard will contribute for seed production as fathers, which causes fertility variation. When we estimate the sibling coefficient Ψ (fertility variation), the number of parents should be known.

Higher gain can be achieved by planting seedlings by family blocks, rather than mixing seeds from a given seed orchard (LI et al., 1999). The family block system redistributes the offspring of the production orchard, but it does not much reduce the genetic diversity found in the orchard because the parental contribution comes from all parents in the orchard since each parent produces both female and male gametes (DUZAN and WILLIAMS, 1988). Thus, genes from all parents in the orchards can be represented in the use of half-sib families. Risk of catastrophic disaster from pests may be slight because genetic variability within a half-sib family block is still large. Even if there is severe pest attack, foresters can salvage the infected blocks in plantations or through rapid screening in the orchards. The most resistant parents can be crossed via supplemental mass pollination and then outplanted where the pest attack is likely to occur. Bishir and Roberds (1999) reported using computer simulation that the number of clone needed decreased as the intensity of pest attack increased. So, it would be better to use a moderate number of clones rather than many.

Several authors found differences in the reproductive success of different fathers in pollen mixtures or supplemental mass pollination (MORAN and GRIFFIN, 1986, CHELIAK et al., 1987; SCHOEN and STEWART, 1986). Differences in the reproductive success will make an increased fertility variation among pollen parents and decrease status number. But, in the other study, YAZDANI and LINDGREN (1991) did not find much difference.

Acknowledgement

This study was supported financially by the Korea Forest Research Institute, the Kempe Foundation, the Föreningen Skogstrådsförädling and the Swedish Institute. This work was done when the first author was a post-graduate student and the second was a visiting scientist at the Department of Forest Genetics and Plant Physiology in the Swedish University of Agricultural Sciences (SLU-Umeå).

Literature cited

ASKEW, G. R. and EL-KASSABY, Y. A.: Estimation of relationship coefficients among progeny derived from wind-pollinated orchard seeds. *Theor. Appl. Genet.* **88**: 267–271 (1994). — BILA, A.: Fertility variation and its affect of gene diversity of forest tree. Ph. D. thesis. The Swedish University of Agricultural Sciences, Umeå, Sweden. *Acta Universitatis Agriculturae Sueciae, Silvestria* 166 (2000). — BISHIR, J. and ROBERDS, J. H.: On numbers of clones needed for managing risks in clonal forestry. *For. Genet.* **6**: 149–155 (1999). — BONDESSON, L. and LINDGREN, D.: Optimal utilization of clones and genetic thinning of seed orchards. *Silvae Genet.* **42**: 157–163 (1993). — CHELIAK, W. M., SKROPPA, T. and PITEK, J. A.: Genetics of polycross 1. Experimental results from Norway spruce. *Theor. Appl. Genet.* **73**: 321–329 (1987). — DUZAN, H. W. and WILLIAMS, C. G.: Matching loblolly pine families to regeneration sites. *South. J. Appl. For.* **12**: 166–169 (1988). — EL-KASSABY, Y. A., RUDIN, D. and YAZDANI, R.: Levels of out-crossing and contamination in two *Pinus sylvestris* seed orchards in northern Sweden. *Scand. J. For. Res.* **4**: 41–49 (1989). — ERIKSSON, G., JONSSON, A. and LINDGREN, D.: Flowering in a clonal trial of *Picea abies* (KARST.). *Stud. For. Suec.* **110**, 45pp. (1973). — FALCONER, D. S. and MACKAY, T. F. C.: Introduction to quantitative genetics. Longman, Malaysia, 1996. p. 48–99 (1996). — HARJU, A.: Genetic functioning of Scots pine seed orchards. Ph. D. thesis. University of Oulu, Finland. *Acta Universitatis Ouluensis* 271 (1995). — KANG, K. S. and LINDGREN, D.: Fertility variation and its effect on the relatedness of seeds in *Pinus densiflora*, *Pinus thunbergii* and *Pinus koraiensis* clonal seed orchards. *Silvae Genet.* **47**: 196–201 (1998). — KANG, K. S. and LINDGREN, D.: Fertility variation among clones of Korean pine (*Pinus koraiensis*) and its implications on seed orchard management. *For. Genet.* **6**: 191–200 (1999). — KANG, K. S., LINDGREN, D. and MULLIN, T. J.: Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies. *Theor.*

Appl. Genet. **103**(6–7): 1099–1107 (2001a). — KANG, K. S., HARJU, A. M., LINDGREN, D., NIKKANEN, T., ALMQVIST, C. and SUH, G. U.: Variation in effective number of clones in seed orchards. *New Forests* **21**(1): 17–33 (2001b). — KJÆR, E. D.: Estimation of effective population number in a *Picea abies* (KARST) seed orchard based on flower assessment. *Scand. J. For. Res.* **11**: 111–121 (1996). — LAI, H.-L. and WANG, Z.-R.: Advance in research on mating system of forest populations. *World Forestry Research* **10**(5): 10–15 (1997). — LI, B., MCKEAND, S. and WEIR, R.: Tree improvement and sustainable forestry – impact of two cycles of loblolly pine breeding in the USA. *For. Genet.* **6**: 229–234 (1999). — LINDGREN, D. and EL-KASSABY, Y. A.: Genetic consequences of combining selective cone harvesting and genetic thinning in clonal seed orchards. *Silvae Genet.* **38**: 65–70 (1989). — LINDGREN, D. and MULLIN, T. J.: Relatedness and status number in seed orchard crops. *Can. J. For. Res.* **28**: 276–283 (1998). — LINDGREN, D., GEA, L. D. and JEFFERSON, P. A.: Loss of genetic diversity monitored by status number. *Silvae Genet.* **25**: 52–59 (1996). — MCKEAND, S. E., CROOK, R. P. and ALLEN, H. L.: Genotypic stability effects on predicted family responses to silvicultural treatments in loblolly pine. *Southern J. of Applied Forestry* **21**(2): 84–89 (1997). — MORAN, G. F. and GRIFFIN, A. R.: Non-random contribution of pollen in polycrosses of *Pinus radiata* D. DON. *Silvae Genet.* **34**: 117–121 (1986). — MÜLLER-STARCK, G.: Reproductive systems in conifer seed orchards. 1. Mating probabilities in a seed orchard of *Pinus sylvestris* L. *Silvae Genet.* **31**: 188–197 (1982). — MUHS, H.-J.: Policies, regulations, and laws affecting clonal forestry. In: *Clonal forestry*, Vol. 2, (eds. M. AHUJA & W. LIBBY). p. 215–227. Springer-Verlag. (1993) — OECD scheme for the control of forest reproductive material moving in international trade, C(74)29 Final of 5th March, 1974. Paris. 24p. (1974). — PAULE, L., LINDGREN, D. and YAZDANI, R.: Allozyme frequencies, out-crossing rate and pollen contamination in *Picea abies* seed orchards. *Scand. J. For. Res.* **8**: 8–17 (1993). — PRAT, D.: Mating system in a clonal Douglas fir (*Pseudotsuga menziesii* (MIRB) FRANCO) seed orchard. II. Effective pollen disposal. *Ann. Sci. For.* **52**: 213–222 (1995). — RUDIN, D., MUONA, O. and YAZDANI, R.: Comparison of the mating system of *Pinus sylvestris* in natural stands and seed orchards. *Hereditas*, **104**: 15–19 (1986). — RUDIN, D. and EKBERG, I.: Genetic structure of open pollinated progenies from a seed orchard of *Pinus sylvestris*. *Silvae Genet.* **16**: 87–93 (1982). — SAVOLAINEN, O.: Background pollination in seed orchards. *Proc. Mtg. Nordic Tree Breeding Group in Sweden*, 1991. p. 6–13 (1991). — SCHOEN, D. J. and STEWARD, S. C.: Variation in male reproductive investment and male reproductive success in white pine. *Evolution*, **40**: 1109–1120 (1986). — YAZDANI, R. and LINDGREN, D.: The impact of self-pollination on production of sound selfed seeds. In: *Biochemical markers in the population genetics of forest trees* (Ed. S. FINE-SCHI, M. E. MALVOLTI, F. CANNATA and H. H. HATTEMER.) p. 143–147 SPB Academic Publishing, Hague, The Netherlands (1991). — YEH, F. C.: Population genetics. In: *Forest conservation genetics – principles and practice*. Eds) A. YOUNG, D. BOSHIER and T. BOYLE. CABI publishing. London. p. 21–37 (2000).

Appendix

According to KANG and LINDGREN (1998), the relationship between sibling coefficient (Ψ) and coefficient of variation (CV) is

$$\Psi = \frac{CV^2(N-1)}{N} + 1 \text{ and thus } CV^2 = \frac{N(\Psi-1)}{N-1}$$

Sibling coefficient (Ψ) describes how much orchard parents vary in fertility. It can be interpreted as the probability for sibs to occur when compared to the situation where parents are equally contributing to the gamete pool.

Since we are dealing with the whole population instead of samples, $(N-1)/N$ can be removed (KANG and LINDGREN, 1999). Hence, $\Psi = CV^2 + 1$ and thus $CV^2 = \Psi - 1$.

In this study, we designate that the corresponding Ψ will be Ψ_w and Ψ_a for fertility variation among orchard fathers (excluding seed parent) and among alien pollen parents outside the orchard, respectively.

Based on the basic and general statistics with a random variable x ,

$$\sum x_i^2 = N * \text{Var}(x_i) + N * \bar{x}^2 \text{ and } \text{Var}(x_i) = \bar{x}^2 * CV^2.$$

Thus, we can get a new formula as

$$\sum_{i=1}^N x_i^2 = N * \bar{x}^2 (CV^2 + 1)$$

Therefore, the squared sum of orchard pollen father's contribution will be

$$\sum_{i=1}^{N_w} n_i^2 = N_w \left[\frac{(1-M-S)N}{N_w} \right]^2 (CV^2 + 1)$$

$$= \frac{\psi_w N^2 (1-M-S)^2}{N_w}$$

From the same way, the squared sum of alien pollen father's contribution becomes

$$\sum_{m=1}^{N_a} a_m^2 = \frac{\psi_a N^2 M^2}{N_a}$$

Comparison of Levels of Genetic Diversity Detected with AFLP and Microsatellite Markers within and among Mixed *Q. petraea* (MATT.) LIEBL. and *Q. robur* L. Stands

By S. MARIETTE^{1*}, J. COTTRELL², U. M. CSAIKL³, P. GOIKOECHEA⁴, A. KÖNIG⁵, A. J. LOWE⁶, B. C. VAN DAM⁷, T. BARRENECHE¹, C. BODÉNES¹, R. STREIFF¹, K. BURG³, K. GROPPÉ⁵, R. C. MUNRO⁶, H. TABBENER² and A. KREMER¹)

(Received 11th June 2001)

Summary

The aim of this study was to compare genetic diversity within and among *Quercus* spp. populations based on two contrasting types of nuclear markers. Seven mixed stands of *Quercus petraea* and *Quercus robur* were analysed using six highly polymorphic and codominantly inherited microsatellite markers as well as 155 dominant AFLP markers. Genetic differentiation and genetic diversity within each population were assessed. The intra- and inter-locus variances were calculated, and the results were used to compare the genetic diversity between populations. Both classes of markers revealed similar results: the genetic diversity within population and the genetic differentiation among populations is greater in *Q. petraea* than in *Q. robur*. The genetic differentiation is generally higher when AFLP markers were used in comparison to microsatellites. For AFLPs, the inter-locus variance is always much higher than the intra-locus variance, and explains why it was not possible to distinguish populations using this marker system. Finally, no significant positive correlation was found between the level of within-population diversity assessed with the two markers.

Key words: microsatellite, AFLP, genetic diversity, genetic differentiation, *Quercus robur*, *Quercus petraea*.

Introduction

The assessment of genetic diversity using nuclear markers can follow two different strategies for the species tested: sampling different populations and individuals as well as analysing an expanding number of loci. The associated components of sampling variance have been termed "intra-locus variance" and "inter-locus variance", respectively, and, from the theoretical point of view, the inter-locus variance is considered to be much higher than the intra-locus component (NEI, 1987). Several PCR-based marker technologies are available now to characterise the genetic diversity of a species directly at the level of DNA (KARP et al., 1997). Microsatellite markers are provided

by sequence-specific primer pairs and this makes their development time-consuming and expensive. However, once developed, they yield a maximum of information with refer to their codominant inheritance which enables to distinguish between both alleles of a single heterozygous locus. In contrast, markers such as Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) are more efficient since no species-specific sequence information is required for the development of PCR primers. They provide information on many loci which are commonly randomly distributed throughout the genome; however, such markers are usually dominantly inherited (BREYNE et al., 1997). Consequently, for a given investment of time and money, multilocus profiling techniques provide information on an enlarged number of loci in contrast to one-locus specific microsatellites, but the genotypic information obtained from each locus is reduced due to the dominant inheritance. Two contrasting sampling strategies can

¹ INRA, Equipe de Génétique et Amélioration des Arbres Forestiers, BP45, F-33610 Cestas, France.

² Forest Research, Northern Research Station, Roslin, Midlothian, Scotland, EH25 9SY, United Kingdom.

³ Österreichisches Forschungszentrum, Seibersdorf Ges. m. b. H., A-2444 Seibersdorf, Austria.

⁴ NEIKER, Dpto Producción y Protección Vegetal, Granja Modelo-Arkaute, Apdo 46, 01080 Vitoria Gasteiz, Spain.

⁵ BFH, Institut für Forstgenetik und Forstpflanzenzüchtung, Sieker Landstrasse 2, 22927 Grosshandorf, Germany.

⁶ Centre for Ecology and Hydrology, CEH, Edinburgh Bush Estate, Penicuik, Midlothian, EH26 0QB, Scotland, United Kingdom.

⁷ Alterra Green World Research, POB 47, Droevendaalsesteeg 3, 6700 AA Wageningen, The Netherlands.

*Corresponding author: STÉPHANIE MARIETTE. Present address: Cemagref, U.R. "Ressources Génétiques et Plantes Forestières", Domaine des Barres, 45290 Nogent-sur-Vernisson, France.