Fertility Variation and its Effect on the Relatedness of Seeds in
Pinus densiflora, Pinus thunbergii and Pinus koraiensis Clonal Seed Orchards

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(Received 26th February 1998)

Abstract

The numbers of female and male strobili were counted in clonal seed orchards with 99 clones of Pinus densiflora and 60 clones of P. thunbergii and in an archive consisting of 180 clones of P. koraiensis, respectively. The observation data showed a great variation in both female and male strobili among clones in the three populations. It was possible to express the expected contribution of genotypes to seed crop as an inverse of the square sum of contribution. The status numbers were calculated to be 69.2 (70% of the initial census number), 54.6 (91%) and 38.8 (22%) in the variances effective population size could be related to the square sum of contribution.

Introduction

The genetic quality of seed crop from a clonal seed orchard is greatly affected by the genetic values and the mating system of the orchard clones. Mating conditions, which are valid for seed orchards in panmicotic equilibrium, are important prerequisites if the orchard crop is to reflect both the genetic superiority and diversity present among the seed orchard clones (MUONA and HARU, 1989; EL-KASSABY and ASKIE, 1991; CHAISSURISSI and EL-KASSABY, 1993; FLAT and CAQUELARD, 1995). The differences in gamete contribution among clones in seed orchards have been shown to be genetic rather than environmental in most investigations (GRIFFIN, 1982; BYRAM et al., 1986; EL-KASSABY et al., 1988; EL-KASSABY and REYNOLDS, 1990; SAVOLAINEN et al., 1993; EL-KASSABY and COOK, 1994; KJÆR, 1996; BURZYCKI and CHALUPKA, 1997; KJÆR and WELLENDORF, 1998). Unequal gamete contributions, therefore, reduce the effective population size so that genetic drift and an increase in inbreeding take place more rapidly than would be predicted from the census number used in a seed orchard (KJÆR, 1996). The number of clones used in clonal seed orchards should be determined to maintain high effective population number and low inbreeding by optimizing the use of the clones with the best breeding values (LINDGREN, 1993). The census number in orchards should thus be adjusted to take into account the quantitative impact of unequal gamete contribution.

Differences among clones in gamete contributions influence the genetic composition of seed orchard crops by over-representing the most productive genotypes (KJÆR, 1996), which might lead to accumulation of coancestry and loss of gene diversity (LINDGREN et al., 1996). Variation in fertility also has important implications in breeding (GRIFFIN, 1982; XIE and KNOWLES, 1992; EL-KASSABY, 1995) and conservation programs (SEIDGLEY and GRIFFIN, 1989). Gamete contributions can be estimated in open-pollinated seeds based on genetic markers such as isozymes (RUDIN and LINDGREN, 1977; SHEN et al., 1981; XIE and KNOWLES, 1994), but these investigations are expensive and it is difficult to get high accuracy on individual gamete contributions. They can also be obtained from the assessment of flowering and seed production (GRIFFIN, 1982; CHAISSURISSI and EL-KASSABY, 1993; EL-KASSABY and COOK, 1994). Flowering phenology may be important in particular cases, but in general the quantitative amount of flowering is most important because the genetic composition of progeny depends most
heavily on the numbers of male and female strobili produced by each clone, which largely overshadow the effects of the other variables such as stage of pollen release and female receptivity (Eriksson et al., 1973; Jonsson et al., 1976; O’Reilly et al., 1982).

The purposes of the present paper are to formulate an expression of expected contribution of orchard genotype to seed crop by a single parameter that is describing the fertility variation, and to calculate status number and variance effective population size relevant for harvests from the idealised clonal seed orchards. It is also to estimate gene diversity of seed crops based on assessment of female and male strobili for orchard clones. The level of relatedness of seed crop and its effects on the gene diversity of the seed orchard crop are also dealt with.

Materials and Methods

The seed orchards

The study was performed in Pinus densiflora S. et Z., P. thunbergii Pal. clonal seed orchards and a P. koraiensis S. et Z. clonal archives. They are all among the most important native species in Korea. The P. densiflora and P. thunbergii orchards were located together in Anmyun (at latitude 36° 3’ N, longitude 126° 2’ E and altitude 35 m), Chuncham-do, Korea. The Forest Genetics Research Institute of Korea established them in 1977 and 1979, respectively. The clone bank of P. koraiensis was located at Gomae experiment forest in Suwon (at latitude 37° 7’ N, longitude 127° 2’ E and altitude 100 m), Kyonggi-do, Korea and was established in 1985. The clones originated from the plus trees selected in natural stands in Korea. The design of the two orchards was essentially random, while the clone bank was a row plantation with 5 ramets per clone. All grafts were planted at 5 m x 5 m spacing.

Although the numbers of ramets per clone differed, we assumed that all clones were equally represented. Seed orchards consisted of 99 clones of P. densiflora, 60 clones of P. thunbergii, and 180 clones of P. koraiensis. We also assumed that the pollen contamination in these seed orchards was negligible and that clones in these orchards were not related and non-inbred.

Assessment of female and male strobili

The numbers of female and male strobili were estimated for the 99 clones in the P. densiflora and 60 clones in the P. thunbergii clonal seed orchards in May 1997. Six ramets per clone were chosen randomly for assessment, avoiding trees growing at the edges of the seed orchards. Clonal variation of flower productions in the P. koraiensis clone bank was assessed by counting 5 ramets per clone in early June 1995. Female and male strobili of the sampled grafts of P. densiflora and P. koraiensis were counted individually over the whole crown. As P. thunbergii is a species producing many female and male strobili, the total numbers of strobili were estimated by multiplying the average numbers of strobili per branch by the total number of branches bearing strobili.

Expression of expected contribution by a single parameter

The cumulative contribution to orchard crop by the seed orchard genotypes which were ranked according to their relative contribution can be described by a function of type, \( F(x) = x^a \) (Bila and Lindgren, 1998, the function may be called as the power function), where \( x \) is the fraction of genotypes ranked by relative contribution and \( a \) is a parameter that cannot be less than one. The probability density function, \( f(x) = ax^{a-1} \), corresponds to the relative contribution of seed orchard genotypes if there is a continuum of genotypes. The inverse of the cumulative expression, \( x = F(x)^{1/a} \), can be used for calculating expected contributions as a function of parameter \( a \). The expected contributions for different values of parameter \( a \) in a seed orchard consisting of 100 clones are presented in figure 1.

\[
\text{Parameter } A \text{ (and thus also } a) \text{ are related to the coefficient of variation (C.V.(%)) for flower production of orchard clones as follows,}
\]

\[
A = \left( \frac{C.V. \cdot 100}{100} \right)^2 \times \frac{N - 1}{N} + 1
\]

\[
\text{In principle, } A \text{ can be regarded as a underlying characteristic of the probability density function of fertility while } C.V. \text{ is a normalised observed variance which is dependent on the number of observation.}
\]

Status effective number

Status number \( (N_s) \) was defined as half the inverse of group coancestry, thus \( N_s = 1/20 \) (Lindgren et al., 1996). In this study we used this approach. For the case where orchard clones are neither related nor inbred that we will assume throughout this study, the group coancestry \( (\Theta) \) could be calculated as (Lindgren and Mullin, 1998),

\[
\Theta = \frac{1}{2} \sum_{i=1}^{N} p_{i}^2
\]
And thus, the status number equals to be

\[ N_s = \frac{1}{\sum_{i=1}^{N} p_i^2} \]

where \( p_i \) is the expected contribution from individual genotype \( i \) to the seed orchard crop and \( N \) is the census number of clones. To compare between census number of clones and status effective number in the seed orchards, the relation, \( N_r = \frac{N_s}{N} \) was used.

From the definition of group coancestry and status number, it follows that the integral of squared contributions, parameter \( A \) equals \( \frac{1}{N_r} \). If there is no inbreeding or coancestry among clones in the seed orchard, which is assumed in this study, the status effective number will become

\[ N_s = \frac{N}{A} \]

Variance effective population size

Variance effective population size (\( N_e^{(v)} \)) was calculated with modification of equations from KJÆR (1996) as follows,

\[ N_e^{(v)} = \frac{N}{(A - 1)} \]

The variance effective population size describes the size of sample that would give the same drift in gene frequencies as the seed crop compared to the seed orchard clones (LINDGREN and MULLIN, 1998). A large number of seeds are collected from clonal seed orchards, thus we can neglect stochastic variations. The variance effective population size depends on the generations between which the rate of change is considered (i.e., it is defined as a measure of rate but not of state).

Gene diversity of seed crop

For the seed crop, gene diversity (\( GD \)), which can also be termed an expected heterozygosity of founder genes, was calculated (WRIGHT, 1969; NEI, 1973; LACY, 1995) as follows,

\[ GD = 1 - \frac{1}{2 N_s} \]

Gene diversity is a direct function of group coancestry, but the coancestry concept requires a reference where trees can be regarded as unrelated. Here we regarded the wild populations from which plus trees were selected as the reference. Every gene in the reference population was regarded as unique by descent.

Results

A great variation in both female and male strobili production among clones in three species was observed (Table 1). In the \( P. \ densiflora \) seed orchard, the female strobilus production per clone ranged between 0 and 105 (averaging 25.1) and that of male strobili ranged between 0 and 1,540 (averaging 628.2). The mean numbers of female and male strobili in the \( P. \ thunbergii \) clonal seed orchard were 159.3 (range 63 to 9,556) and 765.4 (range 120 to 45,923), respectively.

The differences of male strobilus production among clones in the \( P. \ koraiensis \) clone bank were far more extreme than differences of female strobilus production, and than those for strobilus production in \( P. \ densiflora \) and \( P. \ thunbergii \). The mean numbers of female and male strobili in the \( P. \ koraiensis \) clone bank were small. On the other hand, the coefficients of variation were large. The effects of large flowering variation on genetic diversity in the seeds that are resulted from this differential production of male and female gametes may be important (O’REILLY et al., 1982). The correlations between female and male strobili production were weak in the three orchards and none of them was significantly different from zero, therefore, we did not take these into account.

Calculations of parameter \( A \) and \( a \), status number (\( N_s \)), variance effective population size (\( N_e^{(v)} \)) and relative gene diversity (\( GD \)) were based on the counts of female and male strobili production per clone (Table 2). For total flowering, those were calculated on the basis of the relative expected contribution of female and male. Parameter \( A \) and \( a \) were smaller in the species with lower coefficient of variation (see Table 1). \( N_s \) was estimated as 69.2 (70%), 54.6 (91%) and 38.8 (22%) in the expected crops of the assumed clonal seed orchards of \( P. \ densiflora \), \( P. \ thunbergii \) and \( P. \ koraiensis \), respectively.

Relative status numbers (\( N_r \)) varied if clonal contribution was estimated by female, male and total strobili production in the three populations. \( N_r \) was high for small \( A \) where group coancestries were also low. The highest relative status numbers of female and male parents were calculated to be 0.89 and 0.76, respectively, in the \( P. \ thunbergii \) seed orchard. Clonal variation of strobilus production in \( P. \ thunbergii \) seems to be less variable than in the other species. Relative status numbers were higher in those seed orchards with smaller variances. The

<table>
<thead>
<tr>
<th>Seed orchard</th>
<th>Number of clones</th>
<th>Female Average</th>
<th>C.V.(%)</th>
<th>Male Average</th>
<th>C.V.(%)</th>
<th>Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P. \ densiflora )</td>
<td>99</td>
<td>25.1</td>
<td>93.8</td>
<td>628.2</td>
<td>64.2</td>
<td>-0.018</td>
</tr>
<tr>
<td>( P. \ thunbergii )</td>
<td>60</td>
<td>159.3</td>
<td>35.8</td>
<td>765.4</td>
<td>57.2</td>
<td>-0.125</td>
</tr>
<tr>
<td>( P. \ koraiensis )</td>
<td>180</td>
<td>3.4</td>
<td>112.5</td>
<td>44.0</td>
<td>376.6</td>
<td>-0.095</td>
</tr>
</tbody>
</table>

\(^{a)}\) coefficient of variation per clone.

\(^{b)}\) between number of female and male strobili per clone.
Table 2. – Parameter A and a, group coancestry (Θ), status number (N_s), relative status number (N_r), variance effective population size (N_e) and relative gene diversity (GD) of the predicted orchard crop based on the fertility variation estimated by female, male and total strobili production.

<table>
<thead>
<tr>
<th>Seed orchard</th>
<th>P. densiflora (99)</th>
<th>P. thunbergii (60)</th>
<th>P. koraiensis (180)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.87 1.41 1.43</td>
<td>1.12 1.32 1.10</td>
<td>2.26 15.10 4.64</td>
</tr>
<tr>
<td>a</td>
<td>3.15 2.18 2.21</td>
<td>1.50 1.97 1.43</td>
<td>3.94 29.70 8.75</td>
</tr>
<tr>
<td>Θ</td>
<td>0.00945 0.00714 0.00722</td>
<td>0.00937 0.01099 0.00915</td>
<td>0.00627 0.04195 0.01289</td>
</tr>
<tr>
<td>N_s</td>
<td>52.9 70.0 69.2</td>
<td>53.4 45.5 54.6</td>
<td>79.7 11.9 38.8</td>
</tr>
<tr>
<td>N_r</td>
<td>0.53 0.71 0.70</td>
<td>0.89 0.76 0.91</td>
<td>0.44 0.07 0.22</td>
</tr>
<tr>
<td>N_e(v)</td>
<td>113.7 239.5 230.3</td>
<td>482.6 188.0 610.3</td>
<td>143.1 12.8 49.4</td>
</tr>
<tr>
<td>GD</td>
<td>0.991 0.993 0.993</td>
<td>0.991 0.989 0.991</td>
<td>0.994 0.958 0.987</td>
</tr>
</tbody>
</table>

*) Clone number in parentheses

Figure 2. – Observed and expected gamete contributions (pi) in the seed crop as a function of a in Pinus densiflora (1F, 1M), P. thunbergii (2F, 2M) and P. koraiensis (3F, 3M) clonal seed orchards. Expected gamete contribution can be calculated by the inverse of cumulative expression, x = F(x)^(1/a). F and M represent female and male, respectively.
results also show that as much as 70%, 91% and 22% of total census number of clones could be expected to have the same group coancestry and degree of average relatedness as the seed crop in the gamete gene pools of the Pinus densiflora, P. thunbergii and P. koraiensis seed orchards, respectively. The status numbers in table 2 are somewhat lower but similar in magnitude to the actual number of seed orchard clones. The status number is lower than the census number of clones and reflects differences in flower production among seed orchard clones (LINDGREN and MULLIN, 1998).

Variance effective population sizes \( N_e^{(i)} \) were also estimated based on the observations. The variance effective population sizes differed from status numbers, depending on the population sampled. In general, effective population size decreases as variation in the proportional gamete contribution of orchard clones increases (XIE et al., 1994). The variance effective population sizes were therefore relatively high in the P. densiflora and P. thunbergii seed orchards but low in the P. koraiensis seed orchard (i.e., \( N_e^{(i)} = 230, 610 \) and 49, respectively).

Decreases of gene diversity (GD) in the seed crops from studied orchards, compared to wild reference populations from which the orchard clones were derived, were very small. The values of relative gene diversity compared to the reference populations were quite high in all first generation seed orchards.

Expected gamete contributions of individual clones could be calculated as a function of \( a \) by the inverse of cumulative expression, \( x = \frac{F(x)^a}{a} \). Obtained and expected gamete contributions as a function of \( a \) in three seed orchards were then expressed in figure 2.

Discussion

The productions of male and female strobili were found to vary among clones in the three clonal seed orchards. O'Reilly et al. (1982) reported that the genetic composition of the progeny depended most heavily on the numbers of female and male strobili produced by each clone, overshadowing the effects of contributing variables such as stages of pollen release and female receptivity. Therefore, some seed orchard management options may be proposed such as an equal cone-collection from each clone and supplemental mass pollination to alleviate the effect of parental imbalance and panmictic disequilibrium in the P. densiflora and P. koraiensis seed orchards.

However, the group coancestry seems to be affected weakly by variations in strobilus production although flowering in an orchard is dominated by only a few clones. Promotion of flowering in the P. koraiensis seed orchard is desirable to improve seed production and to increase status effective number, especially making paternal fertility more even. Differences in female and male strobili production between seed orchard clones are largely dependent upon genetic factors (Eriksson et al., 1973; Jonsson et al., 1976; KJÆR, 1996). Thus, it might be possible to identify consistently high- or low-flowering clones, and these clones could be considered selectively in the seed orchard management such as roguing or cone harvesting (CHAI SURISRI and EL-KASSABY, 1993; XIE and KNOWLES, 1994).

The effects of fertility variation on effective population size for forest tree species have been reported in seed orchards (MUONA and HARJU, 1989; CHAI SURISRI and EL-KASSABY, 1993; XIE et al., 1994; KJÆR, 1996; KJÆR and WELLENDORF, 1997). These papers concluded that there were reductions of effective population size due to different contributions of clone to the gamete gene pool. CHAI SURISRI and EL-KASSABY (1993) estimated that the proportion of female effective and actual numbers were 0.45 and 0.50 for seed-cone and filled-seed crops in a Sitka spruce seed orchard. KJÆR (1996) reported that effective population size varied with relative clone flowering in Norway spruce seed orchard, and KJÆR and WELLENDORF (1997) estimated that status number and variance effective population size were 76.0 and 315.2, respectively, for the 100 clones in a Danish Norway spruce seed orchard. These results are similar of those in the present study where relative status effective numbers are calculated 70%, 91% and 22% in P. densiflora, P. thunbergii and P. koraiensis seed orchards, respectively.

Status number is small in the young P. koraiensis seed orchard, indicating that it is likely that status number of the crop may be lower in a young seed orchard than at maturity. It is also indicating that the level of relative effective population size may depend on age of the clonal seed orchard (KJÆR and WELLENDORF, 1997). When the effective population size of an orchard is small, the genetic composition of the seed crop may be different from that expected from the genetic composition of the clones in the orchard. A small ratio of effective to actual population size in the seed orchards could result in low genetic diversity of orchard seeds if the clone number is not high. Therefore, information about different contributions of clones to total gamete production is important when tree breeders make assumptions about the expected genetic composition in orchard seed crops used for reforestation (BURCZYK, 1996).

Parameter \( A \) is describing the fertility variation in the seed orchards. The parameter \( A \) is not dependent on relatedness. If \( A \) equals 1, it means that all clones have equal contributions. Therefore, this parameter \( A \) describes the same fertility variation as real situation of flowering variation for the seed orchards consisting of unrelated clones. But it could differ from the real fertility variation in the sampled breeding population due to genetic drift or relatedness. Formula (2) was formulated on the basis of the sampled population. But this equation can also be used for the entire population with small modification.

We assumed that strobilus counts were a good measure of gamete contributions in the seed crop. Different flowering times, pollination preferences or differential selfing incompatibilities could also affect to gamete contributions of clones in seed orchards. To speak generally, however, the quantitative amount of flowering is most important factor to affect on the genetic composition of progeny.

We have not calculated effective population size in the inbreeding sense, and suggest that it does not make much sense to perform such calculations. Inbreeding effective population size describes how fast a population accumulates inbreeding over generations. If the clones are assumed to be unrelated, inbreeding arises only from selfing and it is more relevant to describe the selfing explicitly, rather than try to base an effective number on it (LINDGREN and MULLIN, 1998). Status number is not informative about the selfing, thus selfing consideration has to be made in addition to status number consideration for managing seed orchards and describing their crops.

Gene diversity is the variance in allele frequencies at a gene locus and is equal to the heterozygosity expected in a population with random union of gametes as in a population in Hardy-Weinberg equilibrium (LACY, 1995). For forest improvement or seed orchards, it seems meaningful to consider gene diversity compared to the wild forest of which a number of unrelated and non-inbred founders are composed, where each pair of alleles in each founder tree are considered as unique. Gene diversity in an orchard can be related to that in the wild
reference population, thus the diversity in the wild forest is setting the unit value 1. Relative gene diversity expressed in that way will be a relevant measure how much gene diversity has been reduced in the domestication process. Group coancestry is a direct measure for the loss of gene diversity. Gene diversity reflects both the number of initial unique alleles and the evenness of their frequencies, and it can be averaged over loci to provide a genome-wide measure of diversity. In the present study, decreases of gene diversity in the crops from first generation seed orchards, compared to the wild reference population from which the plus trees were collected, did not seem alarming (Table 2). As status number is a function of gene diversity, it may also be used when applying the technique to maximize gene diversity at a certain status number (LINDGREN and MULLIN, 1997).

Status effective number based on clonal variation of flowering gives orchard managers good information for management of seed orchards. Status number could also serve as a useful measure for the expected relatedness and gene diversity of orchard crops in the seed orchards, and for deciding of orchard management activity and of orchard composition on the basis of expected group coancestry.

Acknowledgements

This study was supported with grants from the Forest Genetics Research Institute of Korea and the Kempe foundation of Sweden. We thank E. ANDERSSON, L. SANCHEZ, S. RUOTSALAINEN and in particular T. J. MULLIN and an anonymous reviewer who made useful comments, which helped to improve the manuscript.

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