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**Integrating fecundity variation and genetic relatedness in estimating the gene
diversity of seed crops: *Pinus koraiensis* seed orchard as an example**

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Running title: Genetic gain and diversity in seed orchard crops

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Abstract

The genetic gain and gene diversity of seed crops from a 1.5-generation clonal seed orchard of *Pinus koraiensis* were estimated under consideration of parental genetic values and fecundity variation. Fecundity variation among clones was estimated for five consecutive years (2010-2014) as the sibling coefficient, which was drawn from clonal contribution to the total production of seed conelet. In order to monitor gene diversity, status number was estimated by the integration of fecundity variation and group coancestry. Group coancestry was calculated as the average of genetic relatedness (coancestry) among orchard clones. The averages of conelet production were high in 2010 and 2011, moderate in 2013 and 2014, and poor in 2012 with the grand mean of 13.7. Correlation analysis showed that good conelet producer consistently gave good production. Cumulative distribution of clonal conelet production was presented as a function of the total conelet yield, and this distribution indicated deviation from the expected clonal equal production. Group coancestry was 0.0096, indicating minimal loss of gene diversity. Status number and genetic gain were higher in good than in poor conelet production years, highlighting the importance of fecundity variation in determining the genetic gain and gene diversity of seed orchard crops.

Key words: coancestry, fecundity, effective number, gene diversity, relatedness

51 Introduction

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A seed orchard is defined as a population where genetically improved seeds are economically mass-produced (Zobel et al., 1958). Seed orchard populations are composed of selected genotypes that are isolated or managed to reduce pollination from outside sources (gene flow) and produce frequent, abundant, and easily harvested seed crops (OECD, 1974; Feilberg and Soegaard, 1975). The genetic quality of seed orchards' crops is often measured by their genetic gain and diversity estimates which are assessed based on their parental breeding values, gametic contribution, and the extent of parental genetic representation to the seed crop (Funda and El-Kassaby, 2012). Seed orchards are commonly categorized by their generational level (i.e., 1st, 2nd, or advanced generation) depending on the number of improvement cycles (Zobel and Talbert, 2003). Generally, first generation orchards are initiated by parents whose genetic worth are unknown and the trees are generally closely spaced to allow for rouging of poor genotypes while maintaining a fully functioning seed orchard (Zobel and Talbert, 2003).

Determining the genetic composition of seed orchard crops is important as their genetic gain and diversity reflect their commercial values. Therefore, parameters such as genetic relatedness, inbreeding, and genetic diversity should be measured for each seed crop. The number of female and male strobili (reproductive energy) and the conversion rate of female strobili to seed-cones (reproductive success) in a seed orchard are all important information for determining the genetic gain and diversity of its resultant seed crops (Matziris, 1997; Kang and Lindgren, 1998; Kang, 2000; Gomory et al., 2000; Ertekin, 2010). It is commonly observed that seed orchard parents vary in both reproductive energy and success with consistently high and low producers resulting in disproportionate contribution from a reduced subset of parents (El-Kassaby et al. 1989). A cumulative contribution curve, known as parental balance curve, is often used to quantify fecundity variation in seed orchards (Griffin, 1982; El-Kassaby and Reynolds, 1990; Adams and Kunze, 1996).

Pinus koraiensis (Korean pine) is of great ecological and economic importance as it occupies vast territory in Korea. It is one of the major forest tree species in its habitat and produces good quality timber and edible pine nuts, accordingly, it has been the subject of intensive harvesting and consequently the need for breeding and conservation efforts (Wang, 1995; Yi, 2005).

In the present study, we assessed the reproductive output of a total of 52 clones in a *Pinus koraiensis* seed orchard in order to: 1) investigate the yearly production pattern of

85 clonal conelet, 2) estimate clonal fecundity variation and its correlation among clones and
86 between years, and 3) examine how the difference of seed conelet production among clones
87 affects the genetic gain and gene diversity of seed crops.
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89 **Materials and Methods**

90 *Seed orchard and conelet assessment*

91 The studied *Pinus koraiensis* seed orchard is located in Chuncheon, Kangwon
92 province, Southern Korea (latitude 37° 23' N, longitude 127° 38' E, and altitude 80 m). The 1-
93 hectare orchard was established in 1995 with a parental population consisting of 52 clones that
94 were selected after progeny testing. The progeny tests were conducted separately from the
95 establishment of seed orchard. The parental 52 clones were selected from the tested 242 plus
96 trees (21.5% selection, Figure 1). The selection criterion was based on the growth
97 performance of plus trees' progeny at age 23 years. For parental selection, height (H) and
98 diameter at breast height (DBH) were used to determine each tree's volume (= H x DBH²)
99 and accordingly parents were ranked and the selected ones (i.e., plus trees) formed the 1.5-
100 generation seed orchard population. The orchard's parental representation is near equal
101 (average = 13.8; standard deviation = 4.6 ramets/clone). Ramets were randomly distributed
102 across the orchard's grid and planted at 5 x 5 m spacing using 2-year old grafts. Conelet (1-
103 year-old seed cones) count was conducted on all ramets (100% sampling) for five
104 consecutive years (2010~2014) and was used as a fecundity representative.

105 *Fecundity variation and statistical analysis*

106 Clonal fecundity was estimated based on the clonal proportion of total conelet
107 production in the seed orchard. Using clonal fecundity means, Pearson's product-moment and
108 Spearman's rank-order correlations were calculated among the studied years, using the "proc
109 corr" function of SAS program (1990). Parental balance curves were used to identify high-
110 and low- conelet producers (Chaisurisri and El-Kassaby, 1993). Analysis of variance was
111 conducted on clonal conelet production and broad-sense heritability was estimated on the
112 individual ramet and clone level.

113 Fecundity variation was estimated using the sibling coefficient, Ψ (Kang, 2001) as
114 follows,

115
$$\Psi = N \sum_{i=1}^N p_i^2 = CV^2 + 1 \quad (1)$$

116 where N is the number of clones, p_i is the proportional contribution of clone i , and CV is the
117 coefficient of variation of the clonal proportion of conelet production in the seed orchard.

118 *Group coancestry and status number*

Group coancestry (Θ) in a population is the average of all coancestries (genetic relatedness) among its members, including self-coancestry and reciprocals (Cockerham, 1967). When orchard clones are unrelated and non-inbred, the group coancestry was estimated (Θ) as:

$$\Theta = \frac{0.5 N}{N^2} = \frac{0.5}{N} \quad (2)$$

where 0.5 is self-coancestry in the coancestry matrix and N is the census number of clone in the seed orchard.

Status number (N_s) defined as half the inverse of group coancestry (Θ), assuming no fecundity variation (Lindgren et al., 1996). In the presence of fecundity variation among unrelated parents, it is calculated using the sibling coefficient (Kang et al., 2004) as follows:

$$N_s = 0.5(\Theta\Psi)^{-1} \quad (3)$$

Relative status number (N_r) was calculated as $N_r = N_s / N$ to compare census and status numbers in the seed orchard.

Fecundity variation among orchard clones can be described by the sibling coefficient (Ψ) as it not only considers the reproductive output variation (CV) but also accounts for clonal genealogical relationships (Kang and Lindgren, 1998; 1999). When Ψ value reaches 2, it is an indication of increased relatedness and inbreeding to a rate twice of that expected in the reference population from which the clones were selected or originated.

Fecundity variation (i.e., parental reproductive output inequality) in orchards' crops causes an increase of Ψ value and a concomitant of accumulation in group coancestry. Coancestry between two clones is the probability of identity-by-descent and group coancestry is the average of all coancestry among clones' coancestry matrix, including self-coancestry and reciprocals (Cockerham, 1967). The group coancestry is also called "average coancestry" or "average kinship", although users of these terms sometimes disregard self-coancestry.

Analysis of variance and broad-sense heritability estimation

Individual ramet conelet production was assessed using aanalysis of variance (ANOVA) following the GLM procedure (SAS statistic package program) and the broad-sense heritability estimates were calculated for individual ramet ($H^2_{\text{individual}}$) and clone (H^2_{clone}) as follows:

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$$H^2_{individual} = \sigma^2_c / (\sigma^2_e + \sigma^2_c) \text{ and } H^2_{clone} = \sigma^2_c / (\sigma^2_e / \kappa + \sigma^2_c) \quad (4)$$

149 where σ^2_e and σ^2_c represent within and among clonal variation, respectively, and k is the
150 coefficient of variance component.

151 ***Gene diversity and genetic gain calculations***

152 Expected gene diversity (GD) is equivalent to the expected heterozygosity in a
153 population following random mating and is a function of group coancestry (Nei, 1973; Lacy;
154 1995). Gene diversity can be formulated as $GD = 1 - \sum q_i^2$, where q_i is the frequency of allele i
155 and the summation is over alleles at that locus. If all alleles are unique in a large reference
156 population of unrelated and non-inbred individuals, the gene diversity can be set to one and
157 the gene diversity of the descendant, as a proportion of diversity in the reference population,
158 can be estimated as:

159
$$GD = 1 - \Theta = 1 - 0.5N_s^{-1} \quad (5)$$

160 Then, loss of gene diversity is relatively estimated as $1 - GD = 0.5 / N_s$.

161 Yearly genetic gain was estimated in terms of GCA increment after considering
162 clonal contribution of total conelet production as follows: (sum of individual clone conelet
163 contribution multiplied by its GCA value).

164
$$Gain = \sum_{i=1}^N (GCA_i \times p_i) \quad (6)$$

165 where GCA_i is the volume gain of i -th clone and p_i is the conelet proportional contribution of
166 clone i , respectively. The GCA s of base population had a mean of zero and one of variance
167 (see Fig. 1).

Results

Clonal fecundity variation

Years 2010 and 2012 represented the extreme range of conelet production among the studied five years with clonal variation (i.e., CV) ranging from 0.599 to 1.924 and among yearly averages ranging from 24.6 ± 4.17 to 1.6 ± 1.05 , respectively (Table 1). This observation indicated that moderate/good conelet production persisted for two consecutive years (2010 and 2011, and 2013 and 2014) with an intervening poor year (2012) (Table 1). Coefficient of variation (CV) and sibling coefficient (Ψ) inversely mirrored the conelet production across the studied years with low and high values for moderate/good and poor years, respectively (Table 1).

With the exception of 2012 (the year with the poorest clonal conelet production mean), all coefficients of Pearson's product-moment (to test cross-year consistency) and Spearman's rank-order (to test cross-year relative order) correlations were positive and statistically significant (Table 2). It is worth noting that all the significant correlations were positive and none of the negative correlations were significant, so these significant correlations indicate that the conelet production of any specific year was greatly influenced by the production of the other years and that the production rank of individual clone maintained its order over years (Table 2).

Yearly cumulative distribution of clonal conelet production as a function of total conelet contributions showed deviation from the expected equal production among clones with the severest deviation belonging to 2012, the year with the poorest conelet production (Figure 2). The top 25% of the orchard clones (good producers) contributed 30.8% of the conelet production in the best year (2010) while they contributed 46.7% in the poor year (2012), indicating that a few fertile clones dominated the production in poor years coupled with the presence of non-producing clones (two in this particular year).

Analysis of variance and heritability estimation

The analysis of variance revealed high degree of variation in the number of conelet among clones (Table 3). The differences among clones were statistically significant at $p < 0.01$ probability level across the studied five years. The results also showed that the magnitude of variation in conelet production was greater among clones than within clones. Yearly clonal broad-sense heritability estimates varied from 0.17 (2010) to 0.71 (2014) and were much higher than those of individual ramet (range: 0.01 (2010) and 0.15 (2014)), indicating that the reproductive output (conelet production) was under strong genetic control (Table 3).

Group coancestry, status number, gene diversity and genetic gain

Assuming that the clones were unrelated and non-inbred, the group coancestry (Θ) is estimated to be 0.0096 and the status number (N_s) varied from 11.1 to 38.2 for 2012 and 2010, respectively. On average (pooled), N_s was 40.6, representing 78.1% of census number ($N = 52$) (Table 4). The status number was not constant but fluctuated over years. Fertility variation (Ψ) was positively correlated with the status number (N_s). This result implied that the N_s of seed crops will increase if the seeds from difference years are pooled or if equal amount of seed is collected from each clone.

Group coancestry and status number are closely related to the loss of gene diversity relative to the reference population. The decrease in heterozygosity compared to the reference population reflects the accumulation of coancestry and inbreeding associated with variation in fertility among clones. In this context, loss of gene diversity would range from 1.31 to 4.50% in good (2010) and poor (2012) years, respectively.

Expected genetic gain was the summation of clonal contribution of conelet production multiplied by its *GCA* value. The *GCAs* of base population, represented by the parental 242 plus trees, had a mean of zero and variance of one (see also Fig. 1). Across years, the genetic gain estimates mirrored that of status numbers which fluctuated as a result of the yearly variable conelet production (Table 4). Higher and lower genetic gain estimates were observed in the best and the worst conelet production years, respectively (Table 4).

Discussion

Fecundity variation and group coancestry

Maximum gene diversity of seed crops from a seed orchard can only be attained when all parents contribute equally to the gamete gene pool (i.e., H-W equilibrium). This assumption is hardly fulfilled and it is commonly observed that a small portion of orchard parents contribute a disproportionally large amount to the seed crops (El-Kassaby and Cook, 1994).

Fecundity is defined as the potential reproductive capacity of a clone (Gregorius, 1989) and estimated by the proportion of conelet production in the orchard population. Fecundity is known to be under both genetic and environmental control, and it is the major measure of fitness (El-Kassaby et al., 1989; Savolainen et al., 1993; Kjær and Wellendorf, 1998). The present study clearly demonstrated that conelet production fluctuated over time and among genotypes, undoubtedly affecting the gene diversity of resulting seed crops. Therefore, information about the clonal differential contribution to the gamete gene pool is important for the proper estimation of the expected genetic composition and subsequently the level of genetic gain in reforestation seedlots (Burczyk and Chalupka, 1997; Stoeckl and El-Kassaby 1997).

Accumulation of group coancestry and increase of fecundity variation result in loss of gene diversity. The combined effect of these two factors has played a role in the studied orchard crop. The loss of gene diversity due to coancestry can be estimated using average of all coancestry pairs that is equal to $[0.5 \text{ (self co-ancestry)} \times \text{the number of clones } (N)] / \text{the total number of crosses } (N^2) = 0.5/52$ which is 0.0096. From this group coancestry, the status number can be calculated to be the same as the census number. Thus, fecundity variation caused loss of gene diversity as shown in Table 4. Similarly, the status number is decreased as fecundity variation increased.

The observed difference of gene diversity loss, which was caused by fecundity variation and coancestry, indicates that most of the gene diversity loss is attributable to fecundity variation rather than self-coancestry (i.e., inbreeding) as the studied orchard was established with unrelated and non-inbred parents. Therefore, the management of fecundity variation is of vital concern. Optimization techniques such as equal seed harvest, pooling of different seed lots from different years for designing seed crops that maximize the genetic gain at a desired gene diversity level are recommended (Funda et al., 2009).

In the present study, genetic relatedness among the selected clones was not considered. Since the orchard's parental populations originated from a subset of parents (plus trees) selected from different stands, then it is safe to assume that they are unrelated and non-inbred. It is also interesting to point out that when genetic relatedness among clones accumulates, specifically in advanced generation seed orchards, group coancestry and status number will be substantially affected.

Status number and gene diversity of seed crop

The status number (N_s) was higher in a good conelet year (2010) as compared to the lower production years (Table 4). If the status number in a seed orchard is small, the genetic composition of the seed crop may be different from the expectation considering the genetic composition of its parental population. A small ratio (i.e., small N_r) of status number to census number (i.e., actual population size) is indicative of reduced gene diversity, thus it is advisable to avoid establishing seed orchards with reduced number of parents. The relative status number of this orchard increased over time from 0.21 (12 years old (Kang and Lindgren, 1998)) to 0.21-0.74 (15-19 years old, the present study), thus it appears that the age of orchards affects the effective population size as reproductive output tends to increase and the difference of reproductive output among clones are somewhat minimized (Kjær and Wellendorf, 1997).

For first generation seed orchards, it seems reasonable to assess the gene diversity in comparison to the natural populations where their parents were selected from. However, even weak genetic relatedness among plus trees should not be excluded from the genetic assessment, and careful caution is required when multiple plus trees are selected from the same stand for avoidances of genetic relatedness (Chaisurisri and El-Kassaby, 1994; El-Kassaby and Ritland, 1996; Stoehr and El-Kassaby 1997).

Level of gene diversity mirrors both the number of initial unique alleles and their evenness (frequencies), and it can be averaged over loci to provide a genome-wide measure of diversity. The decrease of gene diversity in the studied seed crops compared to their wild populations does not seem alarming (Table 4). As status number is a function of gene diversity, it may also be used by orchard managers to develop methods that maximize gene diversity of seed crops (Lindgren and Mullin, 1998; Funda et al., 2009).

Gene flow from external sources (i.e., pollen contamination) and male fecundity were not considered in this study. Pollen contamination, while unpredictable, is expected to increase the gene diversity. Savolainen (1993) reported that the phenotypic correlation

between female and male fecundity was usually positive but the genetic correlation was negative. Such correlation might increase or decrease total fertility variation in the reproductive success (Kang and El-Kassaby, 2002); however, the correlations between female and male strobilus production over five-year period were not different from zero for another *P. koraiensis* seed orchards (Kang and Lindgren, 1999).

Finally, the estimate of genetic gain of the studied orchard crops appeared to be drastically influenced by clonal conelet contribution variation and their respective genetic values. Estimates of genetic gain in seed orchards are the primary goal of their establishment and management, thus management strategies that maximize genetic gain are required through efficient determination of the orchards' clonal composition accomplished through roguing of inferior clones and/or selective cone harvesting from superior clones.

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Table 1. Average conelet production, coefficient of variation (CV) and sibling coefficient (Ψ) over the 5-year study period in a *Pinus koraiensis* clonal seed orchard.

	2010	2011	2012	2013	2014	Mean
Average	24.6	18.6	1.6	12.7	10.0	13.7
CV	0.599	0.734	1.924	0.861	0.945	0.530
Ψ	1.36	1.54	4.70	1.74	1.89	1.28

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Table 2. Pearson’s product-moment (above diagonal) and Spearman’s rank-order correlations (below diagonal) among clonal conelet mean production over the 5-year study period in a *Pinus koraiensis* seed orchard.

Year	2010	2011	2012	2013	2014
2010	-	0.236**	-0.022	0.132*	0.110*
2011	0.233**	-	-0.120	0.350**	0.337**
2012	-0.024	-0.109	-	-0.099	-0.146
2013	0.162**	0.366**	-0.100	-	0.687**
2014	0.119*	0.340**	-0.139	0.711**	-

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*,** significant at $P<0.5$ and $P<0.01$ level, respectively.

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Table 3. Analysis of variance and broad-sense heritability estimates (H^2) for conelet production in a *Pinus koraiensis* seed orchard.

Source	df	EMS ^a	2010	2011	2012	2013	2014
Among	51	$\sigma_e^2 + k(\sigma_c^2)$	252.6 ^{**}	335.3 ^{**}	12.8 ^{**}	308.7 ^{**}	260.8 ^{**}
Within	644	σ_e^2	210.5	178.2	8.6	104.9	76.1
$H^2_{\text{individual}}$		$\sigma_c^2 / (\sigma_e^2 + \sigma_c^2)$	0.01	0.06	0.04	0.13	0.15
H^2_{clone}		$\sigma_c^2 / (\sigma_e^2/k + \sigma_c^2)$	0.17	0.47	0.33	0.66	0.71

^a Expected Mean Square and σ_e^2 , σ_c^2 and k represent within and among clonal variation, and the coefficient of variance component.

^{**} Statistically significant at the 0.01 probability level.

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Table 4. Status effective number (N_s), relative status number (N_r), gene diversity (GD) and expected gain for gamete gene pool over the 5-year study period in a *Pinus koraiensis* seed orchard.

	2010	2011	2012	2013	2014	Mean
N_s	38.2	33.8	11.1	29.9	27.5	40.6
N_r	0.74	0.65	0.21	0.58	0.53	0.78
GD	0.987	0.985	0.955	0.983	0.982	0.988
Gain*	0.604	0.635	0.513	0.594	0.632	0.613

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* Expected gain was the summation of the values that individual clone conelet contribution multiplied by its GCA value. GCAs of base population had a mean of zero and one of variance (see Fig. 1).

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Figure 1. Ordered genetic value (GCA) of the 242 plus-trees and the selected 52 parents used for establishing the seed orchard. Selection was based on parental growth performance (volume growth: $\text{height} \times \text{DBH}^2$) at age 23 years.

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Figure 2. Yearly cumulative clonal conelet production distribution represented as a function of the total conelet yield in the studied *Pinus koraiensis* seed orchard.