Estimation of Heritabilities and Clonal Contribution Based on the Flowering Assessment in Two Clone Banks of *Pinus koraiensis* Sieb. et Zucc.

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**Sang-Urk Han**  
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**Abstract**—Reproductive characteristics of 161 Korean pine (*Pinus koraiensis* Sieb. et Zucc.) clones were surveyed at two clone banks for 3 years. These clone banks were established at Yongin and Chunchon (mid-Korea) in 1983. Characteristics in female and male strobili were spatial (between locations) and temporal (among investigated times) variables. Broad sense heritabilities were found to vary between 0.20 - 0.46 in females and between 0.34 to 0.56 in males. Among 161 clones, 32 clones (20 percent of the total clones) accounted for 42 to 54 percent of clonal contribution in female strobili and 83 to 96 percent in male strobili, suggesting that the clonal contribution for male parents was severely unbalanced compared to that for female parents. The effective population numbers varied depending on time (year), location and sex. The mean values of relative effective population numbers at gamete levels were 0.56 in females and 0.09 in males, respectively, and that value at the clonal level was 0.27 (0.25 at Yongin and 0.29 at Chunchon). The degree of sexual asymmetry ($A_s$) varied with a range of 0.03 to 0.24 at Chunchon and 0.07 to 0.44 at Yongin. The pattern of gamete production within clones was highly asymmetrical as compared to that of other conifers. This indicates that *P. koraiensis* is extremely low in male gamete production compared to female gamete production.

**Key words:** *Pinus koraiensis*, strobili, clone bank, clonal contribution, broad sense heritability, effective population number, sexual asymmetry

**Introduction**

Korean pine (*Pinus koraiensis* Sieb. et Zucc.) is a five-needle pine (*Pinus* subgenus *Strobus*) belonging to subsection *Cembrae*. The species has a wide natural distribution in the northeastern part of Eurasia. It usually occurs as a mixed forest stand consisting of various broad-leaved tree species and other conifers. Korean pine has been widely planted as a pure stand accounting for about 30 percent of the yearly planting areas in Korea due to its high-quality timber and edible seeds. A breeding program for this species has been conducted since 1959 and has resulted in the establishment of 98 ha seed orchards (Mirov 1967, Chun 1992, Choi 1993, Wang 2001).

The main goal for seed orchards is large-scale production of genetically improved seeds that maintain genetic diversity to prevent inbreeding depression. Thus, the maintenance of random mating among clones is one of the key elements to successful management of seed orchards (Roberds and others 1991, Chaisursri and El-Kassaby 1993, Matziris 1993, El-Kassaby and Cook 1994, Burczyk and Chalupka 1997, Han and others 1999).

The clonal contribution to seed production in a seed orchard is one of the most important factors; genetic composition of the seed produced is determined by the contributions of each clone. Differences in clonal contribution have been previously reported in several studies and have been attributed to genetic rather than environmental factors (Griffin 1982, Schmidling 1983, Askew 1988, Brunet and Charlesworth 1995, Kjaer 1996, Han and others 1999, Nikkanen and Ruotsalainen 2000).

To date, numerous studies have been conducted to obtain information related to reproductive processes such as flowering characteristics, clonal contribution, and sexual asymmetry in seed orchards. Clonal contribution within a seed orchard is commonly depicted by a flowering or cone yield curve. In this method, the clones are ranked from high to low in flower production, and cumulative contribution (in percent) is plotted against the proportion of the clones. Additionally, the concept of effective population number has been recently applied to the estimation of clonal contribution (Griffin 1982, Kjaer 1996, Choi and others 1999, Han and others 2001a, 2001b, Kang 2001).

Our major interest in this study is to quantify the reproductive processes using empirical data from two *Pinus koraiensis* clone banks, to survey the differences of clonal contribution by means of flowering assessments, and to monitor the genetic diversity measured by effective population sizes. These include estimating heritability, gamete contribution, and sexual asymmetry.
Materials and Methods

Reproductive characteristics such as number of male and female strobili were surveyed in the two clone banks of *P. koraiensis*. The two clone banks were established at Chunchon (lat. 37°55’, long. 120°46’) and Yongin (lat. 37°30’, long. 127°20’) in 1983. A total of 167 clones were grafted at Chunchon and 180 clones at Yongin with a space of 4m x 4m. Reproductive characteristics of 161 clones, which the two clone banks have in common, were investigated for 3 consecutive years (1998 to approximately 2000).

The clone banks were not considered as fully mature populations when the numbers of female and male strobili were counted. Generally, Korean pine begins to show strobili at age of 12 or 15 in natural stands. Grafted clones, however, produce strobili earlier than natural clones. In these clone banks, there is not much difference in height (4 to 5m) and DBH. Five ramets per clone were chosen for assessment in early June. The number of female strobili was counted individually from a whole tree. The total number of male strobili was estimated by multiplying the average number of strobili per branch by the total number of branch bearing male strobili.

Analysis of variance (ANOVA) tests and heritability estimates were conducted based on the data for female and male strobili production. The ANOVA was performed using a logarithmic transformation of the original data to normalize the distribution of variances (Steel and Torrie 1980). SAS program (ver. 6.12; SAS Institute Inc., 1996) was used for ANOVA tests and heritability estimation. Broad-sense heritabilities (H²) were estimated on the basis of individual trees (Schmidtling 1983) as:

$$H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_e}$$

Parental balance was assessed using a cumulative gamete contribution curve (Griffin 1982). The numbers of female and male strobili were ordered by clone from high to low strobilus production, and the cumulative contribution percentages were plotted against the proportion of the clones (Kang 2000).

The maleness index (Ai) is defined as the proportion of a clone’s reproductive success that is transmitted through its pollen (Kang 2000). Maleness index based on strobilus production was estimated as follows:

$$Ai = \frac{ai}{gi + ai}$$

where ai and gi are the proportions of ith clone of which male and female strobili contribute to the whole population. A high maleness index of a clone indicates that the clone is contributing more as a paternal, rather than maternal parent.

The effective population numbers at gamete level (Eq. 1 and Eq. 2) and clonal level (Eq. 3 and Eq. 4) and the sexual asymmetry (Eq. 5) were estimated using Choi and others’ (1999) methods as follows:

$$\mu_g = \sum_{i=1}^{n} \frac{x_i(q_i)^2}{(1 - q_i)^2}$$

$$\mu_m = \sum_{i=1}^{n} \frac{x_i(r_i)^2}{(1 + r_i)^2}$$

$$\mu_a = \frac{1}{2} (\mu_g + \mu_m)$$

$$\mu_b = \frac{1}{2} (\frac{1}{2} (\mu_g + \mu_m) + x_i(q_i)^2)$$

$$A_s = \frac{\mu_a}{\mu_b - \mu_a}$$

where $n$ is the total number of clones, $\mu_g$ is the female effective population number, and $x_i(q_i)$ is the proportion of the female strobili of the ith clone to the whole production of females. $\mu_m$ and $x_i(r_i)$ in males correspond to those for females. $\mu_a$ is the arithmetic mean of the two measures ($\mu_g$ and $\mu_m$) and $\mu_b$ is based on the relative frequency of $x_i(q_i)$ and $x_i(r_i)$. In this study, we used the relative effective population number instead of effective population number for easy comparison with those of other studies.

Results and Discussion

Reproductive Characteristics and Heritability

Large variations in both female and male strobilus production among clones were observed at both Yongin and Chunchon (table 1). The differences of male strobilus production among clones were far more extreme than that of female strobilus production. It seems that this phenomenon is a typical character of Korean pine from our experience of

| Table 1—Mean, standard deviation (S.D.) and coefficient variation (C.V.) for the number of female and male strobili at Yongin and Chunchon during the period of 1998 to 2000. |
|---|---|---|---|---|---|---|---|
| | Yongin | | Chunchon | | | |
| | Female | Male | Female | Male | Female | Male | Female | Male |
| Mean | 5.3 | 64 | 13.6 | 240 | 9.1 | 36 | 2.2 | 146 | 12.5 | 393 | 5.8 | 307 |
| S.D.(±) | 4.6 | 184 | 12.8 | 975 | 9.2 | 111 | 2.1 | 514 | 9.3 | 988 | 5.1 | 982 |
| C.V.(percent) | 87 | 298 | 94 | 406 | 101 | 305 | 97 | 353 | 75 | 251 | 87 | 320 |

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orchard management. The average female strobilus productions per clone ranged between 0 and 46.7 at Yongin and between 0.1 and 63.5 at Chunchon. The production of female strobili in Yongin was consistently greater than that in Chunchon, while male strobili production showed an opposite trend. During this study, the production of female and male strobili was most abundant in 1999.

The ANOVA results and broad sense heritabilities for reproductive characteristics are presented in table 2. The number of female and male flowers was significantly different among clones within a clone bank, while those for ramets within a clone did not show any significant differences. These results showed that the reproductive characteristics are under genetic influences rather than environmental influences. Similar results have been reported in other conifers such as *P. taeda* (Byram and others 1986), *P. densiflora* (Han and others 1999), *P. thunbergii* (Han and others 2001b) and *Picea abies* (Nikkanen and Ruotsalainen 2000).

The values of broad sense heritabilities for female strobili ranged from 0.21 to 0.20 in a poor flowering year (1998) and they varied between 0.46 and 0.27 in a good flowering year (1999). Temporally those values for male strobili varied between 0.21 in 1999 and 0.42 in 1998 at Chunchon and 0.20 in 1998 and 0.34 in 2000 at Yongin. The values for males (0.22 to 0.56) were higher than females (0.20 to 0.51) for all years studied. This indicates that the genetic influence determining the reproductive characteristics is stronger in males than in females.

The two-way ANOVA results and estimated heritabilities for reproductive characteristics in the two clone banks are presented in table 3. The differences in the number of female and male strobili among clones were statistically significant for 3 years excluding that of males in 1998 and that of females in 2000. Significant differences in reproductive characteristics between the two locations were observed for females in 1998, and for females and males in 2000. In 1999, the flowering characteristics for both sexes were significantly different among clones. The interaction of clone and location effects was significant in all years, implying that clones should be selectively chosen when production (in other words, seed orchards) and/or breeding populations are established at the different sites.

The heritabilities for female and male strobili in each year showed maximum values of 0.59 and 0.77, respectively. The minimum values for heritabilities were 0.02 for female in 2000 and 0.23 for male in 1998.

### Clonal Contribution

We used two types of measures, cumulative contribution curves and relative effective population number, for demonstrating the clonal contribution. The cumulative contribution curves of 161 clones for female and male strobili are presented in figure 1. Thirty-two clones (20 percent of the total clones investigated at both locations) accounted for 42-54 percent of clonal contribution in female and 83 to 87 percent in male strobili.

#### Table 2—Analysis of variance and broad sense heritability ($H^2$) for the number of female and male strobili at Yongin and Chunchon during the period of 1998 to 2000.

<table>
<thead>
<tr>
<th>Location</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Yongin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among clones</td>
<td>0.37**</td>
<td>2.06**</td>
<td>0.69**</td>
</tr>
<tr>
<td>Within clones</td>
<td>0.17</td>
<td>0.49</td>
<td>0.15</td>
</tr>
<tr>
<td>$H^2$</td>
<td>0.21</td>
<td>0.42</td>
<td>0.46</td>
</tr>
<tr>
<td>Chunchon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among clones</td>
<td>0.46**</td>
<td>5.99**</td>
<td>0.60**</td>
</tr>
<tr>
<td>Within clones</td>
<td>0.12</td>
<td>0.47</td>
<td>0.17</td>
</tr>
<tr>
<td>$H^2$</td>
<td>0.20</td>
<td>0.51</td>
<td>0.27</td>
</tr>
</tbody>
</table>

**: Significant at 1 percent level.

#### Table 3—Two-way ANOVA and broad sense heritabilities—($H^2$) for the number of female and male strobili at Yongin and Chunchon during the period of 1998 to 2000.

<table>
<thead>
<tr>
<th></th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Clone</td>
<td>0.48**</td>
<td>3.40</td>
<td>0.90**</td>
</tr>
<tr>
<td>Location</td>
<td>33.55**</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>Clone x Location</td>
<td>0.31**</td>
<td>3.03**</td>
<td>0.37**</td>
</tr>
<tr>
<td>Error</td>
<td>0.13</td>
<td>0.47</td>
<td>0.17</td>
</tr>
<tr>
<td>$H^2$</td>
<td>0.38</td>
<td>0.23</td>
<td>0.59</td>
</tr>
</tbody>
</table>

**: : Significant at 1 percent level.
*: Significant at 1 percent and 5 percent level, respectively.
96 percent in male strobili. The curves for male strobili were severely distorted compared to those for female strobili. Alternately, the clonal contributions of female and male strobili for each year were 49 percent and 89 percent in 1998, 44 percent and 92 percent in 1999, and 54 percent and 96 percent in 2000, respectively at Yongin, while those for Chunchon were 51 percent and 92 percent in 1998, 42 percent and 86 percent in 1999, and 47 percent and 83 percent in 2000, respectively. The biased contribution of a small number of clones to the whole clonal contribution was greater for pollen parents than female parents.

Park and others (1987) reported that 19 percent of the total clones in a *P. koraiensis* clone bank accounted for 63 percent of male strobili production and 58 percent of female strobili production. This study was conducted at Chunchon where our study was also conducted. However, they studied a 4 to 5 year old clone bank. Alternately, Han and others (1997) conducted a similar study in a *P. koraiensis* clone bank at Yongin, our other study site. In that study, they reported that 20 percent of the total clones investigated accounted for 49 to 65 percent of female strobili production, while 8 to 15 percent of the total clones accounted for over 80 percent of male strobili production. The differences in results between the above studies and our study might be due to plantation age. Regardless, these comparisons show that clonal contribution to strobili production is more balanced in female than that in male reproduction.

When compared to other conifers, Korean pine appears to have a more unbalanced clonal contribution in seed production. Han and others (1999) observed that the contribution of 33 percent of 99 *P. densiflora* Ait. clones varied between 46 percent and 70 percent in female and 40 percent and 87 percent in male, and the degree of contribution increased with age. In *P. radiata* D. Don, 23 percent of the total clones accounted for 50 percent of seed production (Griffin 1982). Adams and Kunze (1996) found that 49 percent of the total clones in *Picea mariana* (Mill.) B.S.P. and 43 percent of the clones in *P. glauca* (Moench.) Voss. accounted for a total of 80 percent of seed production.

The relative effective population numbers estimated at gamete and clonal levels are shown in table 4. The relative effective population number for sexes were extremely different with $m_f = 0.56$ and $m_m = 0.09$. The values of relative effective population number at the gamete level did not differ significantly by year or location. In a *Pinus sylvestris* L. clonal seed orchard at the age of 17-19, Burczyk and Chaluka (1997) found that the effective population number (0.76) in males was only slightly lower than that (0.96) in females. In contrast, Han and others (2001a) observed slightly higher values in males (mean 0.63 with a range of 0.24 - 0.94) than those in females (mean 0.55 with a range of 0.28 - 0.83) in a *P. densiflora* clonal seed orchard.

The values at the clonal level ($\mu_b$) ranged from 0.19 - 0.38. The values of $\mu_b$ between the two locations ranged from 0.24 in 1998 to 0.38 (mean: 0.29) in 1999 at Chunchon and from 0.19 in 1999 to 0.28 (0.25) in 2000 at Yongin. Interestingly, the values of $\mu_b$ are lower than those of $\mu_F$ and $\mu_m$ in all observations. It is generally known that the values of $\mu_b$ are...
Table 4—Relative effective population number at the gamete level and the clonal level in *Pinus koraiensis* clone banks investigated for 3 consecutive years.

<table>
<thead>
<tr>
<th></th>
<th>Yongin</th>
<th>Chunchon</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_\varphi$</td>
<td>161</td>
<td>0.52 (83.7)a</td>
</tr>
<tr>
<td>$\mu_\psi$</td>
<td>161</td>
<td>0.10 (16.1)</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>161</td>
<td>0.31 (49.9)</td>
</tr>
<tr>
<td>$\mu_b$</td>
<td>161</td>
<td>0.28 (45.1)</td>
</tr>
</tbody>
</table>

* Effective population number in parenthesis

always larger than those of $\mu_a$ and similar to or larger than those for $\mu_\varphi$ and $\mu_\psi$. For instance, Han and others (2001a) showed that the value for $\mu_\varphi$ (0.69) was higher than those for $\mu_a$ (0.58) in a *P. densiflora* seed orchard. The reason for the contrary tendency as shown in this study was explained in elsewhere (Choi and others 1999).

**Sexual Asymmetry**

The degrees of sexual asymmetry between female and male strobili were shown in Table 5. The degree of sexual asymmetry (0.03 to approximately 0.24 with a mean of 0.17) for Chunchon was lower than that of Yongin (0.0 to approximately 0.44 with a mean of 0.20). The degree of sexual asymmetry ($A_p$) was variable depending on time and location.

Table 5—Estimation of the degree of sexual asymmetry ($A_p$) at *Pinus koraiensis* clone banks for 3 years.

<table>
<thead>
<tr>
<th></th>
<th>Yongin</th>
<th>Chunchon</th>
</tr>
</thead>
<tbody>
<tr>
<td>$</td>
<td>\mu_a - \mu_b</td>
<td>$</td>
</tr>
<tr>
<td>$A_p$</td>
<td>0.10</td>
<td>0.44</td>
</tr>
</tbody>
</table>

The degree of sexual asymmetry in this study was higher especially when it was compared to that of *P. densiflora* (Han and others 2001a). In this species, the difference between two types of effective population number at clonal levels $\mu_a$ and $\mu_b$ were large because a majority of clones did not bear male flowers while most of them bore female flowers, therefore contributing to sexual asymmetry (see also Choi and others 1999). In contrast, most conifer species (*P. densiflora* and *P. thunbergii*) had similar effective population numbers between sexes.

Male index estimates are showed in figure 2. The distribution pattern of maleness indices in the present study deviated from the normal distribution pattern found in other pine trees (Burczyk and Chalupka 1997). Generally, most clones in other pines such as *P. densiflora*, *P. thunbergii* and *P. sylvestris* had maleness index of 0.8 to 0.2. Our study demonstrated a bimodal distribution, with the majority of Korean pine clones maleness indices above 0.8 or below 0.2. For instance, more than 80 percent of clones had values above 0.9 or below 0.1 regardless of year or location. In 2000 at Yongin and in 1998 at Chunchun, more than 95 percent of clones had maleness indices above 0.9 or below 0.1. On the other hand, the sexual balance within clones was highly asymmetrical in *P. koraiensis* as compared to that of other pine species such as *Pinus densiflora*, *P. thunbergii* Parl., and *P. sylvestris* (Han and others 2001a, 2001b, Burczyk and Chalupka 1997). This tendency is due to the extreme...
difference in effective population number between sexes and high degree of sexual asymmetry as already shown above.

Our study indicates potential problems in the seed orchard management of *P. koraiensis*. These problems are: 1) differential fertility variation, 2) inadequate pollen supply, 3) panmictic disequilibria, and 4) parental unbalance. Such problems relate to both the amount of seed produced and the genetic diversity of seed crops (Kang 2001). Thus, some management options, such as supplemental mass pollination, flower stimulation and equal seed harvest, should be considered in the clonal seed orchard of *P. koraiensis*.

References


Part III: Genetic Diversity and Conservation

Collage by R. Berdeen
1. Underside of *Ribes bracteosum* leaf exhibiting blister rust infection.

2. Dorena crew members laying out *Ribes* leaves in preparation for inoculation.

3. Pine seedlings under racks of *Ribes* leaves during inoculation.

4. Inoculation chamber at 100% RH with mist system engaged.

5. Infected pine seedling exhibiting numerous needle lesions.

6. Infected pine seedling with several stem cankers.

7. Infected pine seedling exhibiting
   a) a needle lesion
   b) an incipient stem canker at a needle fascicle
   c) a bark reaction

8. Frames of pine seedlings showing a high rate of mortality due to blister rust infection.

Collage by: R. Berdeen