

Estimation of Effective Population Size in a Clonal Seed Orchard of *Chamaecyparis obtusa*

K. S. Kang^{1*}, S. G. Son² and C. S. Kim¹

¹Department of Forest Genetic Resources, Korea Forest Research Institute, Suwon 441-350, Korea

²Warm-Temperature Forest Research Center, Korea Forest Research Institute, Seogwipo 697-050, Korea

Abstract : Clonal differences in fertility (expressed as the number of female and male strobili) were determined for five consecutive years (2002-2006) in a clonal seed orchard of *Chamaecyparis obtusa*. Fertility varied among clones and among years with producing five-year averages of 378.8 and 871.2 for female and male strobili per ramet, respectively. Correlation between female and male strobilus production was positive over the five years and statistically significant. Based on the observed fertility variation, the effective population sizes (estimated by status numbers, N_e) were calculated and varied from 24.3 to 47.9 (48.6% to 95.8% of census number, N) among the five studied years. On average (pooled), the relative effective population size was 82% of the N . Variation in female fertility was higher than that in male fertility, and this variation was reflected on female and male parents' status numbers. Pooled N_e estimated from the five years was higher than that from poor seed production years. From our results, it was concluded that genetic diversity collected from good flowering years would be higher than that from poor flowering years.

Key words : fertility variation, sibling coefficient, status number, effective number, flowering, *Chamaecyparis obtusa*

Introduction

Chamaecyparis obtusa (common name, Hinoki), native to southern Japan and Taiwan, was introduced to Korea in the early 19th century from Japan. It has been traditionally planted on various sites in southern parts of the country. Hinoki means "fire tree", referring the wood to make fire by friction. The various cultivars of *C. obtusa* are used as specimens and for hedging, screening, and windbreaks (Yamashita *et al.*, 2004). The species is valued for appearance-grade lumber, and is also cultivated as an ornamental species in warm-temperate climates. On the steep topography that is typical for forest land in Korea, *C. obtusa* is grown on the upper slopes or hilly areas which are poor in soil fertility, whereas *Cryptomeria japonica* (sugi) is more commonly established on rich middle slopes or plains.

The Korean breeding program for *C. obtusa* initiated in 1960 with plus-tree selection of superior genotypes in artificial plantations. A total of 114 plus trees were selected phenotypically and used, by rooted cuttings, to establish the clonal seed orchards. At present, the total of 48 ha *C. obtusa* seed orchards have been established at

15 sites in two places in Jeju Island and the average of 130 kg improved seed is produced annually from the seed orchards.

To ensure expected genetic gain and diversity of seed orchard seeds, the attainment of maximal reproductive phenology synchronization, output equality, and minimal inbreeding and pollen contamination is an important prerequisite for seed orchard management. However, research on seed orchard genetics has proven that these conditions are not fully met in most cases (Eriksson *et al.*, 1973; El-Kassaby, and Askew, 1991; Gömöry *et al.*, 2003). As a system, the seed orchards have also been proven to be robust and acted as a viable, reliable source for the production of genetically improved seed for reforestation programs (El-Kassaby, 2000).

Seed orchard managers should focus not only on biological aspects (flowering, diversity, insects etc.) but also on economical aspects (gain, production, cost etc.). Genetic variation, selfing rate and gene flow (e.g., pollen contamination) of *C. obtusa* have been studied (Seido *et al.*, 2000a; Seido *et al.*, 2000b; Shiraishi *et al.*, 2001), but little is known about reproductive output and genetic diversity of seed crops from the seed orchards.

The main purposes of the study are to determine clonal reproductive output (production of female and male strobili) variation, and to estimate effective popu-

*Corresponding author
E-mail: kangks@foa.go.kr

lation size based on clonal fertility variation among clones in a clonal seed orchard of *C. obtusa*.

Materials and Methods

1. Seed orchard and data collection

The study was conducted in a *C. obtusa* clonal seed orchard located in the southernmost part of Korea, Seogwipo, Jeju Island (33°17'N, 126°38'E and 550m above sea level). The seed orchard covered 6-ha area and was established by rooted cuttings in 1982, which was planted in a random design with 5m × 5m spacing. Clones originated from a total of 56 plus trees selected from plantations. The numbers of ramets established per clone varied from 1 to 154 (averaging 43.5 per clone). At present, the seed orchard is being managed by the Warm-Temperature Forest Research Center of the Korea Forest Research Institute.

The numbers of female and male strobili were counted for 50 clones (89.3% of the total) out of 56 clones. Clones with a few ramets were excluded from the assessment. Reproductive output assessment was conducted with five ramets per clones randomly chosen and surveyed over five consecutive years (2002-2006), avoiding ramets growing at the edges of the seed orchard. The numbers of female and male strobili for the sampled five ramets were estimated by multiplying the average number of strobili per branch by the total number of branches bearing strobili.

2. Fertility variation

Female and male fertility variations were estimated following the sibling coefficient developed by Kang and Lindgren (1999). The female (ψ_f) and male (ψ_m) fertility variations can be estimated by either relative contribution of clones or the coefficient of variation (CV) to strobilus production as follows:

$$\psi_f = N \sum_{i=1}^N \left(\frac{f_i}{\sum f_i} \right)^2 = CV_f^2 + 1, \psi_m = N \sum_{i=1}^N \left(\frac{m_i}{\sum m_i} \right)^2 = CV_m^2 + 1 \quad (1)$$

where N is the census number, f_i and m_i are the numbers

of female and male strobili of the i^{th} clone and CV_f and CV_m are the coefficients of variation in female and male strobilus production among clones, respectively.

Total fertility variation was estimated by the sibling coefficient (Ψ) (Kang and Lindgren, 1999; Kang and El-Kassaby, 2002; Bilir *et al.*, 2004) as follows:

$$\Psi = N \sum_{i=1}^N \left(\frac{0.5f_i}{\sum f_i} + \frac{0.5m_i}{\sum m_i} \right)^2 \quad (2)$$

Total fertility variation means clone fertility variation considering both female and male fertility variation.

2. Effective population size (status number)

Effective population size was estimated by the concepts of status number (Lindgren and Mullin, 1998) and effective parent number (Kang and Lindgren, 1999). Effective population size was calculated based on the fertility variation of female, male and clone levels, respectively, as follows:

$$N_{e(f)} = \frac{\psi_f}{N}, N_{e(m)} = \frac{\psi_m}{N} \text{ and } N_e = \frac{\Psi}{N} \quad (3)$$

where $N_{e(f)}$, $N_{e(m)}$, and N_e are the effective population sizes of female, male and clone, respectively. To compare between census number (N) and effective population size (N_e), relative effective population size (N_r) was calculated as $N_r = N_e/N$.

Results

1. Strobilus production

Numbers of female and male strobili varied considerably among clones and among years, which was expressed as the coefficient of variation and the sibling coefficient (Tables 1 and 2). Over the 5-year assessment period, all clones produced female and male strobili at least once more but there was a large variation in the average strobilus production ranged from 26.7 to 809.1 in female and from 100.0 to 1,583.3 in male.

Average female and male strobilus production reached

Table 1. Average strobili per ramet, coefficient of variation (CV) and correlation coefficient (r) between female and male strobilus production over five years in a clonal seed orchard of *Chamaecyparis obtusa*.

	2002		2003		2004		2005		2006		Pooled	
	female	male	female	male	female	male	female	male	female	male	female	male
Average ¹	56.6	111.9	100.2	794.2	379.9	1248.5	1339.3	2013.8	279.3	834.0	378.8	871.2
CV	0.991	1.029	0.856	0.502	0.611	0.414	0.276	0.208	0.574	0.511	0.512	0.441
r	0.65**		0.44		0.77**		0.66**		0.50*			

¹: the number of strobili per ramet

** and * : statistically significant at the 0.01 and 0.05 probability level ($df=48$)

Table 2. Sibling coefficient (ψ_f , ψ_m and Ψ), effective population size [$N_{e(f)}$, $N_{e(m)}$ and N_e], and relative effective population size (N_r) in a clonal seed orchard of *Chamaecyparis obtusa*

	2002			2003			2004			2005			2006			Pooled (average)		
	♀	♂	clone	♀	♂	clone	♀	♂	clone	♀	♂	clone	♀	♂	clone	♀	♂	clone
ψ_f , ψ_m and Ψ	1.98	2.06	1.84	1.73	1.25	1.34	1.37	1.17	1.23	1.08	1.04	1.05	1.33	1.26	1.22	1.26	1.19	1.22
$N_{e(f)}$, $N_{e(m)}$ and N_e	25.2	24.3	27.1	28.9	39.9	37.3	36.4	42.7	40.5	46.5	47.9	47.7	37.6	39.7	41.0	39.6	41.9	41.1
N_r^*	0.50	0.49	0.54	0.58	0.80	0.75	0.73	0.85	0.81	0.93	0.96	0.95	0.75	0.79	0.82	0.79	0.84	0.82

* : Relative effective population size, $N_r = N_e / N$

its peak in 2005 where the coefficient of variation (*CV*) in strobilus production among clones was lower (Table 1). On average (pooled), each ramet produced 378.8 and 871.2 strobili in female and male, respectively (Table 1). The correlation coefficients (*r*) between female and male strobilus production were positive over the studied five years and were statistically significant, except only in 2003 (Table 1).

Clonal differences in strobilus production indicated that a few prolific clones could contribute a large proportion of the seed, particularly to seedlots harvested during the poor seed years (see also Figure 1).

2. Fertility variation and effective population size

Estimated fertility variation (i.e., sibling coefficient) varied among years and between genders within years, and female fertility variation (ψ_f) was higher than male fertility variation (ψ_m) except in 2002 (Table 2). The effective population size for female, male, and clone level were highest in 2005, good flowering year, where the fertility variation were lowest.

Overall clone fertility variation (Ψ) within the studied seed orchard was slightly lower comparing to that found in other seed orchards. Kang *et al.* (2003) reported the sibling coefficient value of 2 in good or moderate flowering years of mature seed orchards, which is equal to $CV = 100\%$. In the present study, clone fertility variation (Ψ) for the pooled data (average) was lower than that observed for most single year (Table 2), implying that the genetic diversity of seed crops from the studied seed orchard could be reasonably high.

Yearly changes in the effective population sizes (i.e., status numbers) of female, male, and clone mirrored those in the seed production (Figure 1). Seed production showed a peak in 2005 and effective population size was also highest in 2005. In general, fertility variation tends to be small in good seed production years (Reynolds and El-Kassaby, 1990; Matziris, 1993; Burczyk and Chalupka, 1997; Nikkanen and Ruosalainen, 2000). In 2005, the seed production was highest, and as expected, the fertility variation was lowest (Table 1) and the differences among effective population sizes ($N_{e(f)}$, $N_{e(m)}$ and N_e) were smallest (Figure 1).

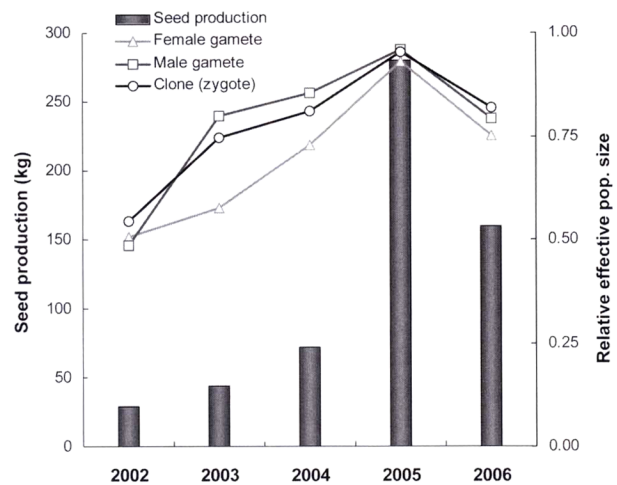


Figure 1. Seed production, and female and male relative status numbers in a clonal seed orchard of *Chamaecyparis obtusa* for the five consecutive years.

Discussion

Close observation of clonal reproductive output indicated the presence of consistently high or low fertility clones during the studied period (2002-2006). In an ideal situation, it is expected that the flowering is synchronized between genders and the seed productivity is also equal among clones. In a real seed orchard, however, there is a large variation in flowering and seed production. This is also reported previously in several seed orchards (Eriksson *et al.*, 1973; El-Kassaby *et al.*, 1989; Gömöry *et al.*, 2003) as well as natural populations (Linhart *et al.*, 1979; Xie and Knowles, 1994). Additionally, it should be noted that reproductive phenology asynchrony, pollen viability and compatibility, and clone size (ramet numbers/clone) all affect clonal gametic contribution (El-Kassaby and Askew, 1991; Nikkanen, 2001).

Consistently high or low fertility clones require special consideration during seed orchard rouging. The main purpose of rouging is to cut out some clones which have lower breeding value so to transfer the higher valued genes to the next generations. The aim of seed orchard is to produce genetically improved seeds as many as possible. In general, the main selection criterion of the orchard rouging is clonal genetic value (breeding value)

of orchard clone (El-Kassaby and Barclay, 1992). However, clonal fertility propensity should also be considered, especially when information on clonal genetic value is not available as in the studied seed orchard or when the clonal fertility variation is very severe.

Variation in reproductive output and phenology as well as rate of pollen contamination, all act individually or in concert in accumulating genetic relatedness and subsequently raising inbreeding level in the progeny and consequently affect the genetic property of the seed (Ying *et al.*, 1985; Nikkanen and Ruosalainen, 2000; Gömöry *et al.*, 2003). Clonal selfing rate of 2.3% and high pollen contamination were reported in a clonal seed orchard of *C. obtusa* by Seido *et al.* (2000a), supporting that high pollen contamination could drastically reduce the quality of the seed crops.

The sibling coefficient ($\Psi \geq 1$) expresses how fertility varies among clones as the coefficient value means the increase of the probability that sibs occur compared to the ideal situation where clones have equal fertility (Kang and Lindgren 1999). When Ψ is equal to one, all clones have equal fertility, and thus theoretically, the sibling coefficient cannot be smaller than one. It should be noted that the sibling coefficient represents the same information as the coefficient of variation (see the formula (1)); however, Ψ is mainly based on a probabilistic aspect while CV is based on a variance aspect.

In general, the effective population size at clone level is estimated as the intermediate value between female and male effective population sizes. But, the effective population sizes at clone level in 2002 and 2006 were higher than those at female and male gamete levels (Figure 1). This may be due to the intermediate correlation between female and male strobili production (seed Kang and El-Kassaby, 2002), and may need to further study.

Small effective population size causes the accumulation of group coancestry in the following generation (Cockerham, 1967), which in turn causes the loss of genetic diversity that is proportional to the accumulation rate. Reduction in genetic diversity in seed crops will affect the level of diversity in seedlings and subsequently of the plantations.

Acknowledgements

This study was supported by the Korea Forest Research Institute (KFRI). The authors thank Dr. Yoshinari Moriguchi, who is the post-doctoral fellow in the KFRI, for his review, discussion and valuable comments on the manuscript.

Literatures Cited

1. Bilir, N., Kang, K.S., Zhang, D., and Lindgren, D. 2004. Fertility variation and status number between a base population and a seed orchard of *Pinus brutia*. *Silvae Genet.* 53: 161-163.
2. Burczyk, J. and Chalupka, W. 1997. Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard. *Ann. Sci. For.* 54: 129-144.
3. Cockerham, C.C. 1967. Group inbreeding and coancestry. *Genetics* 56: 89-104.
4. El-Kassaby, Y.A. 2000. Effect of forest tree domestication on gene pools. In: *Forest Conservation Genetics: Principles and Practice*. Commonwealth Scientific and Industrial Research Organisation (CSIRO) (Young, A., D. Boshier and T. Boyle, eds.). CSIRO Publishing-CABI Publishing, Canberra, Australia. Chapter 13: 197-213.
5. El-Kassaby, Y.A. and Askew, G.R. 1991. The relation between reproductive phenology and reproductive output in determining the potential gametic pool profile in a Douglas-fir seed orchard. *For. Sci.* 37: 827-835.
6. El-Kassaby, Y.A. and Barclay, H.J. 1992. Cost of reproduction in Douglas-fir. *Can. J. Bot.* 70: 1429-1432.
7. El-Kassaby, Y.A., Fashler, A.M.K., and Crown, M. 1989. Variation in fruitfulness in a Douglas-fir seed orchard and its effect on crop-management decisions. *Silvae Genet.* 38: 113-121.
8. Eriksson, G., Jonsson, A., and Lindgren, D. 1973. Flowering in a clone trial of *Picea abies* Karst. *Stud. For. Suec.* 110. 45pp.
9. Gömöry, D., Bruchanik, R., and Longauer, R. 2003. Fertility variation and flowering asynchrony on *Pinus sylvestris*: Consequences for the genetic structure of progeny in seed orchards. *For. Ecol. Manage.* 174: 117-126.
10. Kang, K.S. and El-Kassaby, Y.A. 2002. Considerations of correlated fertility between genders on genetic diversity: *Pinus densiflora* seed orchard as a model. *Theor. Appl. Genet.* 105: 1183-1189.
11. Kang, K.S. and Lindgren, D. 1999. Fertility variation among clones of Korean pine (*Pinus koraiensis*) and its implications on seed orchard management. *For. Genet.* 6: 191-200.
12. Kang, K.S., Bila, A.D., Harju, A.M., and Lindgren, D. 2003. Fertility variation in forest tree populations. *Forestry* 76: 329-344.
13. Lindgren, D. and Mullin, T.J. 1998. Relatedness and status number in seed orchard crops. *Can. J. For. Res.* 28: 276-283.
14. Linhart, Y.B., Mitton, J.B., Bowman, D.M., Sturgeon, K.B., and Hamrick, J.L. 1979. Genetic aspects of fertility differentials in ponderosa pine. *Genet. Res. Camb.* 33: 237-242.
15. Matziris, D.I. 1993. Variation in cone production in a clonal seed orchard of Black pine. *Silvae Genet.* 42:

- 136-141.
16. Nikkanen, T. 2001. Reproductive phenology in a Norway spruce seed orchard. *Silvae Fenn.* 35: 39-53.
 17. Nikkanen, T. and Ruosalainen, S. 2000. Variation in flowering abundance and its impact on the genetic diversity of grafts of *Pinus sylvestris*. *For. Ecol. Manage.* 19: 35-40.
 18. Reynolds, S. and El-Kassaby, Y.A. 1990. Parental balance in Douglas-fir seed orchards-cone crop vs. seed crop. *Silvae Genet.* 39: 40-42.
 19. Seido, K., Maeda, H., and Shiraishi, S. 2000a. Determination of the selfing rate in a hinoki (*Chamaecyparis obtusa*) seed orchard by using a chloroplast PCR-SSCP marker. *Silvae Gene.* 49: 165-168.
 20. Seido K, Maeda H, Murakami H, Ida K., and Shiraishi, S. 2000b. Contribution of clones as the pollen parent in a hinoki (*Chamaecyparis obtusa*) seed orchard estimated using a chloroplast DNA marker. *J. Japanese For. Soc.* 82: 105-108.
 21. Shiraishi, S., Maeda, H., Toda, T., Seido, K., and Sasaki, Y. 2001. Incomplete paternal inheritance of chloroplast DNA recognized in *Chamaecyparis obtusa* using an intraspecific polymorphism of the trnD-trnY intergenic spacer region. *Theor. Appl. Genet.* 102: 935-941.
 22. Xie, C.Y. and Knowles, P. 1994. Mating system and effective pollen immigration in a Norway spruce (*Picea abies* (L.) Karst) plantation. *Silvae Genet.* 43: 48-52.
 23. Yamashita, T., Kasuya, N., Nishimura, S., and Takeda, H. 2004. Comparison of two coniferous plantations in central Japan with respect to forest productivity, growth phenology and soil nitrogen dynamics. *For. Ecol. Manage.* 200: 215-226.
 24. Ying, C.C., Murphy, J.C. and Andersen, S. 1985: Cone production and seed yield of lodgepole pine grafts. *For. Chron.* 61: 223-228.

(Received April 30, 2007; Accepted June 20, 2007)