

Fertility variation and gene diversity in a clonal seed orchard of *Pinus sylvestris*

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Abstract

Clonal differences in fertility (expressed as the number of female and male strobili) were determined for three years (2001, 2005 and 2006) in a clonal seed orchard of Scots pine (*Pinus sylvestris*). The seed orchard consisted of 29 clones (total of 1,265 ramets), and was established at northern part of Turkey (latitude 40°52'N, longitude 30°42'E, altitude 120m) in 1986. Grafts originated from plus trees selected in a stand and planted at spacing 7m x 7m.

The numbers of female and male strobili were counted for 25 clones. The number of ramets per clone varied from 11 to 58 (average 44 per clone). Reproductive output assessment was conducted for five ramets per clones randomly chosen and surveyed over the three years, avoiding ramets growing at the edges of the seed orchard. The number of female strobili for the sampled ramets was counted from a whole crown, but the number of male strobili was estimated by multiplying the average number of strobili per branch by the total number of strobili bearing branches.

Based on the reproductive output assessment, female and male fertility variation was estimated following the concept of sibling coefficient developed by Kang and Lindgren (1999). The female and male fertility variation was estimated by either relative contribution of clones or the coefficient of variation (CV) to strobilus production. Clonal fertility variation was estimated from the female and male fertility variation (Kang and El-Kassaby, 2002). Based on the observed clonal fertility variation, the status numbers (Ns, measure of genetic diversity) were calculated. The clones were similar fertile on both female and male sides. Pearson's correlation coefficients between female and male strobili production were positive for all studied years. Variation in female fertility was higher than that in male fertility in 2001, but it was opposite situation in 2006. Such variation was reflected on female and male parents' status numbers. Clonal fertility variation measured as the sibling coefficient was 1.10, 1.01 and 1.04 for the three years. The status numbers varied from 22.95 to 24.69 among the three studied years. On average (pooled), the status number (Ns) was more than 90% of census number (N), implying that most clones show near equal contribution. This even distribution of fertility by the clones is in contrast to the literature review by Kang et al (2001) who reported a typical value slightly above 2.0 (CV=100%) in mature clonal seed orchards.

Clones were grouped according to their female strobili production (i.e., seed production) into 5, 10, 15, 20 and 25 clones and then fertility and status number were estimated. This alternative might be used as selective harvest from fertility clones. But, fertility variation was similar for female and male parents and total gametic gene pool for clone groups and years. Establishment and management of seed orchards were also discussed in the light of the results reported from this study.

Key words: Fertility variation, sibling coefficient, status number, flowering, Scots pine.

Introduction

Number of clones is one of the most important factors both economically (e.g. selection of plus trees, harvested seed orchard crop) and genetically (e.g. gene diversity of orchard crop). But, there were large differences among species and countries used on clone numbers in seed orchards. If many clones were used, genetic diversity would be high. But seed orchard cost would also be high. When a small number of clones are used, some rare alleles in a base population may be lost in a seed orchard due to sampling effect (Bilir *et al.*, 2004). For gene diversity of orchard crops, the number of clones may be more important than the equal number of ramets among clones (Kang, 2001). Many works were carried out on clone numbers (e.g., Kang *et al.*, 2001; Bilir *et al.*, 2004; Lindgren and Prescher, 2005) recently, while much previous discussion about inbreeding and genetic diversity in seed orchards on the number of clones required such as by Lindgren (1974), and Moran *et al.* (1980). Establishment of seed orchard and estimation of seed demand for the plantations are important stages in breeding programs. Gene diversity in seed crop used in plantations plays an important role for sustainable forestry such as transmission of gene diversity from current generation to next. Estimation of gene diversity in seed orchard crop by fertility variation is a cheap method. Parental fertility of a tree is proportional to the number of female and male gametes produced by the tree (Gerigori, 1989). Differences in gamete contribution among clones could be genetic (Eriksson *et al.*, 1973), environmental (Hedergart, 1976), and management of orchard (Zobel and Talbert, 1984). Besides, years could impact on flowering differences among clones (Lindgren *et al.*, 1977). For instance, good seed years appear one time in two or three years (Ata, 1995).

The purpose of the study is to estimate fertility variation and gene diversity in seed orchard crop based on years and different census numbers of clones, and to discuss management and establishment of seed orchards.

Material and methods

Descriptions of seed orchard and data collection

The study was carried out in a clonal seed orchard of *P. sylvestris*. The seed orchard originated from plus trees, and was established at Sogutlu-Adapazari (latitude 40°52'N, longitude 30°42'E, altitude 120 m), northern part of Turkey in 1986. Grafts were planted at spacing 7 m x 7 m, and the seed orchard is composed of 29 clones (total of 1265 ramets) (Anonymous, 2001). Numbers of female and male strobili were counted in six ramets chosen randomly of each twenty-five clones in April of 2001, May of 2005 and 2006. The clones were grouped according to their female strobili production to 5, 10, 15, 20 and all (25).

Fertility variation

The female fertility (ψ_f) and male fertility (ψ_m) variation were estimated as (Kang and Lindgren, 1999):

$$\psi_f = N \sum_{i=1}^N f_i^2 = CV_f^2 + 1, \psi_m = N \sum_{i=1}^N m_i^2 = CV_m^2 + 1 \quad [1-a\&b]$$

where N is the census number, f_i is the female fertility of the i^{th} individual, m_i is the male fertility of the i^{th} individual and CV_f and CV_m are the coefficients of variation in female and male fertility among individuals, respectively.

Total fertility variation (Ψ) was estimated as (Kang, 2001):

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

Where N is the census number, f_i and m_i are female and male fertility, respectively.

Status number and gene diversity

If the clones are unrelated and non-inbred, the status numbers of female ($N_{s(f)}$) and male parents ($N_{s(m)}$) are calculated as:

$$N_{s(f)} = \frac{1}{\sum_{i=1}^N p_{i(f)}^2}, N_{s(m)} = \frac{1}{\sum_{i=1}^N p_{i(m)}^2} \quad [3-a\&b]$$

where $p_{i(f)}$ is the female fertility of the clone i , $p_{i(m)}$ is the male fertility of the clone i and N is the census number in the seed orchard. Here, fertility is estimated based on the strobilus assessment. Status number on total fertility was calculated following as Kang (2001):

$$N_s = \frac{4N}{[\psi_f + \psi_m + 2 + 2r\sqrt{(\psi_f - 1)(\psi_m - 1)}]} \quad [4]$$

where N is census numbers of clone, ψ_f and ψ_m are the fertility variation of female and male parents, and r is the correlation coefficient between female and male fertility.

Gene diversity (GD) was estimated based on total fertility (Ψ) and census number of clones (N) as:

$$GD = 1 - \frac{0.5\Psi}{N} \quad [5]$$

Results and discussion

Strobili production

As seen from Table 1 and Figure 1, there were large differences for strobili production and coefficient of variation among years and clone numbers. 20% of the most productive clones produced 31% of total female strobili, 29% of male strobili in 2001, 23% of both female and male in 2005, 25% of female and 27% of male in 2006.

Table 1. Averages, coefficient of variation, correlation (r) of female and male strobili production for number of clones (N) and years.

N	2001			2005			2006		
	\bar{x}_f & \bar{x}_m	CV_f & CV_m	r^*	\bar{x}_f & \bar{x}_m	CV_f & CV_m	r	\bar{x}_f & \bar{x}_m	CV_f & CV_m	r
5	299 & 737	0.05 & 0.14	0.73	293 & 804	0.12 & 0.17	0.83	404 & 738	0.06 & 0.10	0.78
10	263 & 631	0.15 & 0.15	0.75	277 & 784	0.10 & 0.10	0.79	378 & 641	0.09 & 0.09	0.80
15	243 & 585	0.19 & 0.24	0.73	270 & 771	0.09 & 0.11	0.77	359 & 607	0.11 & 0.18	0.79
20	217 & 553	0.28 & 0.24	0.72	264 & 756	0.09 & 0.10	0.75	343 & 600	0.13 & 0.19	0.72
all	193 & 522	0.38 & 0.27	0.69	253 & 734	0.12 & 0.12	0.78	324 & 561	0.17 & 0.24	0.74

*; statistically significant at 0.01 probability level.

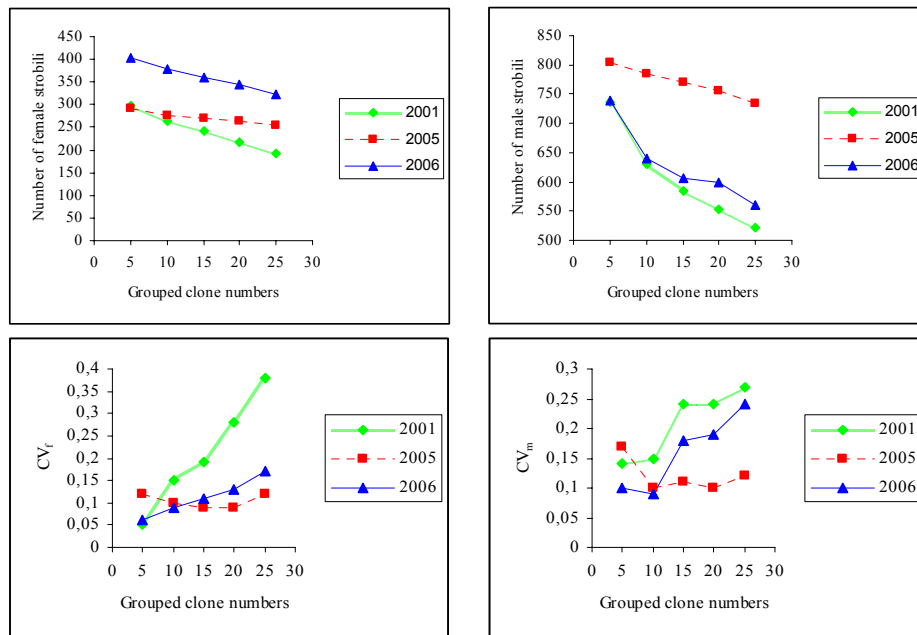


Figure 1. Averages, and coefficient of variation of female and male strobili.

Significant relation was found between female and male strobili in all year and clone number (Table 1). It was also reported by Jonsson *et al.* (1976), Bhumibhamon (1978), Burczyk and Chalupka (1997), and Bilir *et al.* (2002) in the species.

Large differences for average strobili and coefficient of variation among census number of clones and years were found (Table 1). But, there were significant relation between female and male strobili for both all year and clone numbers (Table 1).

Fertility variation, status number, relative status number and gene diversity

Although there were large differences among year and clone numbers for average strobili (Table 1 and Figure 1), parental and total fertility were similar (Table 2 and Figure 2). So, even year was one of the important factors on strobili production (e.g good seed year appears one time in

two or three years in the species; Ata, 1995), it was not effective on fertility variation. Similar results were reported in natural stands of Brutian pine by Bilir et al. (2005). Maximum gene diversity of seed orchards crop can only be attained when all parents contribute equally to the gamete gene pool (Kang, 2001).

Table 2. Female and male fertility variation (ψ_f & ψ_m), total fertility variation (Ψ), status number (Ns) and relative status number (Nr) and gene diversity for number of clones (N).

N	2001					2005					2006				
	5	10	15	20	all	5	10	15	20	all	5	10	15	20	all
ψ_f	1.00	1.02	1.03	1.07	1.14	1.01	1.00	1.01	1.01	1.01	1.00	1.00	1.01	1.02	1.03
ψ_m	1.02	1.05	1.05	1.06	1.07	1.02	1.01	1.01	1.01	1.01	1.00	1.03	1.03	1.04	1.06
Ψ	1.01	1.03	1.04	1.06	1.09	1.02	1.01	1.01	1.01	1.01	1.00	1.02	1.02	1.02	1.04
$N_{s(f)}$	4.99	9.81	14.53	18.64	22.00	4.94	9.91	14.88	19.83	24.65	4.98	9.93	14.84	19.68	24.32
$N_{s(m)}$	4.92	9.57	14.25	18.95	23.34	4.89	9.87	14.84	19.79	24.67	4.96	9.71	14.56	19.30	23.70
N_s	4.98	9.73	14.45	18.93	22.95	4.92	9.90	14.88	19.83	24.69	4.98	9.85	14.73	19.59	24.13
$N_{r(f)}$	0.99	0.98	0.97	0.93	0.88	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.97
$N_{r(m)}$	0.98	0.96	0.95	0.95	0.93	0.98	0.99	0.99	0.99	0.99	0.99	0.97	0.97	0.97	0.95
N_r	0.99	0.97	0.96	0.95	0.92	0.98	0.99	0.99	0.99	0.99	0.99	0.98	0.98	0.98	0.97
GD	0.900	0.949	0.965	0.974	0.978	0.898	0.950	0.966	0.975	0.980	0.900	0.979	0.966	0.975	0.979

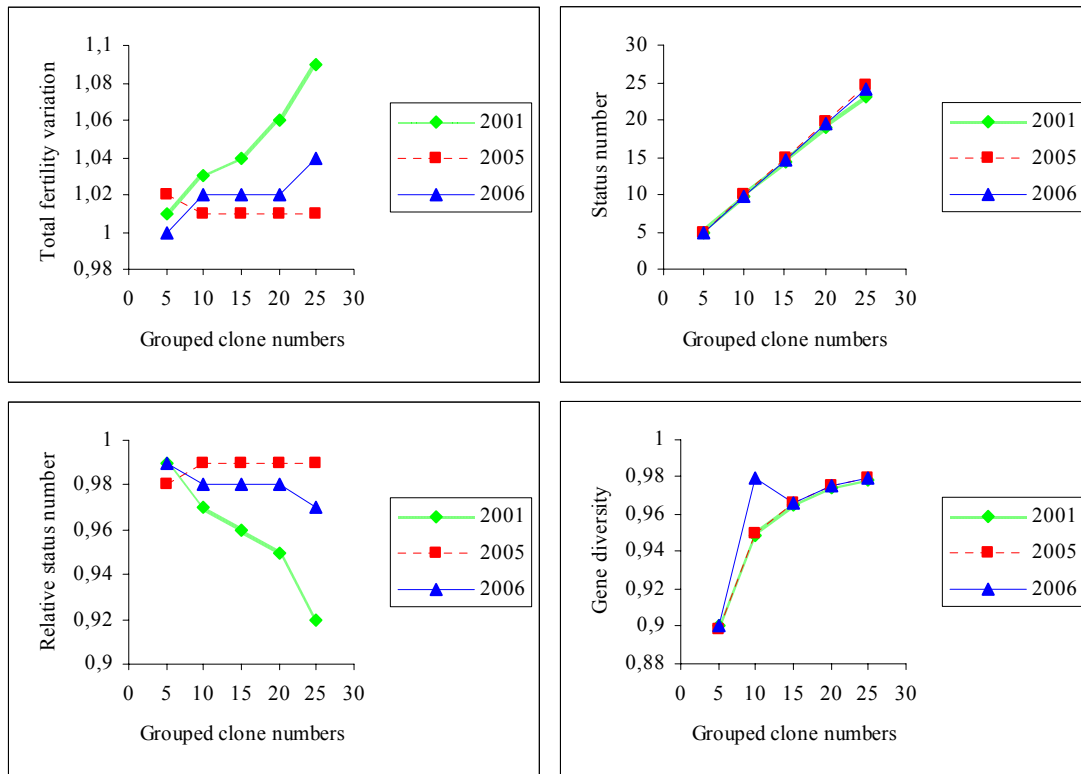


Figure 2. Total fertility variation, status number, relative status number and gene diversity.

It showed that less clones (e.g., 5-10 clones) could be used in establishment of seed orchards both economy and balance among clones (Table 2). Bilir et al. (2004) reported that even though the production of strobili is assumed to be proportional to the successful number of gametes, the

number of 30 clones could hardly deliver entire gene diversity of the base population into the seed orchard. When used less clones, probably some rare alleles could also be missing when the base population transmits gene pool onto the seed orchard. But most breeding program does not focus mainly on the rare alleles that are not important for economic traits (Bilir et al., 2004). Maximum gene diversity of seed orchards crop can only be attained when all parents contribute equally to the gamete gene pool (Kang, 2001). The relation between gene diversity (GD) and fertility variation (Ψ), and census number of clones (N) can be showed as: $GD = 1 - (0.5 \Psi/N)$. It is also estimated based on status number (Ns) as: $GD = 1 - 1/(2*Ns)$ (Kang, 2003). When used many clones in establishment of seed orchard, fertility variation among and within clone will be high (e.g. CV) and then balance of fertility by seed orchard manager will be hard. Besides, effective number of clone can be low. So, fewer clones (e.g., 5-10 clones) should be used at establishment of seed orchard. it could be reduced to 20 or fewer clones after genetic rouging (Lindgren and Prescher, 2005).

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