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Fertility Variation and Gene Diversity in Clonal Seed Orchards of Pinus brutia, Pinus nigra and Pinus sylvestris in Turkey

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Summary

Clonal variation in the production of female and male strobili was studied in seed orchards of *Pinus brutia* in southern, and those of *P. nigra* and *P. sylvestris* in northern Turkey. Large differences in female and male fertility among clones were found. The most abundant quartile of clones in *P. brutia*, *P. nigra* and *P. sylvestris* seed orchards produced 44.1%, 40.5%, 33.9% and 37.8% of total female, and 76.1%, 62.6%, 36.7% and 34.1% in total male strobilus production, respectively. There were positive correlations between female and male strobilus production in all seed orchards. The status numbers (N_s) were calculated as 19.3 (64% of census number) and 23.2 (66%) in two *P. brutia* seed orchards, 29.2 (94%) in a *P. nigra* seed orchard and 25.7 (92%) in a *P. sylvestris* seed orchard. Male fertility variation was larger than female fertility variation in *P. brutia* and *P. nigra* seed orchards, while it was an opposite situation in the *P. sylvestris* seed orchard. The effect of fertility variation was discussed for the establishment of new seed orchards and the management of current orchards in Turkey.

Key words: Pinus brutia, *Pinus nigra*, *Pinus sylvestris*, seed orchard, fertility variation, status number, gene diversity.

Introduction

A seed orchard is an important seed source for forest plantation, and it keeps generally high genetic diversity compared to natural forests where orchard parents were selected (SAVOLAINEN and KÄRKKÄINEN, 1992). *Pinus brutia* TEN., *P. nigra* ARNOLD. and *P. sylvestris* L. are among most important native *Pinus* species in Turkey, and they occupy about 3.7 (26.4%), 2.5 (17.9%) and 0.7 (5.0%) million ha of the total 14 million ha of high forest area in Turkey, respectively (CALISKAN, 1998).

Large clonal differences in female and male fertility have been reported in seed orchards (KJAER, 1996; KANG and LINDGREN, 1998; NIKKANEN and RUOTSALAINEN, 2000), in natural populations (BILA and LINDGREN, 1998), and in plantation areas (BILA *et al.*, 1999). Research on cone and seed properties, phenology, and pollen production in seed orchards have also been studied (YAZDANI and LINDGREN, 1991; MATZIRIS, 1993, 1997, 1998; FRIES, 1994; YAZDANI *et al.*, 1995; KESKIN, 1999). Such fertility variation will cause rapid accumulation of group coancestry and potential inbreeding in the following generation (BILA, 2000; KANG, 2001). To monitor and determine the genetic diversity of seed crops, some genetic parameters such as genetic relatedness, inbreeding, and gene diversity should be calculated.

Effective population size and status number have been widely used to express accumulated inbreeding, random genetic drift and group coancestry (CROW and KIMURA, 1970; LINDGREN *et al.*, 1996; KJAER and WELLENDORF, 1997). A key difference between effective population size and status number is how to describe the loss of gene diversity (inbreeding and group-coancestry). The former expresses it as dynamic over generations, while the latter does as static in each generation separately (LINDGREN and MULLIN, 1998). Seed orchards are generally used only for one generation. So, the status number is a good tool for monitoring gene diversity of orchard crops.

The purpose of this study is to evaluate fertility variation among orchard parents and its effect on gene diversity of seed (i.e., status number), and to provide genetic information to guide the "National Tree Breeding and Seed Production Programme for Turkey (KOSKI and ANTOLA, 1993)". New seed orchards in Turkey will be established vegetatively with grafts after progeny tests according to the programme.

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Materials and Methods

Descriptions of seed orchards

The study was carried out in two clonal seed orchards of *P. brutia* and one clonal orchard of *P. nigra* and *P. sylvestris*, respectively. The seed orchards of *P. brutia* originated from plus trees, which were selected from seed stands at Bucak (orchard 1) and Serik (orchard 2), were established in 1985, at Antalya (latitude 37°20'N, longitude 30°35'E, altitude 310 m), southern part of Turkey. Grafts were planted at spacing 8 m x 8 m, and the seed orchards 1 and 2 are composing of 30 and 35 clones (1,620 and 1,080 ramets), respectively. The seed orchard of *P. nigra* originated from a plus tree stand at Tavsanli and that of *P. sylvestris* originated from a plus tree stand at Dirgine were established in 1986, at Adapazari (latitude 40°55' N, longitude 30°45' E, altitude 120 m), northern part of Turkey. Grafts were planted at spacing 8 m x 8 m in the *P. nigra* seed orchard consisted of 31 clones (1,340 ramets), and 7 m x 7 m in the *P. sylvestris* seed orchard consisted of 29 clones (1,265 ramets), respectively (ANONYMOUS, 2001).

Data collection and parental-balance curve

The numbers of female and male strobili were counted from six ramets chosen randomly from each clone in each orchard. Strobili of the sampled grafts were counted individually over the whole crown. All clones in the studied seed orchard were surveyed at the beginning of April in 2001.

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

Status number

We used the concept of status number (N_s, LINDGREN *et al.*, 1996). 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

$$N_{s(f)} = \frac{1}{\sum_{i=1}^{N} p_{i(f)}^{2}} \qquad [1\text{-a}]$$

$$N_{s(m)} = \frac{1}{\sum_{i=1}^{N} p_{i(m)}^{2}} \qquad [1\text{-b}]$$

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.

The central issue for describing fertility variation is the square sum of contribution as in formulae [1-a] and [1-b]. KANG and LINDGREN (1998) defined and developed the sibling coefficient (Ψ) that expresses the fertility variation in a population using a coefficient of variation (CV) in strobilus production among clones, which is independent on the census number (N). Ψ is related to CV as $\Psi = N\Sigma p_i^2 = CV^2 + 1$. In principle, Ψ is regarded as a characteristic of the probability density function of fertility, while CV is a normalized observed variance that is dependent on the number of observation (KANG and LINDGREN, 1998). When the total fertility (i.e., average of female and male fertility) is estimated, the correlation between female and male fertility is also important (KANG and EL-KASSABY, 2002).

Status number on total fertility was calculated following KANG (2001) and as:

$$N_s = \frac{4N}{[\Psi_f + \Psi_m + 2 + 2r\sqrt{(\Psi_f - 1)(\Psi_m - 1)}]} \qquad [2]$$

where Ψ_f and Ψ_m are the fertility variation of female and male parents, which are equivalent to $CV_f^2 + 1$ and $CV_m^2 + 1$, respectively (KANG, 2001). Here, CV_f and CV_m are the coefficients of variation in female and male fertility, and r is the correlation coefficient between female and male fertility.

Results and Discussion

Female and male strobilus production

The average, coefficient of variation (CV), range of female and male strobilus production and phenotypic correlation coefficients between female and male strobili are presented in *Table 1*.

Table 1. – Average, coefficient of variation (CV), range and correlation (r) in female and male strobilus production in the studied seed orchards in Turkey.

	*Pinus brutia*1		*Pinus brutia* 2		*Pinus nigra*		*Pinus sylvestris*	
	female	male	female	male	female	male	female	male
Average	89.0	289.8	93.6	317.0	78.3	187.4	201.4	531.3
CV	0.491	1.277	0.493	1.036	0.247	0.350	0.375	0.257
Range	0-368	0-2472	0-333	0-2720	14-210	0-600	0-910	136-1768
r a)	0.278		0.701		0.387		0.774	

a) phenotypic correlation coefficient between clonal female and male strobilus production

As shown in *Table 1*, there was a large difference in the production of female and male strobili among clones. The fertility variation (CV) in *Pinus brutia* was larger than *P. nigra* and *P. sylvestris*. Variation in male fertility was higher than in female fertility in *P. brutia* and *P. nigra* seed orchards. Also, the most abundant quartile of clones in *P. brutia* (orchards 1 and 2), *P. nigra* and *P. sylvestris* seed orchards produced 44.1%, 40.5%, 33.9% and 37.8% of total female, and 76.1%, 62.6%, 36.7% and 34.1% of total male strobilus production, respectively. Positive correlations between female and male fertility were found in all seed orchards (*Table 1*).

Parental-balance curves are shown in *Figure 1* by means of cumulative gamete contribution. Cumulative contribution of female and male parents showed that male fertility variation was very large in *P. brutia* seed orchards, compared to the other species. In the seed orchard of *P. britua* 1, six clones (20%) did not produce any male strobili, meaning that those clones do not contribute to gamete production as fathers. The curves in *P. nigra* and *P. sylvestris* seed orchards were less distorted than in *P. brutia*, implying that clones in *P. nigra* and *P. sylvestris* seed orchards contribute more equally to gamete production (KANG, 2000).

The *P. brutia* seed orchards were 16 years old at the time of the study, which is rather young in age to peak strobilus production. It is reported that fertility variation is higher when seed orchards are young (KANG and LINDGREN, 1999; BILA, 2000). In the present study, data on strobilus production were collected from only one year. KANG (2001) reported that clonal fertility was fluctuated over many years in conifer seed orchards. Therefore, it is needed to collect more data on fertility variation to draw accurate conclusion. It should be emphasised in this study that there is the immediate need for fertility

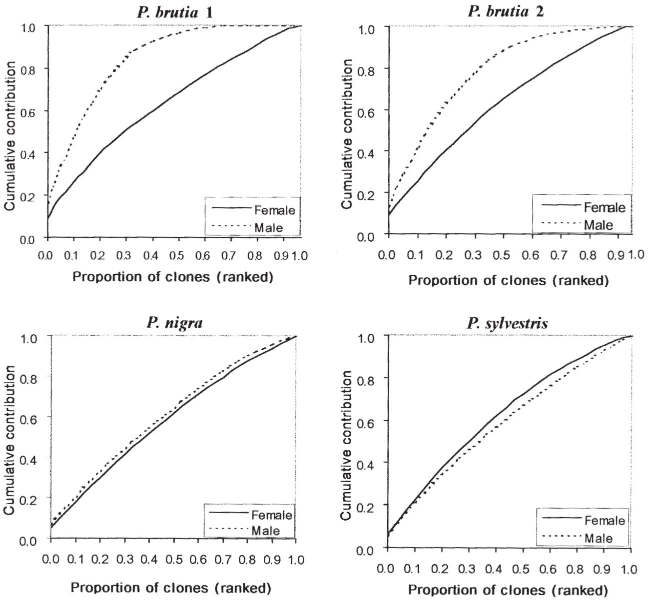

Figure 1. – Parental-balance curves in the seed orchards of *P. brutia, P. nigra* and *P. sylvestris* in Turkey. Proportion of clones was decreasingly ranked according to strobilus production.

information on young seed orchards to adjust orchard management options.

Flowering data were collected from six randomly selected ramets per clone. Many of the first-generation clonal seed orchards are established with intention to have nearly equal number of ramets for each clone. However, there could be ramet variation among clones because intentional (*e.g.,* roguing) and unintentional (*e.g.,* graft availability, graft-incompatibility) variations in ramet numbers among clones occur in the seed orchards (KANG *et al.*, 2001). We did not consider pollen contamination in the present study. Pollen contamination is a main source of gene migration into seed orchard, which affects genetic quality of orchard crops (KANG, 2001). While ramet variation will decrease status number, gene migration from trees outside of the orchards will increase gene diversity (LINDGREN and MULLIN, 1998).

Fertility variation, status number and relative status number

Fertility variation, status number and relative status number for female and male strobili are presented in *Table 2*, and those factors for total fertility are shown in *Table 3*.

Table 2. – Fertility variation (Ψ_f & Ψ_m), status number ($N_{s(f)}$ & $N_{s(m)}$) and relative status number ($N_{r(f)}$ & $N_{r(m)}$) in gametic gene pool for female and male parents.

	P. brutia 1 (30) a)		*P. brutia* 2 (35)		*P. nigra* (31)		*P. sylvestris* (28)	
	female	Male	female	male	female	Male	female	male
Ψ_f & Ψ_m b)	1.24	2.63	1.24	2.07	1.06	1.12	1.14	1.07
$N_{s(f)}$ & $N_{s(m)}$	24.2	11.4	28.2	16.9	29.2	27.6	24.5	26.3
$N_{r(f)}$ & $N_{r(m)}$	0.81	0.38	0.80	0.48	0.94	0.89	0.88	0.94

a) census number of clones (*N*) in parentheses

b) *f* and *m* represent female and male, respectively.

The status number and relative status number of female parents were higher than those of male parents in most seed orchards, except only in *P. sylvestris* (*Table 2*). The relative status numbers for male strobili of *Pinus brutia* (orchards 1 and 2) were very low, implying that some management options (*e.g.,* supplemental mass pollination) should be applied.

Fertility variation (Ψ) in *P. brutia* seed orchards was higher than that in *P. nigra* and *P. sylvestris* seed orchard (*Table 3*). Status number (N_s) for the total fertility was moderately high in all seed orchards (*Table 3*). But the relative status number (N_r) was low in *P. brutia* seed orchards, indicating that orchard may not be functioning efficiently. Relative gene diversity (*GD*) was maintained high (*Table 3*). Gene diversity of orchard crop can be estimated relative to the reference population where trees are unrelated and non-inbred (KANG and LINDGREN, 1999). However, if the parents are related or inbred, *GD* in the studied seed orchard should be lower.

Table 3. – Fertility variation (Ψ), status number (N_s), relative status number (N_r) and relative gene diversity (*GD*) in total gametic gene pool in the studied seed orchards.

	P. brutia 1 (30) a)	*P. brutia* 2 (35)	*P. nigra* (31)	*P. sylvestris* (28)
Ψ	1.55	1.51	1.06	1.09
N_s	19.3	23.2	29.2	25.7
N_r	0.64	0.66	0.94	0.92
GD b)	0.974	0.978	0.983	0.981

a) census number of clones (*N*) in parentheses

b) $GD = 1- 1/(2*N_s)$

Based on genetic information from progeny tests, new seed orchards will be established in Turkey according the "Turkish Breeding Programme". Our results show that there is lack of pollen production in *Pinus brutia* seed orchard, which may cause to low seed production. Therefore, special attention should focus on the increase of pollen production in future orchards of this species. In the seed orchard of *P. sylvestris*, female fertility variation was higher than male fertility variation. So, equal seed harvest or mixing seed equally from clones may improve the female fertility variation and thus status number as suggested by KANG (2001).

Fertility variation may be controlled somewhat by ramet number when new seed orchards are established or existing orchards are rogued (HODGE and WHITE, 1993; KANG *et al.*, 2001). In advanced-generation seed orchards, the main criteria for the selection of orchard parents will be the genetic values of the candidates. However, fertility variation should also be considered to increase status effective number, especially in *P. brutia* seed orchards.

Turkish seed orchards have been established with about 30 clones originated from plus trees from a single seed stand (ANONYMOUS, 2001). The genetic variation is therefore quite narrow, especially if orchard parents are related or somewhat inbred. The existing seed orchards do not have enough clones for genetic roguing based on progeny-test results in the future. Thus, the existing orchards should be managed by refreshment or infusion of new genetic materials. New orchards should also be established with more clones, which are genetically unrelated each other. Based on the Turkish breeding programme, the new seed orchards would be established in *P. brutia*, *P. nigra*, and *P. sylvestris*. In the establishment of new seed orchards or management of existing orchards, the effective number (*e.g.,* status number) rather than census number should be addressed, considering relatedness and fertility variation, for the monitoring of gene diversity in orchard crops.

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Inbreeding in *Pinus Radiata*

III. The Effect of Inbreeding on Age-age Correlation and Early Selection Efficiency

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Abstract

A breeding strategy involving inbreeding followed by crossbreeding of inbreds requires that the production of superior inbred lines must be possible, but crosses between lines should exhibit heterosis, inbreeding should not substantially delay reproduction, and early selection between lines to be effective. Age-age correlation and the effectiveness of early selection have been extensively reported for outcrossed populations of different species, but there are no reports for inbred populations. In this study, age-age correlations based on both family means and individual trees were investigated and compared in radiata pine populations with five different inbreeding levels ($F = 0$, 0.125, 0.25, 0.5 and 0.75). Trends in additive genetic variance, environmental variance, heritability and age-age additive genetic correlations were estimated from an outcrossed population ($F = 0$).

For cross-sectional area at breast height, additive genetic variance increased from 3.7% at age 3 to 29.4% at age 5, remained at about 30% up to age 10, then declined to 15.6% at age 13.

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