

Fertility variation, seed collection and gene diversity in natural stands of Taurus cedar (*Cedrus libani*)

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Research

Keywords: Forest reproductive material, Effective population size, strobilus production, Parental balance curve, Equal cone harvest

Posted Date: May 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-25470/v1>

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Version of Record: A version of this preprint was published at European Journal of Forest Research on October 17th, 2020. See the published version at <https://doi.org/10.1007/s10342-020-01324-1>.

Abstract

Background

Seed stand is one of the important seed sources that is essential for global seed production programs. The primary objective of seed stands is to produce seeds as fast as possible. Characteristics of seed stand seeds depend mainly on stand quality, fertility variation and mating system. Individual fertility is an ability to produce progeny to next generation. Prediction of fertility variation is useful for seed production, genetic resources management and gene conservation. In this study, we report how to estimate fertility variation and gene diversity, and discuss its effect on the management of natural stands of Taurus cedar.

Results

Fertility variation and gene diversity were estimated based on the differences of strobilus production in female and male parents from three seed stands of Taurus cedar. A total of 50 trees were randomly chosen from each seed stand, and the female and male strobili were counted for three consecutive years. The coefficients of variation for female and male strobilus production were subjected to estimation female and male fertility variation. The total fertility variation (Ψ) was then estimated from the female and male fertility variation. The effective number of parents (N_p) was calculated based on the Ψ . The mean of female strobili ranged from 31 to 150, and that of male strobili ranged between 77 and 828. The Ψ in a good crop year was smaller than in a poor year and the N_p varied from 34.7 to 44.2. The Ψ was improved and the N_p was increased when strobilus productions were pooled across three years or populations. The equal cone harvest could mitigate the fertility variation among individuals but caused loss of seed production.

Conclusions

The effective number of parents could estimate gene diversity of seeds from natural stands, which was based on the function of correlated fertility variation between female and male parents. Mixing seeds from different years could improve the fertility variation among individuals and increase the gene diversity of seeds. However, a balancing between the effective number of parents and the number of mixed years or populations should be carefully considered for maintaining the gene diversity.

Background

Taurus cedar (*Cedrus libani* A. Rich), also called as Lebanon cedar, is a valuable timber species and quite striking specimen plants in the landscape. It is endemic to elevated mountains around the Eastern Mediterranean in Lebanon, Syria, and Turkey. Taurus cedar is classified as one of the economically important species for Turkish forestry and the 'National Tree Breeding and Seed Production Programme' (Koski and Antola 1993) because of its valuable wood product and the largest natural distribution on the Taurus Mountains in southern Turkey (Boydak 2003).

Taurus cedar forests cover about 463,000 ha according to Turkish forest inventory, while Boydak (2003) reported that the suitable plantation area for the species was 600,000 ha. Annual seed production is about 139 tones

based on forestry inventory between 2012 and 2018 from the seed stands of Taurus cedar (General Directorate of Forestry 2015). The seeds are produced from 21 seed stands selected at 1,313.5 ha core area and 2,791.9 total area included protection zones. A total of 8 seed orchards (54.1 ha) were established but they are not productive yet because of young age (General Directorate of Forestry 2020).

Seed stands are one of the important seed sources and they are essential for global seed production programs. The primary objective of seed stands is to produce seeds as fast and urgent as possible, but they can also act as breeding and conservation populations. Seed stands have been designated and utilized to produce seeds for reforestation in many countries, and become an important global seed source (Zobel and Talbert 2003). The seed stands are one of the most common and cost-effective means of making available a stable supply of seeds for artificial planation.

Genetic quality of seed stands and the vitality and performance of the resulting seeds depend on many factors, including individual fertility, flowering synchronization, mating system, and gene flow (Muller-Starck and Ziehe, 1984; Zobel and Talbert 2003; Bilir 2011). In any seed stands, some trees produce more flowers, pollen or seeds than others. The seeds from seed stands are often derived from a limited number of parents. It is reasonable to assume that the seed lot from one crop year does not represent the total gene pool of the respective seed stand. It is common that a small portion of the seed parents contribute a disproportionately large amount to the progeny. This unequal contribution leads to an increase in genetic relatedness and gene diversity loss in the resultant seeds.

Estimation of fertility variation is one of the important procedures of breeding and conservation programs, which is defined as a difference of the ability to give progeny (i.e. reproductive success) among individuals (Bila 2000; Kang 2001). It is a useful tool for different purposes such as gene conservation, seed production programs, managing forest genetic resources, and evolutionary and physiological study. Many studies which most of them on estimation practices have been carried out on fertility variation among genotypes in managed populations (Kang et al. 2003; Bilir et al. 2005; Kamalakannan et al. 2015; Bilir and Ozel 2017; Yazici and Bilir 2017). Knowledge of fertility variation estimated based on reproductive characters is getting importance because of its easy, cheap and light survey. The fertility variation should be quantified and its impacts on the population should also be evaluated and mitigated for maintaining gene diversity (Kang et al. 2003). However, theoretical development on fertility variation and its related genetic parameters has been limited in natural forest populations.

A census (minimum) number of adult trees should be required to form approved seed stands in the category "Selected" for wind-pollinated tree species in the scheme of forest reproductive materials (OECD 1974). In German, for example, a number of 40 adult trees, an age of 40 years and an area of 0.25 ha are required as the minimum for Douglas-fir while a minimum area of 2.5 ha is required for Norway spruce and Scots pine (Wojacki et al. 2019). The census number does not give sufficient genetic information while the effective number size would give better information for the characteristics of reproductive materials (Wright, 1931; Falconer and Mackay, 1996; Lindgren and Mullin, 1998).

An effective population size is one of the key parameters in conservation and population genetics (Allendorf et al. 2013). The concept of effective population size is central to ecological, quantitative and evolutionary genetics (Charlesworth and Charlesworth 2010) and plant breeding (Falconer and Mackay 1996). It quantifies the magnitude of genetic drift and inbreeding in real populations. A substantial number of extensions to the basic theory and predictions have been made such as the inbreeding effective population size, the variance effective

population size (Wright 1931), the selection effective population size (Lynch 2007) and the status number (Lindgren and Mullin 1998).

The main purposes of the present study are 1) to survey the female and male strobilus production for three consecutive years in three seed stand populations of Taurus cedar, 2) to estimate the female and male fertility variation among individuals based on the flowering survey, 3) to estimate the effective number of parents in order to monitor gene diversity of seeds harvested from the seed stands, and 4) to discuss its effect on forest management practices (i.e. gene diversity manipulation) for supplying reproductive materials in the natural seed stands of Taurus cedar.

Methods

Flowering survey

Three seed stands were set to be surveyed flowering at three different locations where the species have optimal natural distributions from southern part of Mediterranean region in Turkey (Table 1). The fifty healthy individual trees were randomly chosen from each seed stands. The numbers of female (N_f) and male (N_m) strobili were surveyed for three consecutive years (2016–2018) in three seed stand populations. The production of female strobilus was counted entirely from the individuals. The production of male strobilus was estimated by using number of male strobili in a branch sampled from each direction and multiplying the branch number of each individual. We did not collect data of flowering time or female receptivity to describe the overlapping of flowering periods. Pollen contamination was ignored in the study.

Table 1
Geographic information (latitude, longitude and altitude), age, size, mean DBH and stand density of the seed stands of Taurus cedar

Population	Latitude (N)	Longitude (E)	Altitude (m)	Age (year)	Size (ha)	Mean DBH (cm)	Density (trees/ha)
Senirkent (P ₁)	38°05'23"	30°42'20"	1,600	53	77.0	22.0	800
Egirdir (P ₂)	37°44'47"	30°49'21"	1,600	113	48.9	36.0	650
Kizildag (P ₃)	37°51'00"	31°19'10"	1,550	144	23.0	50.0	450

The coefficients of variation (*CV*) for female and male fertility were calculated and applied to estimate the fertility variation as female and male parents, respectively (see the Eq. 2-a and 2-b). The individual contribution (fertility) to strobilus production was proportionally calculated from the surveyed flowering data in each population.

Genetic variation and parental balance

The following ANOVA model was used to analyses the difference of strobilus production among populations and years by the SAS software (SAS Inst. Inc. 1988) in the present study.

$$Y_{ijk} = \mu + F_i + B_{j(i)} + FB_{(ij)} + e_{ijk} \quad (1)$$

where Y_{ijk} is the observation from the k^{th} tree of the j^{th} population in the i^{th} year, μ is the overall mean of strobilus production, F_i is the effect of i^{th} year, B_j is the effect of the j^{th} population in the i^{th} year, $FB_{(ij)}$ is the effect of the interaction between i^{th} year and j^{th} population, and e_{ijk} is the random error.

The correlation between female and male strobilus production was analyzed by the Pearson's product moment correlation coefficients. The correlation coefficients (r) were implemented to estimate the total fertility variation (see below Eq. (4)).

The parental balance curve was used to characterize high or low productive individuals for female and male strobili. Individual trees were ranked from high to low for the production of female and male strobilus production, and then cumulative percentage calculations are plotted against the total number of individuals in the studied population (Chaisurisri and El-Kassaby 1993). Female and male genetic contributions can be explained by the parental balance curves, and the cumulative percentage curve is often used to quantify fertility variation in forest populations (Griffin 1982; Kang, 2001).

Theoretical frameworks on fertility variation and effective number

Fertility variation estimation

Female and male fertilities of the i^{th} individual were defined as the ability to produce female and male strobili, respectively and estimated as the relative proportion of female and male strobilus production to the entire population (Muller-Starck and Ziehe 1984). The variations of female and male fertility (ψ_f and ψ_m) among individuals, also called female and male gametic fertility variations, were estimated by the coefficient of variation (CV) for female and male strobilus production suggested by Kang and Lindgren (1998) as:

$$\psi_f = N \sum_{i=1}^N f_i^2 = CV_f^2 + 1 \quad (2-a)$$

$$\psi_m = N \sum_{i=1}^N m_i^2 = CV_m^2 + 1 \quad (2-b)$$

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

Combined fertility variation both female and male leads to the total fertility variation, designated as a symbol of Ψ and called as a sibling coefficient. The total fertility variation (Ψ) can be calculated by Kang (2001) as:

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

$$= 0.25N \sum_{i=1}^N (f_i^2 + m_i^2 + 2f_i m_i) \quad (3)$$

where N is the census number; p_i is the total fertility as a whole parent of i^{th} individual; f_i and m_i are the fertilities as female and male parents of the i^{th} individual, respectively.

Sibling coefficient (Ψ) expresses the probability that two alleles drawn randomly from the gamete gene pool originate from the same parent compared to the probability when the parents have equal representation (Kang and Lindgren 1998; Kang et al. 2003). Fertility variation (sibling coefficient) is a standardized measure that does not depend on the census number (N) of parents, but only on how variable their fertility is. Thus, the fertility of each parent is given as a fraction of all gametes.

The Eq. (3) was improved under the correlation (last term of the equation) between female and male fertility. The new equation was implemented for the estimation of total fertility variation (Ψ) as a whole parent in the present study as;

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

$$= 0.25 (\psi_f + \psi_m) + 0.5 [1 + r \sqrt{(\psi_f - 1)(\psi_m - 1)}] \quad (4)$$

where N is the census number; p_i is the total fertility as a whole parent of i^{th} individual; f_i and m_i are the fertilities as female and male parents of the i^{th} individual; ψ_f and ψ_m are the fertility variations of female and male parents; r is the correlation coefficient between female and male strobilus production in the population, respectively.

If there is no correlation between female and male fertility, the Eq. (4) is simplified as:

$$\Psi = 0.25 (\psi_f + \psi_m) + 0.5 \quad (5)$$

An equal seed harvesting among individuals is imposed in a seed stand population, the Eq. (5) is then described, $\Psi = 0.25\psi_m + 0.5$, as the female fertility does not have variation. However, note that the equalizing female fertility should be given to the most productive parents.

Effective number of parents

The effective number of parents (N_p) is the number of individuals in which an idealized population would produce the same number of sibs (offspring) as the real population (Kang 2001). The effective number of parents is one of

the concepts of effective population size that comes from the fertility variation among population members. It can estimate loss of gene diversity at the state rather than generation transition (Kang 2001).

The effective number of parents (N_p), the effective number of female ($N_p^{(f)}$) and male ($N_p^{(m)}$) gametic parents were calculated based on the total fertility variation (Ψ), female (ψ_f) and male (ψ_m) fertility variations, respectively. The relative effective number of parents (N_r) and the relative effective numbers of female and male parents, $N_r^{(f)}$ and $N_r^{(m)}$, were connected to the N_p , $N_p^{(f)}$ and $N_p^{(m)}$ as (Kang et al. 2003; Bilir 2011; Kamalakannan et al. 2015):

$$N_p = \frac{\Psi}{N}, \quad N_p^{(f)} = \frac{\psi_f}{N}, \quad N_p^{(m)} = \frac{\psi_m}{N} \quad (6)$$

$$N_r = \frac{N_p}{N}, \quad N_r^{(f)} = \frac{N_p^{(f)}}{N}, \quad N_r^{(m)} = \frac{N_p^{(m)}}{N} \quad (7)$$

Results

Strobilus production and parental balance

The production of female and male strobili varied among populations and years, and also between individuals within a population or a year (Table 2). The mean of female strobili was ranged from 31 in 2017 to 150 in 2018 in the Kizildag population (P_3), and that of male strobili was ranged between 77 in 2017 (P_1) and 828 in 2018 (P_3). The Egirdir population (P_2) was poorest in female and male strobilus production among the seed stand populations for all studied years (on average of N_0 55 and N_0 98). The average of female and male strobilus production was highest (on averaged of N_0 109 and N_0 514) in the P_3 population. On average (pooled) of three years across populations, the year 2016 was good female production year, while the year 2017 was poor year (Table 2). On the other hand, the male strobilus production was highest in 2018 and poorest in 2017.

Table 2
Mean of female (N_f) and male (N_m) strobilus production, coefficient of variation (CV) and range of strobilus production in three populations for the studied years

	P₁			P₂			P₃			Pooled	
Year	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV
2016											
N_f	120	0.746	40–420	75	0.687	25–322	147	0.496	36–323	114	0.417
N_m	396	0.721	45–870	105	0.536	35–300	626	0.832	60–2020	376	0.497
2017											
N_f	43	0.556	20–135	54	0.586	13–140	31	0.519	9–75	43	0.412
N_m	77	0.561	25–218	84	0.496	26–225	88	0.362	15–205	83	0.288
2018											
N_f	46	0.549	18–115	37	0.595	20–135	150	0.481	22–342	77	0.383
N_m	351	0.793	50–950	105	0.843	22–550	828	0.865	80–2080	428	0.651
Pooled											
N_f	70	0.523	33–193	55	0.396	20–143	109	0.353	30–204	78	0.877
N_m	275	0.662	58–608	98	0.390	39–228	514	0.574	140–1351	295	1.407

The coefficient of variation (CV) of strobili production changed over years and varied among populations, and the CV values between female and male were not much larger than expected in natural seed stands (Table 2). When pooled, the CV s were improved for female and male fertility, compared to a single year or population. The value of CV ranged from 0.288 in 2017 (male) to 0.651 in 2018 (male) when crossed populations in a year, and varied from 0.353 in P_3 (female) to 0.662 in P_1 (male) for pooled three years in a population (Table 2). However, the CV values were increased (0.887 and 1.407) for grand mean of three years and three populations, implying that female and male fertility variation among individuals could worsen when the entire strobilus production were completely pooled.

There was fluctuation of female and male strobilus production among years and populations (Fig. 1). In seed stands of Taurus cedar, the production of female (seed) and male (pollen) strobili seemed to be low, especially in the poor flowering year. The observed parental balance curves for female and male strobili production were deviated significantly from the ideal situation (equal production among individuals) in all seed stands (Fig. 2). It was clearly showed that the female and male parents contributed unequally to the gamete gene pool. Thus, specific individuals may consistently produce high- or low-strobili based on genetic tendencies. The most

abundant five individual trees (10% of total) produced 29.9% and 21.3% of female and male strobili in 2016, 23.3% and 23.2% in 2017, and 22.0% and 25.0% in 2018 in the P_1 population. These were the similar trend as in the P_2 and P_3 populations (Fig. 2).

Table 3
Analysis of variance for female (N_f) and male (N_m) strobilus production among years and populations in the seed stands of Taurus cedar

Source	Variable	df	Sum square	Mean square	F
Year (Y)	N_f	2	379,562	189,781	**
	N_m	2	10,369,646	5,184,823	**
Population (P)	N_f	2	235,498	117,749	**
	N_m	2	13,055,300	6,527,650	**
Y x P interaction	N_f	4	306,182	76,546	**
	N_m	4	7,263,984	1,815,996	**
Error	N_f	441	1,180,390	2,677	
	N_m	441	46,954,148	106,472	
Total	N_f	450	4,839,589		
	N_m	450	116,923,598		

The significant difference of female and male strobilus production among populations and years were supported by the analysis of variance (ANOVA). The ANOVA for female and male strobilus production showed highly significant differences ($p < 0.01$) among populations and years (Table 3).

The Pearson's correlation coefficients showed positive and significant relationships between female and male strobilus production for all populations within a year ($r = 0.491, 0.454$ and 0.581) (Table 4). The correlation coefficients (r) between female and male strobilus production were applied to estimate the total fertility variation (Ψ) and the effective number of parent (N_p) (see Eq. (4)).

Table 4
Pearson's correlation coefficients (r) among female (N_f) and male (N_m) strobilus productions based on pooling of three populations within a year

Year	2016		2017		2018	
	N_f	N_m	N_f	N_m	N_f	N_m
2016	N_f	-				
	N_m	.491**	-			
2017	N_f	.159 ^{NS}	-.126 ^{NS}	-		
	N_m	.268**	.207*	.454**	-	
2018	N_f	.284**	.403**	-.216**	.049 ^{NS}	-
	N_m	.159 ^{NS}	.380**	-.253**	.005 ^{NS}	.581**

* and ** indicate statistically significant at the probability levels of 0.05 and 0.01.
NS indicates non-significant.

Fertility variation and effective number of parents

Fertility variations of female (ψ_f) and male (ψ_m) parents varied among populations and years (Table 5). The ψ_f ranged from 1.23 (P_3 in 2018) to 1.55 (P_1 in 2016) and the ψ_m ranged 1.13 (P_1 in 2017) to 1.73 (P_1 in 2018), showing that the difference of fertility between female and male was not so large. The variation also indicated that the fertility variation among individuals in a good crop year was smaller than in a poor year (Table 5).

Table 5

Female (ψ_f) and male (ψ_m) fertility variation and the female ($N_p^{(f)}$) and male ($N_p^{(m)}$) effective number in the seed stand populations for the three consecutive years

Year	P_1		P_2				P_3					
	ψ_{f^*}	ψ_m	$N_p^{(f)}$	$N_p^{(m)}$	ψ_f	ψ_m	$N_p^{(f)}$	$N_p^{(m)}$	ψ_f	ψ_m	$N_p^{(f)}$	$N_p^{(m)}$
2016	1.56	1.52	32.1	32.9	1.47	1.29	34.0	38.8	1.25	1.69	40.1	29.5
2017	1.31	1.31	38.2	38.0	1.34	1.25	37.2	40.1	1.27	1.13	39.4	44.2
2018	1.30	1.63	38.4	30.7	1.35	1.71	36.9	29.2	1.23	1.75	40.6	28.6
Pooled	1.27	1.44	39.2	34.8	1.16	1.15	43.2	43.4	1.12	1.33	44.5	37.6

*; f and m represent female and male, respectively.

Table 6

Total fertility variation (Ψ) and the effective number of parent (N_p) in the seed stand populations for three studied years

	P₁		P₂		P₃		Pooled	
Year	Ψ	N_p	Ψ	N_p	Ψ	$N_e^{(p)}$	Ψ	N_p^*
2016	1.40	35.8 (0.72)	1.25	40.1 (0.80)	1.32	38.0 (0.76)	1.59	94.4 (0.63)
2017	1.26	39.6 (0.79)	1.21	41.2 (0.82)	1.13	44.2 (0.88)	1.22	123.3 (0.82)
2018	1.27	39.5 (0.79)	1.44	34.7 (0.69)	1.31	38.1 (0.76)	1.91	78.4 (0.52)
Pooled	1.27	39.5 (0.79)	1.10	45.5 (0.91)	1.13	44.2 (0.88)	2.03	221.5 (0.49)
(): parentheses indicates the relative effective number of parents (N_p) to the census number (N).								
*: note that the census number (N) increased when populations were pooled across a year.								

The total fertility variation (Ψ) ranged from 1.13 to 1.44 across populations and years (Table 6). The Ψ was smallest in the population P_3 in 2017 and largest in the population P_2 in 2018. This was inversely mirrored to the effective number of parents (N_p), and thus the N_p was smallest (69% of census) in the population P_2 in 2018 and largest (88%) in the population P_3 in 2017 (Table 6).

Equal cone harvest simulation

The census number (N) to collect cones could be taken to achieve satisfactory gene diversity of seeds when the fertility variation among individuals is qualified in a population (Kang et al. 2003). The practice of equal cone harvest was simulated for a good crop year (2018) in Kizildag population (P_3). The equalizing of female fertility was preferentially applied to the most fertile female parents. When the equal cone harvest was proportionally increased, the effective number of parents was also increased (Fig. 3).

The simulation of equal cone harvest, however, resulted in loss of seed-cone production. Thus, a balancing among proportion of cone harvest (census number), production of cone (seeds) and effective number of parents (gene diversity) should be considered for the implementation of equal cone harvest. In the simulation, the over-represented female parents were the first concern for the equalizing of female fertility in the seed stand of Taurus cedar.

Discussion

Strobilus production variation

The quantity of strobilus and coefficient of variation (CV) showed a large difference among populations and years, and also between individuals within a population (Table 2). This was supported by yearly fluctuation of strobilus production (Fig. 1) and ANOVA results (Table 3). These results were consistent with the previous reports that a large difference of strobilus production was found among individuals and years within a population and among populations in the same species (Bilir and Ozel 2017; Yazici and Bilir 2017), and in other forest tree species (Bila 2000; Bilir et al. 2005; Kamalakannan et al. 2015; Park et al. 2017). Boydak (2003) reported that a good crop

year in natural stands of the same species was once in two or three years. It should be emphasized that selecting populations and harvesting years are important for collecting seeds and managing seed stand populations. There could be genetical (Eriksson et al. 1973) and environmental factors such as tree form, growth, resistance, slope, aspect, altitude and age (Bilir 2011; Yazici and Bilir 2017) for selecting and managing the seed stands.

The production of cones, flowers, pollen, fruits and seeds have been used to estimate fertility variation among individuals in many plant species (Savolainen et al. 1993; Yazici and Bilir 2017). We did not consider the gene flow from outside populations and flowering synchronization that are certainly contributing to gene diversity of seeds from the seed stand populations. The progress of a seed production program depends on a plentiful delivery of viable seed. The final seed yields may be influenced by breakdown of any one of the processes of pollination, pollen grain germination, pollen tube growth, fertilization and embryo development (Brown 1971; Sarvas 1962). For maximum yield of seed, the female strobili must be pollinated when they are fully receptive to pollen. Empty seeds and low viability may result from a breakdown in embryogeny, and a further loss of seeds is occasioned by premature abscission of seed cones.

Positive and significant correlations were found between female and male strobilus production (Table 4). This result was also reported in the same species (Yazici and Bilir 2017) and in the similar conifer species (Kang and Lindgren 1998; Kang and El-Kassaby 2002; Bilir et al. 2005; Kamalakannan et al. 2015). The correlation could be used for different purposes such as total fertility variation and the effective number of parents as the Eq. (4) in the present study.

Fertility variation and effective number of parents

Female and male fertility variations varied among populations and years (Table 5, Fig. 1). When pooled across years in a population, the female and male gametic fertility variations (ψ_f and ψ_m) were improved (Table 5). When pooled years or populations, the total fertility variation (Ψ) and the effective number (N_p) were improved, respectively (Table 6), indicating that pooling seeds from three years could increase the gene diversity of seeds from the Taurus cedar seed stands.

On the other hand, the mixing all populations and years increased the Ψ value, meaning that fertility variation got worse compared to a single population (Table 6). When pooling three populations, it should be noted that the census number (N) increased as 150 and thus the N_p was larger than a single population. However, the relative effective number of parents (N_r) decreased. Therefore, a balancing between the effective number of parents (gene diversity) and the number of populations and years should be carefully considered for management the seed stands of Taurus cedar. This result might be due to the boosted strong positive correlation (covariation) among populations and years by pooling strobilus productions (see Eq. 4 and Fig. 3).

Sibling coefficient (Ψ) expresses how fertility varies among parents as the increase in the probability that sibs occur compared to the situation where parents have equal fertility (Kang 2001). The Ψ cannot be smaller than one. If $\Psi = 1$, all individuals have the equal fertility, and $\Psi = 2$ means that the probability that two individuals share a parent is twice as high, compared to when the parental fertility is equal across the population. As a heuristic rule of thumb, Kang et al. (2003) suggested that the Ψ equals 3 in natural seed stands and 2 in managed populations as seed orchards. Total fertility variation (Ψ) was 2.03 in totally pooled populations and years, which was smaller than the thumb rule (Table 6).

Effective population size theory has been based on the rate of change in gene frequency variance (genetic drift) and the rate of inbreeding. The effective population size (N_e) is defined as a hypothetical population with simplifying characteristics where genetic drift is the only factor in operation, and the dynamics of allelic and genotypic frequencies across generations depend merely on the census number (N) of population (Crow and Kimura 1970). The effective size of a real population is then defined as the number of individuals in an idealized population, which would give rise to the same rate of inbreeding ($N_e^{(i)}$) or the same change in the variance of gene frequencies ($N_e^{(v)}$) observed in the population under consideration (Allendorf 2013), and status number (N_s) (Lindgren and Mullin 1998).

Effective number of parents (N_p) was proposed in the present study, which was based on the function of correlated fertility variation (Ψ) between female and male parents. The N_p is the same as the status number (N_s) where the parents are not related nor inbred (Lindgren and Mullin 1998). The N_s is the function of group ancestry (Θ) while the N_p is based solely on the fertility variation (Ψ) among members in the population of interest (Kang 2001). Both N_p and Ψ parameters can estimate loss of gene diversity between parents and their gametic progeny. Recently, Park et al. (2017) proposed to use the integration of fertility variation (Ψ) and genetic relatedness (Θ) in estimating the gene diversity of seed crops.

Gene diversity (GD) of seeds was estimated based on the effective number of parents (N_p) as: $GD = 1 - 0.5/N_p$ (Kang and Lindgren 1998; Park et al. 2017). High genetic diversity is important for natural regeneration and sustainability of a species, and for adaptation to climate change and to resistance to biotic factors. The loss of gene diversity in the seeds from the populations could be estimated to be less than 2% which was not alarming.

Implication on management of seed stands

The use of bulked seeds derived from commercial harvests can influence the estimate of effective number and gene diversity. Adult trees with insufficient yield of seed cones are generally not climbed during the commercial harvest. Therefore, these adults could only contribute to the seeds as male parents. The variation in reproductive success of individual trees is amplified by this harvesting procedure (Wojacki et al. 2019). In contrast, the equal seed collection per mother tree may have an artificial balancing influence.

Seeds from seed stands must reflect the gene diversity of their parents and keep gene diversity sufficiently for future generations. One of the management options to reduce loss of gene diversity due to fertility variation is to restrict the parental contribution to the next generation (Bila 2000; Kang et al. 2003). This restriction is more likely to be applied to the maternal contribution (e.g., equal cone harvest). The equal utilization of seeds among trees is often proposed to mitigate the effect of unbalanced contribution among parents in seed stands and orchards (Kang and Lindgren 1998; Bila 2000). By keeping the female contribution equal among individuals, the gene diversity is optimized in the seeds from seed stands. The balancing between gene diversity and seed collection would be more important in genetic resource conservation of *Taurus cedar*

Mixing of seeds from a few seed stands or different years was suggested as a means to mitigate the imbalance of gamete contributions among individuals (Bila 2000; Kang et al. 2003; Wojacki et al. 2019). When seeds were mixed for three years, the fertility variation (Ψ) was improved and the effective number of parents (N_p) was increased (Table 6). However, a certain loss of gene diversity during cone harvest seems inevitable (Fig. 3), and mixing seeds from too many populations or years might be deteriorate fertility variation and gene diversity. Thus,

choice of population numbers, structural characteristics of seed stands, and spatial distribution of seed trees need to be optimized in a way that keeps the gene diversity loss to a minimum.

Stimulation of strobilus production would be necessary to increase the seed and pollen production in seed stands of Taurus cedar. Silvicultural thinning based on the survey of strobilus production would be proposed for spacing, aeration and pollen flow. Organic fertilizing may also be effective to produce more female strobili in the seed stands of Taurus cedar.

Conclusions

The effective number of parents estimates gene diversity of seeds from natural stands, which is based on the function of fertility variation between female and male parents. The fertility variation, called as a sibling coefficient (Ψ), expresses how fertility varies among parents as the increase in the probability that sibs occur compared to the situation where parents have equal fertility. The gene diversity of seeds could be estimated by the effective number of parents (N_p) that is the number of individuals in which an idealized population would give the same number of sibs (relatives) as the real population. The equal cone harvest improved fertility variation and effective number but resulted in loss of seed production in natural stands of Taurus cedar. The mixing seeds from different years also improved the fertility variation among individuals and increased the gene diversity of seeds. However, a balancing between the effective number of parents and the number of mixed years or populations should be carefully considered for maintaining the gene diversity.

Abbreviations

N_p : Effective number of parents; $N_p^{(f)}$: effective number of female parents; $N_p^{(m)}$: effective number of male parents; N_r : relative effective number of parents; CV : coefficient of variation for fertility; r : Pearson's product moment correlation coefficient; Y : sibling coefficient

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the R&D program of Korea Forest Service (Korea Forestry Promotion Institute, project No. 2020185A00-2022-AA02), and partly carried out when Prof. Kang was a visiting faculty to UBC, Canada.

Author contributions

NB conducted fieldwork and data analyses with assistance from K-S and regional forest directorate in Turkey. The manuscript was written by K-S and NB. K-S also developed new equations.

Acknowledgements

Authors thank to the graduate students for their help during data collection and the regional forest directorate in Turkey.

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Figures

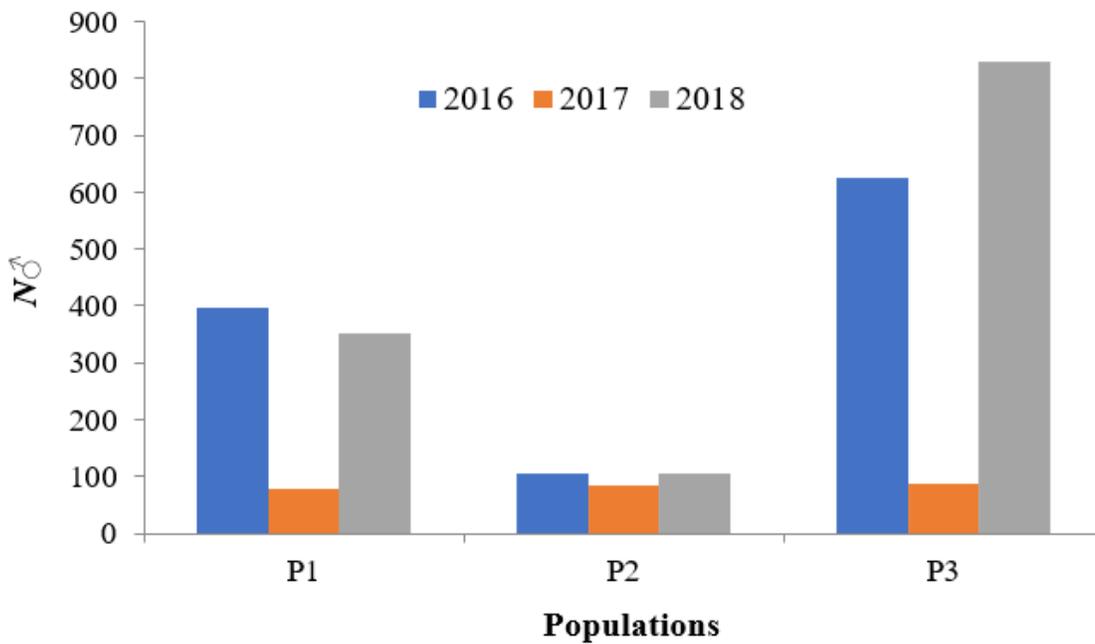
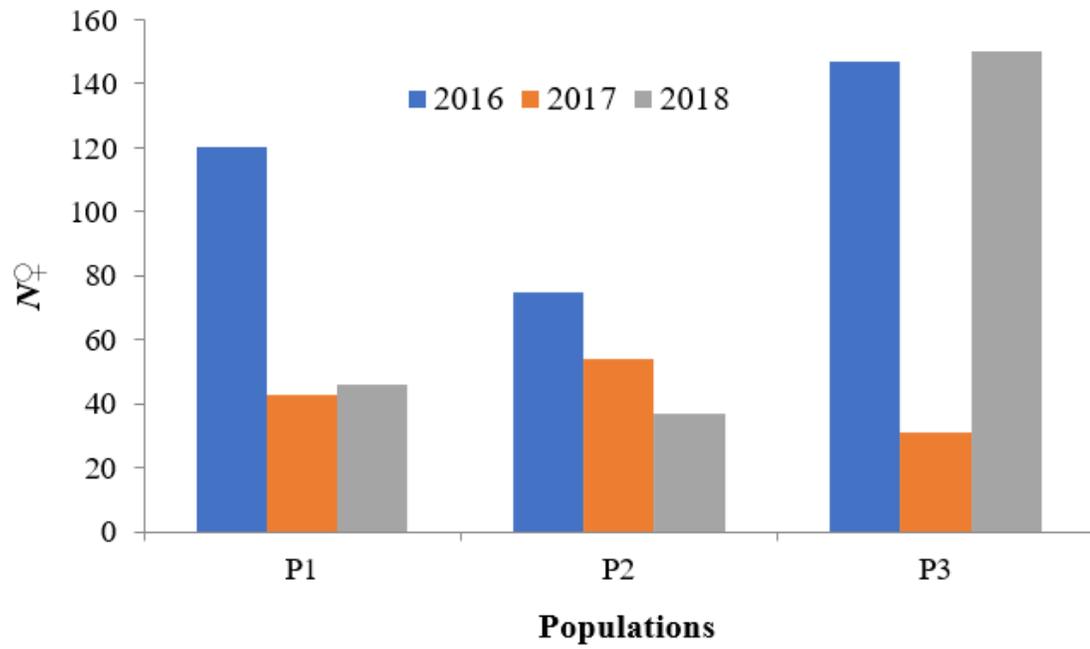


Figure 1

Average of female ($N_{\text{♀}}$) and male ($N_{\text{♂}}$) strobilus productions for three consecutive years in three seed stand populations of Taurus cedar

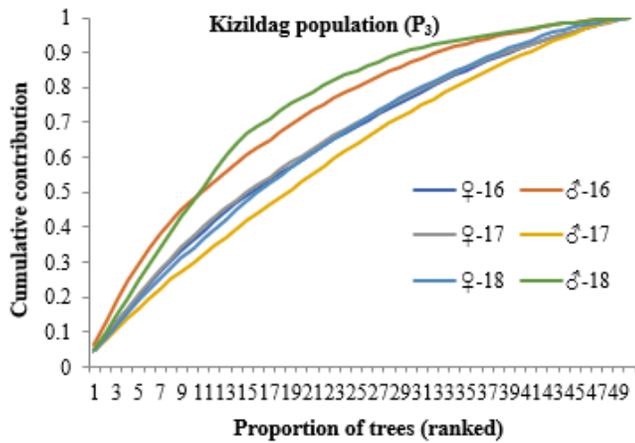
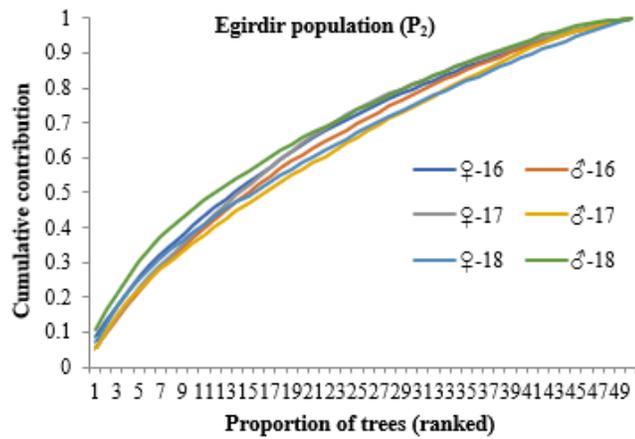
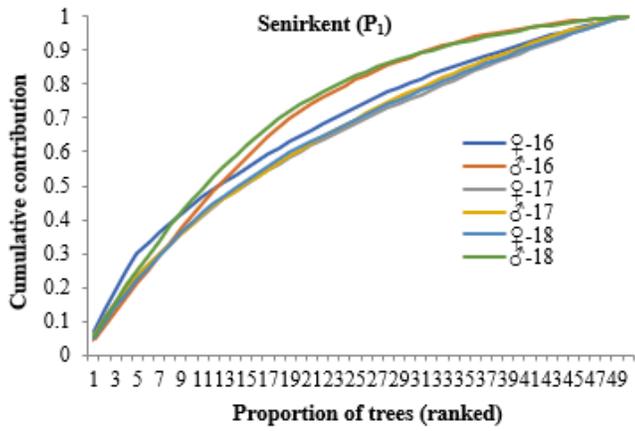


Figure 2

Parental-balance curve of female and male strobilus productions in the seed stand populations for 2016 to 2018

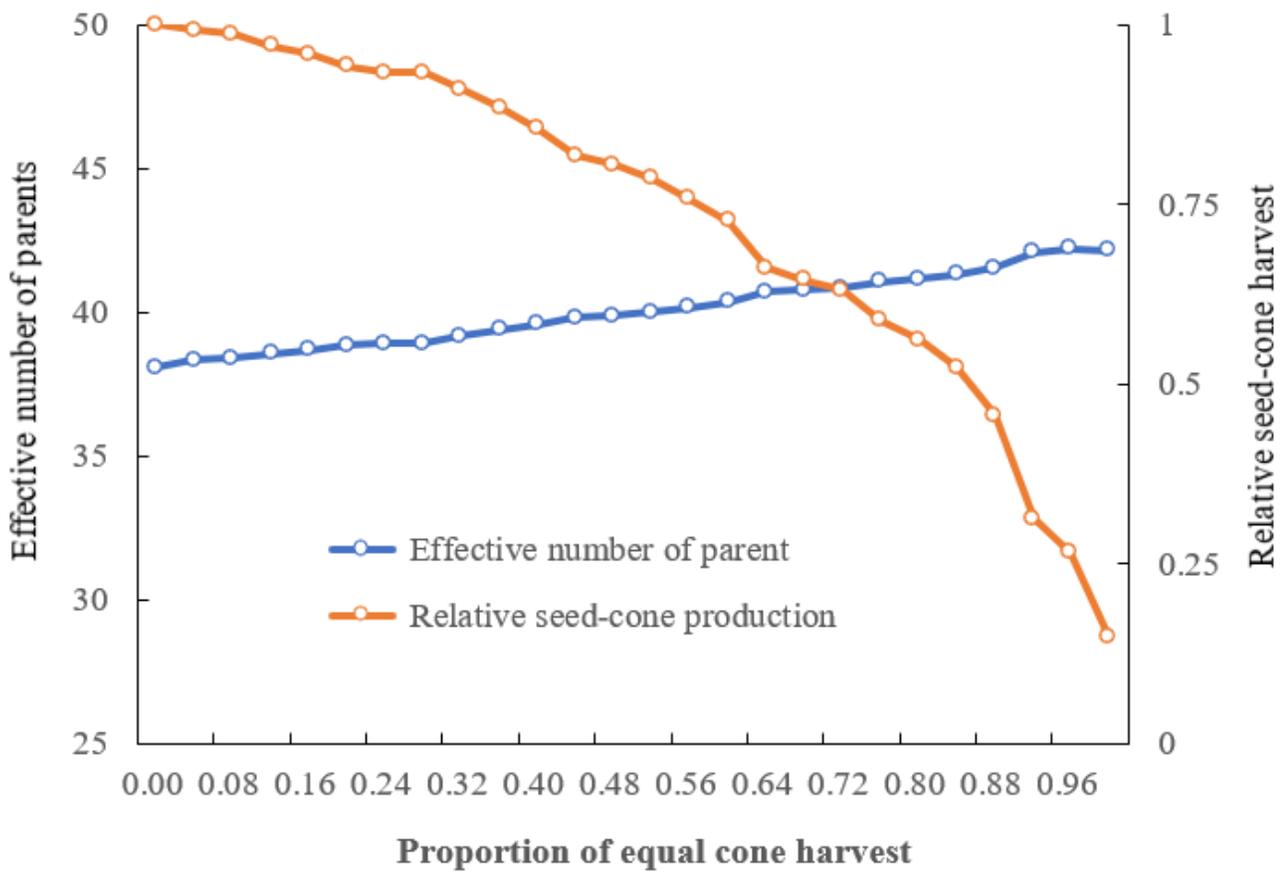


Figure 3

Balancing among proportion of equal cone harvest, relative production of cones and effective number of parents in a seed stand of Taurus cedar simulated for a good crop year (2018) in Kizildag population (P3)