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EFFECTIVE POPULATION NUMBER ESTIMATION OF THREE SCOTS PINE (*PINUS SYLVESTRIS* L.) SEED ORCHARDS BASED ON AN INTEGRATED ASSESSMENT OF FLOWERING, FLORAL PHENOLOGY, AND SEED ORCHARD DESIGN

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ABSTRACT

Male and female gametic contributions of clones were estimated on the basis of observations of male strobili and cone numbers, reproductive phenology, and clone dislocation in three Scots pine seed orchards in central Slovakia. Among the ramets in the investigated seed orchards, 15–35% are planted on wrong positions and 12–27% represent alien genotypes. A large variation of gamete contributions and sexual asymmetry was found. Inbreeding effective population numbers varied from 28.6% to 59.3% of the census number of clones, whereas the relative status numbers ranged from 48.4% to 72.4%. A concept of ideal status number of the seed orchard crop was developed. Relative status numbers of the crop varied between 45.3% and 69.4%. The smallest effective population sizes were observed in the youngest seed orchard Kolkáreň, despite the highest census number of clones.

Keywords: *Pinus sylvestris*, seed orchard, effective population size, status number, phenology, pollen dispersal

INTRODUCTION

Panmixis is generally used as a reference of functioning of seed orchards. The conditions of panmixis, as defined by WEINBERG (*ex* MÜLLER-STARCK *et al.* 1982) are the following ones: Mendelian inheritance of traits, random mating, i.e. equal probability of mating between any pair of the population members (absence of any mating preferences, equal fertility) and equal viability of all zygotes independent of their genotypes. The latter two conditions are known to be frequently violated. Differences in floral phenology may cause unequal probabilities of mating between particular parental pairs. Genetic incompatibilities result in different viability of embryos – the best known form in case of generally outcrossing plant species like Scots pine is the partial self-incompatibility – viability of inbred (especially selfed) embryos is only approx. 30% as compared to non-inbred ones (YAZDANI & LINDGREN 1991).

In most seed orchard studies, the potential parental contributions of individual clones are estimated on the basis of fecundity, measured by cone crop, seed crop, eventually female and male strobili scores (EL-KASSABY& COOK 1994, MUONA & HARJU 1989, KJÆR 1996, KJÆR & WELLENDORF 1997, REYNOLDS & EL-KASSABY 1990). In some cases, the role of reproductive phenology is recognized (ASKEW 1988, O'REILLY *et al.* 1982, XIE *et al.* 1994). The effects of the localization of individual clones on the genetic composition of seed orchard crop is, however, generally neglected. In the studies of natural forests, distance between parents proved to be an important factor affecting the probability of mating between them (ADAMS *et al.* 1992, MÜLLER(-STARCK) 1977). In clonal seed orchards, repetition of clones in several ramets, and differences in floral phenology are supposed to overlay the effects of distance between mates as a determinant of mating success (ADAMS & BIRKES 1991). On the other hand, in a study of a Douglas-fir seed orchard (BURCZYK & PRAT 1997), the effect of distance proved to affect significantly the male mating success. Maybe in well-established seed orchards, where there are no mistakes in the position of individual clones and the representation of clones is balanced, distance actually need not be considered, but with increasing proportions of wrongly labelled or wrongly planted ramets this factor might become affecting considerably the genetic composition of seed orchard crop.

There are further factors, which can affect mating between a particular pair of ramets, like wind direction during the mating season, genetic incompatibilities etc. Their assessment or modelling is, however, quite difficult.

Another factor, which must be taken into consideration in case of Scots pine, is sexual asymmetry. As shown by SAVOLAINEN *et al.* (1993) or MÜLLER-

STARCK & ZIEHE (1984), male and female gamete production are not correlated, or even negatively correlated.

For the quantification of the genetic diversity of seed orchard outputs, various concepts of effective population sizes (or numbers) are most frequently used. The concept of effective population size is characterized by two principal features: a concept of an ideal population (mostly a panmictic Mendelian population), and a characteristic variable, like inbreeding coefficient or variance of gene frequencies (GREGORIUS 1991). A good overview of effective population numbers and their specific meanings in case of clonal seed orchard crops is given in KJÆR & WELLENDORF (1997).

The use of effective population numbers for monitoring the genetic diversity in seed orchard outputs was criticized by LINDGREN & MULLIN (1998). To their opinion, the traditional definitions of effective population numbers were developed to describe a process, not a state of a population. Effective population number expresses the relationship between the orchard and its crop in a single number, so that it is neither a characteristic of orchard alone nor its seed crop alone. Therefore, they propose utilization of so called status (effective) number developed by LINDGREN *et al.* (1996), which refers to the concept of group coancestry, i.e. the likelihood that two genes taken at random from the gene pool are identical by descent. Status number is defined as half the inverse of group coancestry (N_S = 0.5/Θ). It measures the number of equally fertile clones in an ideal seed orchard that – following random mating in the seed orchard progeny – gives raise to the same amount of inbreeding as will be experienced following random mating in the present seed orchard progeny.

The objective of this study was to assess the effects of variation in male and female fecundity, phenological differences, and dislocation of clones on parental balance in three Scots pine seed orchards, and subsequently to assess the increase of coancestry and decrease of the genetic diversity in seed orchard crops by means of inbreeding effective population size and status number.

Reduction of status number in the seed orchard crop

In first-generation seed orchards, where there is no information on kinship relationships between clones, only self-coancestry can be taken into account. In case of monoecious species, non-inbred and unrelated clones, and absence of pollen contamination, status number is calculated simply as:

$$N_S = \left[\sum_{i=1}^{N} r_i^2\right]^{-1} \qquad [1]$$

where N_S is the status number, N is the census number of clones, and $r_i = ½(m_i + f_i)$ is the average of the male (m_i) and female (f_i) gamete contribution of clone *i*. LINDGREN *et al.* (1996) defined also the relative status number as the percentage of the census number ($N_r = N_S/N$).

In fact, status number of seed orchard clones is related to the genetic composition of the orchard population itself, but the genetic composition of the seed orchard crop is better characterized by relative status number of the crop itself. Unequal gamete contributions of clones results in unequal representation of parental combinations (*i.e.*, reduced genetic diversity), and higher coancestry in seed orchard crop, and leads subsequently to higher levels of inbreeding in further generations.

Let us consider a seed orchard consisting of N clones. In case of panmixis, all parental combinations are equally probable, and result in progenies of equal size a. The census number of the seed orchard crop is then N^2a. There are N progenies, which are inbred, since they originate from selfing (progenies originating from matings $♂_1♀_1$, $♂_2♀_2$ ... $♂_N♀_N$). The total number of coancestry relationships within the crop (including self-coancestries and reciprocal matings) will be $(N^2a)^2$.

Following LINDGREN & MULLIN (1998), the average coancestry is a weighted average of coancestries of all possible parental pairs, including self-coancestry:

$$\Theta = \sum_{i=1}^{N} p_i^2 0.5(1 + F_i) + \sum_{i=1}^{N}\sum_{j\neq i}^{N} p_i p_j \theta_{ij} \qquad [2]$$

where F_i is the coefficient of inbreeding of individual *i* in case of self-coancestry (progeny from selfing of non-inbred individual has $F_i = 0.5$), p_i and p_j are the probabilities that two randomly sampled genes originate from parents *i* and *j*, respectively. Assuming these probabilities are equal for all individuals ($p_i = p_j = 1/N^2a$), coancestries can be simply averaged over all possible pairs of individuals in the seed orchard crop, as shown in Table 1.

The average coancestry can be summed as follows:

$$\begin{aligned}\Theta = {} & [0.75 + 0.75(a-1) + 0.375 \times 4(N-1)a + 0.5(N-1) \\ & + 0.25(N-1)(2a-1) + 0.125 \times 4(N-1)(N-2)] / N^3 a \\ = {} & 0.25\,(2N^2 a + 2Na + N - a - 1)/N^3 r \qquad [2]\end{aligned}$$

The status number is then:

$$N_S = \frac{0.5}{\Theta} = \frac{2N^3 a}{2N^2 a + 2Na + N - a - 1} \qquad [3]$$

Table 1. Coancestries in the seed crop of a seed orchard.

Relatedness of parents	Frequency of coancestries in a seed orchard crop		Coancestry[1]
	ideal[2]	real	
Self-coancestry of inbred individuals	$Na/(N^2a)^2 = 1/N^3a$	$\Sigma_i\, a'_{ii}/N_c^2$ $i=1...N$	$\theta_{AA} = 0.5(1 + F_A) =$ $0.5(1 + 0.5) = 0.75$
Full-sib coancestry between inbred individuals[3]	$N(a^2-a)/(N^2a)^2$ $= (a-1)/N^3a$	$\Sigma_i\, (a'^2_{ii}-a'_{ii})/N_c^2$ $i=1...N$	$0.25(2\theta_{AB} + \theta_{AA} + \theta_{BB}) =$ $0.25\times4\times[0.5(1 + 0.5)] = 0.75$
Half-sib coancestry, one of the individuals inbred[3]	$4N(N-1)a^2/(N^2a)^2$ $= 4(N-1)/N^3$	$(\Sigma_i\Sigma_j\, a'_{ii}a'_{ij} +$ $\Sigma_i\Sigma_j\, a'_{ii}a'_{ji})/N_c^2$ $i \neq j,\ i, j=1...N$	$0.25(\theta_{AA} + \theta_{AB} + \theta_{AC} + \theta_{BC}) =$ $0.25[2\times0.5(1 + 0.5) + 2\times0] =$ 0.375
Self-coancestry of non-inbred individuals	$(N^2 - N)a/(N^2a)^2$ $= (N-1)/N^3a$	$\Sigma_i\Sigma_j\, a'_{ij}/N_c^2$ $i \neq j,\ i, j=1...N$	$\theta_{AA} = 0.5(1 + 0) = 0.5$
Full-sib coancestry between non-inbred individuals	$(N^2-N)(2a^2-a)/(N^2a)^2$ $= (N-1)(2a-1)/N^3a$	$(\Sigma_i\Sigma_j(a'^2_{ij}-a'_{ij}) +$ $\Sigma_i\Sigma_j\, a'_{ij}a'_{ji})/N_c^2$ $i \neq j,\ i, j=1...N$	$0.25(2\theta_{AB} + \theta_{AA} + \theta_{BB}) =$ $0.25[2\times0 + 2\times0.5(1 + 0)] = 0.25$
Half-sib coancestry, both individuals non-inbred	$8N\begin{pmatrix} N-1 \\ 2 \end{pmatrix} a^2/(N^2a)^2$ $=4(N-1)(N-2)/N^3$	$(\Sigma_i\Sigma_j\Sigma_k\, a'_{ik}a'_{jk} +$ $\Sigma_i\Sigma_j\Sigma_k\, a'_{ki}a'_{kj} +$ $\Sigma_i\Sigma_j\Sigma_k\, a'_{ik}a'_{kj} +$ $\Sigma_i\Sigma_j\Sigma_k\, a'_{ki}a'_{jk})/N_c$ $i \neq j \neq k,\ i, j, k=1...N$	$0.25(\theta_{AA} + \theta_{AB} + \theta_{AC} + \theta_{BC}) =$ $0.25[0.5(1 + 0) + 3\times0] = 0.125$

[1] Following LINDGREN *et al.* (1996) and LINDGREN & MULLIN (1998).

[2] see Appendix I.

[3] Inbred individuals originate from selfing, *i.e.* parent A and parent B are identical, $\theta_{AB} = \theta_{AA} = \theta_{BB} = 0.5(1 + 0.5)$

a – constant progeny size in an ideal seed orchard, a'_{ij} – size of a progeny in a real seed orchard originating from mating of male i and female j, N – census number of clones, N_c – seed crop number.

The derived status number can be used as a comparison standard for real seed orchards, where the average coancestry is higher due to unbalanced matings, resulting in higher inbreeding levels in further generations.

MATERIALS AND METHODS

Three clonal Scots pine seed orchards, situated in Central Slovakia, were object of investigation. A detailed description is given in Table 2. Visual assessment of cone and seed morphology indicated that the true lay-out of clones does not correspond to that given in the seed orchard plans. Therefore, clone identity was verified using 7 polymorphic isozyme loci (BRUCHÁNIK & LONGAUER in preparation).

In all seed orchards, aborted ramets were successively replanted. As a consequence, there is a considerable variation among ramets in age as well as crown size, which affects the pollen and seed production. Therefore, crown diameters were measured for each ramet, which served for the calculation of crown surface.

Assessment of gamete contribution of clones

In all three seed orchards, cone crops of individual ramets were scored in five consecutive years (in 1995 and 1996, cone crops were weighted for each ramet, from 1997 to 1999, crop was estimated using the experience from the first two years). Length and width of two randomly chosen cones were measured for each ramet. Numbers and sizes of male strobili were assessed in 1997 and 1998. Like in case of cone crop, numbers of strobili were exactly counted on a set of ramets with different flowering intensities to improve the precision of ocular estimation. Since we did not have any information about the variation of the pollen-grain size, the production of male gametes was estimated by the weight of the produced pollen. According to SARVAS (*ex* JONSSON *et al.* 1976), Scots pine male strobili produce 0.028 g of pollen per 1 cm of strobilus length. To estimate the ovule production on the basis of cone crop, lengths, widths, and weights of 200 cones of various sizes were measured and number of seeds was counted, and the following regression equation between

Table 2. Description of the investigated Scots pine seed orchards.

Characteristic	Seed orchard		
	Kolkáreň	Háj	Sýkorová
Geographical coordinates	48°46'N 19°16'E	48°15'N 19°19'E	48°29'N 18°42'E
Altitude	450 m	350 m	475 m
Year of establishment	1982/1987	1979	1979
Census number of clones	43	30	35
Coefficient of variation of clone representation[1]	40.8%	43.0%	59.4%
Number of ramets	589	574	372
Number of wrongly planted ramets	117	199	56
Number of alien genotypes	160	63	45
Number of alien ramets	160	81	45
Spacing	5 m × 7 m	8 m × 8 m	7 m × 8 m
Average cone yield per ramet over the period 1995 to 1999 (fresh weights)	0.47 kg	2.38 kg	2.80 kg
Average cone yield per ramet in 1999 (fresh weights)	0.89 kg	2.93 kg	3.17 kg

[1] Without alien genotypes

Table 3. Description of the scored phenological phases and dates of phenological observations.

Male strobili			Female strobili		
score	description of modal state	relative pollen shedding	score	description of modal state	relative receptivity
0	not visible	0	0	bud closed, completely covered by scales	0
1	developing, closed in integuments	0	1	ovuliferous scales visible at the apex	0
2	yellow, fully shedding	100	2	strobili developed, starting to open	25
3	brownish, weakly shedding	40	3	open, fully receptive	100
4	dry, brown, not shedding	0	4	open, scales sickle-shaped	30
			5	closed, starting to form cones	0

Seed orchard	Year	Observation dates
Kolkáreň	1996	May 13, 16, 18, 20, 22
	1997	May 14, 16, 19, 22, 26
	1998	May 12, 15, 18, 21, 25
Háj	1997	May 11, 15, 20
	1998	May 9, 12, 18
Sýkorová	1997	May 15, 20, 23
	1998	May 12, 15, 18, 21

cone dimensions (in centimetres) and seed production was derived:
$number\_of\_seeds = -26.27 + 3.06 * cone\_length + 11.76 * cone\_width$ ($R^2 = 0.6332$).

Assessment of floral phenology followed JONSSON *et al.* (1976). Phenological phases were scored on three to five days in each seed orchard in 1997 and 1998. Table 3 describes the scored phenological phases and scoring dates in the three seed orchards. There is a within-ramet variability in phenology, and even within a single strobilus, ovules do not reach receptivity at the same moment (JONSSON *et al.* 1976, KRIŽO 1989). Therefore, modal phenological stage (valid for most flowers) was assessed for each ramet, to which a relative female receptivity and/or pollen shedding was attributed (Table 3, estimated based on own observations and KRIŽO 1989).

A complete dataset (male strobili, cones, phenology) was available only for the year 1997. As follows from Table 2, it was a year of quite an abundant flowering.

To describe the dependence of mating success from the distance between mates, the model according to ADAMS & BIRKES (1991) was used:

$$\varphi_{ij} = e^{-\beta d_{ij}} / \sum_{k=1}^{N_{ram}} e^{-\beta d_{ik}}$$

where φ_{ij} is the mating success between female i and male j, d_{ij} is the simple aerial distance between female i and male j, N_{ram} is the number of ramets, and β is the distance parameter. The authors recommended for Scots pine the value of $\beta = 0.02$. However, we verified this model for Scots pine on the basis of the data published by MÜLLER(-STARCK) (1977), and the value of $\beta = 0.04$ was found more appropriate. For self-pollination within the crown of one ramet ($i = j$), we did not use $d_{ii} = 0$. Ramets have systematically been top-pruned and pruned, so that the crown form is kept approximately globular. Male and female flowers are generally located on the crown surface. Therefore, we estimated d_{ii} as the average distance of two randomly chosen points on a globular surface. A simulation has shown that this distance is approximately equal to 1.3-multiple of the crown radius. The distance between ramets was estimated as the distance between crown centers.

For the assessment of the gametic contributions of individual clones, a modified method following ASKEW (1988) and XIE *et al.* (1994) was used. Relative gamete contribution of the pair of ith male ramet and jth female ramet was estimated as:

$$GC_{ij} = \Sigma_k\, \varphi_{ij} P_i p_{ik} O_j o_{jk} / \Sigma_i \Sigma_j \Sigma_k\, \varphi_{ij} P_i p_{ik} O_j o_{jk}$$

where P_i is the pollen gamete contribution of the ith ramet, O_j is the ovule contribution of the jth ramet, p_{ik} is the relative pollen shedding rate of the ith ramet at date k, o_{jk} is the relative receptivity of female flowers of jth ramet at date k (p_{ik} and o_{jk} depend from the phenological phase of male and female strobili, respectively, see Table 3), φ_{ij} is the distance-dependent mating success of the ith male ramet with the jth female one.

It is only male (pollen) gametes, which compete in the fertilization. Unless some clones are completely desynchronized, so that there is no pollen available when their female strobili are receptive, all available ovules have almost equal chance to be fertilized. This assumption is not absolutely realistic, but it is quite difficult to quantify the differences between clones in the proportions of fertilized ovules. Therefore, the total proportion of female gametes contributed by clone j was estimated based on the produced ovules: $f_j = O_j / \Sigma_k O_k$, whereas the total proportion of male gametes contributed by clone i was estimated as $m_i = \Sigma_j GC_{ij}$ ($\Sigma_i m_i = \Sigma_j f_j = 1$).

Statistical analysis of flowering

Analysis of variance was used for evaluation of the variation in male and female gamete production. The following model was applied:

$$P_{ijk} (O_{ijk}) = \mu + C_i + Y_j + (CY)_{ij} + \varepsilon_{ijk}$$

where P_{ijk} and O_{ijk} is the pollen and ovule production per m^2, respectively, μ is the overall mean, C_i is the effect of clone, Y_j is the effect of year (j = 1995 ... 1999 for ovules, j = 1997, 1998 for pollen), $(CY)_{ij}$ is the interaction between clones and year, ε_{ijk} is the error term. Both clone and year are considered random effects. Because the model is unbalanced, GLM procedure of the SAS STAT® package (SAS 1988) was used.

Under the assumption of isogamy, there should be a significant positive correlation between male and female gamete production. To test this hypothesis, the procedure following SAVOLAINEN *et al.* (1993) was employed. Replication of clones in several ramets allowed the estimation of the within- and between-genotype components of variances and covariance of male and female gamete production, which served for estimation of phenotypic, genotypic, and environmental correlations. Procedure NESTED (SAS 1988) was used for calculations.

Status number and effective population size estimation

Because effective population number is a concept commonly used for evaluation of deviations from idealized panmictic breeding, we used it for comparison. Inbreeding effective population number was calculated using the formula derived by KJÆR & WELLENDORF (1997):

$$N_e^{(i)} = \left[s + w \cdot t \sum_{i=1}^{N} f_i \cdot m_i \right]^{-1}$$

where N is the census number of clones, s is the selfing rate, t is the outcrossing rate, w is the relative fitness of selfed zygotes compared to outcrossed ones, f_i and p_i are the relative female and male contributions of the ith clone, respectively. We have not made any own experiments concerning the survival rate of selfed zygotes, but based on data published by YAZDANI & LINDGREN (1991) for Scots pine, an estimate of $w = 0.35$ was used. KJÆR & WELLENDORF's (1997) formula considers selfing rate s based on estimation employing genetic markers, *i.e.* on embryos surviving the postzygotic viability selection. We estimated selfing rate as $s' = \Sigma_i GC_{ii}$, *i.e.* based on the frequency of pollination events before selection. Therefore, for the calculation of $N_e^{(i)}$, we reduced this selfing rate by the relative fitness of selfed embryos ($s = s' \cdot w$).

Status number of clones was estimated according to LINDGREN & MULLIN (1998) (Eq. 1). For the estimation of the status number of seed crop, coancestries were summarized over individual coancestry types as indicated in Table 2. Sizes of progenies a'_{ij} originating from the mating of male clone i and female clone j were calculated as $a'_{ij}=O_j GC_{ij}/\Sigma_j GC_{ij}$. The actual status number of seed crop was compared with that of the crop of an ideal seed orchard, calculated using Eq. 3, whereby the average progeny size was used: $a = \Sigma_i \Sigma_j a'_{ij} / N^2$.

RESULTS

Fertility variation

Genotype verification based on isozyme markers high

Table 4. Analysis of variance (F-tests, degrees of freedom in parentheses) and variance components of male and female gamete production in individual seed orchards.

	Source					
	Clone		Year		Clone × year	
	F-test	V.C.	F-test	V.C.	F-test	V.C.
Kolkáreň – verified ramets:						
Male gametes	4.87*** (42, 42)	31.85	27.16*** (1, 42)	10.12	3.65*** (42, 768)	11.14
Female gametes	8.49*** (42, 168)	21.25	39.44*** (4, 168)	21.73	2.79*** (168, 1920)	7.57
Kolkáreň – all ramets:						
Male gametes	4.54*** (201, 201)	33.84	101.04*** (1, 201)	10.12	1.24* (201, 774)	12.27
Female gametes	7.38*** (201, 804)	26.85	110.14*** (4, 804)	10.98	0.96*ns* (804, 1935)	14.22
Háj – verified ramets:						
Male gametes	12.94*** (29, 29)	27.00	21.59*** (1, 29)	2.95	1.18*ns* (29, 985)	0.03
Female gametes	9.77*** (29, 116)	8.58	66.08*** (4, 116)	12.11	1.19*ns* (116, 2315)	0.20
Háj – all ramets:						
Male gametes	10.10*** (92, 92)	28.16	46.62*** (1, 92)	3.24	0.59*ns* (92, 962)	0.02
Female gametes	6.47*** (92, 368)	8.41	88.77*** (4, 368)	12.06	1.10*ns* (368, 2405)	0.16
Sýkorová – verified ramets:						
Male gametes	29.18*** (34, 34)	43.62	19.28*** (1, 34)	1.51	0.47*ns* (34, 584)	0.02
Female gametes	11.23*** (34, 136)	11.49	226.85*** (4, 136)	36.56	1.03*ns* (136, 1460)	0.54
Sýkorová – all ramets:						
Male gametes	12.59*** (79, 79)	42.67	10.67*** (1, 79)	0.92	0.62*ns* (79, 584)	0.02
Female gametes	9.16*** (79, 316)	11.87	383.91*** (4. 316)	37.73	0.70*ns* (316, 1460)	0.01

V.C. – variance component (%)

(BRUCHÁNIK & LONGAUER, in preparation) revealed a proportion of ramets, which could not be classified to any original orchard clone (see Table 2). Therefore, except the analyses of all represented ramets, separate analyses were performed for the sets of ramets with verified identity.

In all three seed orchards, a portion of individuals had to be replanted due to a relatively high mortality of ramets. The ages and also crown sizes of individual ramets vary considerably. To avoid the effects of crown size, male and female gamete productions were recalculated per square meter of the crown surface. A significant variation in pollen and ovule production was found between clones as well as between years, what means that despite a temporal variation in flowering, there are clones which contribute systematically over several years more or less to the gamete production than the average (Table 4).

Interaction between clone and year was found significant only in the youngest seed orchard Kolkáreň. In the older seed orchards Háj and Sýkorová, the ranking of clones in flowering intensity over years is stable.

In general, there is a bigger variation in pollen production than in ovule production. In Háj and Sýkorová, the between-clone component of variance is around 10% for female gametes, but it ranges from 27% to almost 44% for male gametes. In both seed orchards, five clones which were the most successful in mating in 1997 contributed by only 22.5% and 26.9% of female gametes, but even by 43.1% and 37.8% of male gametes, respectively. In Kolkáreň, where the proportion of replanted ramets is the highest among the investigated seed orchards, the between-clone components of variance are comparable for male and female gametes. However, the male and female contributions are unbalanced similarly as in the previous seed orchards: in 1997, the five first-ranking clones contributed by 28.3% of ovules but 36.3% of pollen. Except for Háj, alien ramets contribute more pollen by than by ovules. Summary contribution by female gametes in Kolkáreň, Háj, and Sýkorová was 11.97, 17.77, and 12.13%, respectively, whereas male contributions were 23.27, 12.73, and 13.78%, respectively. It means that well-synchronized heavy pollen producers are represented among alien genotypes in the seed orchard Kolkáreň, causing a severe internal contamination of seed crop.

The genetic correlation between pollen and ovule production is significantly positive only in Kolkáreň (Table 5). Again, this is probably due to a lower age – there are clones which have started neither male nor female flowering yet. In the older seed orchards, the genetic correlations are low and non-significant (even negative in Sýkorová). Both orchards are thus sexually asymmetric, the gamete contributions of clones through pollen and ovules are unequal.

Table 5. Phenotypic (r_p), genetic (r_g), and environmental (r_e) correlations of male and female gamete productions of verified ramets in three Scots pine seed orchards in 1997.

Seed orchard	r_p	r_g	r_e
Kolkáreň	0.476***	0.664***	0.341*
Háj	0.273*ns*	0.170*ns*	0.309*
Sýkorová	0.193*ns*	–0.016*ns*	0.284*

Status number and effective population number

A complete dataset (flower assessment, phenology, corresponding cone weights) was avaiable only for the year 1997 (i.e. cone collection in the spring 1999). As can be seen from Table 2, this was a year of a relatively abundant flowering in all the investigated seed orchards.

In all three seed orchards, there is a considerable proportion of alien genotypes. Therefore, we considered three situations: (a) alien ramets will be removed (census number of clones is equal to the planned number), (b) alien ramets will not be removed, but no cones will be collected from them (census number is equal to the total number of genotypes, i.e. planned clones plus alien genotypes, since alien ramets contribute to pollination), (c) cones will be collected from all ramets (census number is equal to the total number of genotypes).

Selfing rate (as estimated from mating probabilities) ranged between 3.9% and 7.1% (Table 6). Under the assumption that the survival rate of selfed embryos is approximately three times lower than that of outcrossed embryos, it corresponds to selfing rates of 1.3% to 2.5%, which would be estimated on living embryos. This corresponds roughly to multilocus estimates based on isozyme markers (e.g., BURCZYK 1991, EL-KASSABY *et al.* 1989, HARJU & MUONA 1989), so that our estimates seem to be quite realistic.

Male as well as female contributions of alien ramets exceed 10% in all cases. Harvesting cones from alien ramets, and even leaving alien ramets in the seed orchard leads thus to a substantial contamination of the gene pool of the seed crop. It is questionable, if this disadvantage is balanced by increased genetic diversity in the crops, since effective numbers increase by 3.5 to 6.5 as compared with the situation (a).

In the youngest seed orchard Kolkáreň, gametic contributions seem to be most unbalanced. Even in the most favourable situation, if all alien genotypes were

Table 6. Effective population numbers and status numbers in the three Scots pine seed orchards in 1997.

Seed orchard	Kolkáreň			Háj			Sýkorová		
Situation[1]	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
Census number of genotypes	43	203	203	30	93	93	35	80	80
Estimated selfing rate	0.0672	0.0519	0.0480	0.0711	0.0623	0.0592	0.0445	0.0424	0.0386
Inbreeding effective number	26.63	34.36	38.40	26.65	30.36	35.57	34.28	38.25	42.37
Relative inbreeding effective number	61.9%	16.9%	18.9%	88.8%	32.6%	38.2%	97.9%	47.8%	53.0%
Status number of clones	20.82	27.18	32.34	21.73	25.18	27.89	22.31	25.95	29.05
Relative status number of clones[2]	48.4%	13.4%	15.9%	72.4%	27.1%	30.0%	63.7%	32.4%	36.3%
Group coancestry in the crop	0.0263	0.0197	0.0165	0.0248	0.0213	0.0190	0.0234	0.0201	0.0178
Real status number of crop	19.03	25.36	30.36	20.14	23.51	26.28	21.35	24.86	28.02
Ideal status number of crop	42.03	201.97	201.97	29.05	92.01	92.02	34.04	79.02	79.02
Relative status number of crop[3]	45.3%	12.6%	15.0%	69.4%	25.5%	28.6%	62.7%	31.5%	35.5%

[1] (a) – alien ramets removed, (b) – alien ramets not removed but not harvested, (c) – all ramets harvested

[2] Related to the census number of genotypes

[3] Related to the ideal status number of the seed orchard crop

removed from the seed orchard, the effective population numbers would range from 48% to 62% of the census number of clones. In Sýkorová, the selfing rate is lower (probably due to a better spatial distribution of clones, or asynchrony of male and female flowering within clones), what results in a higher relative inbreeding effective population number (97.9% of the census number of clones) as compared with Háj (88.8%). On the other hand, the representation of clones (as measured by coefficient of variation of the number of ramets per clone – Table 2) is more irregular in Sýkorová, therefore, the relative status numbers of clones as well as seed crop are higher in Háj (Table 6).

DISCUSSION

In contrast to seed stands, clonal seed orchards are generally composed of a limited number of genotypes, but the seeds they produce are used for reforestation of vast areas. Much greater attention must therefore be paid to the genetic composition of seed orchard crops.

The presented effective population number estimates are generally comparable with those reported for conifer seed orchards. Of course, only the results for situation (a) can be compared, since a massive presence of alien material in seed orchards has not been mentioned in the cited studies. Muona & Harju (1989) estimated the inbreeding effective population size of two Scots pine seed orchard in Finnland at 60% and 93% of the census number of clones, respectively. Relative status number of seed orchards of three East Asian pine species ranged from 0.22 (*P. koraiensis*) to 0.91 (*P. thunbergii*) (Kang & Lindgren 1998). In a Danish Norway spruce seed orchard, inbreeding effective population number varied from 54% to 73% of the census number of clones in different years (Kjær 1996). In all these studies, male and female gamete contributions of individual clones were estimated simply on the basis of strobili counts. The effects of phenological variation and pollen dispersal were not considered. The opinions about the contribution of phenology to the variation in reproductive success are controversial. On one hand, O'Reilly *et al.* (1982) consider the effect of reproductive phenology on the genetic composition of a black spruce seed orchard progeny negligible, on the other hand, the importance of phenology was stressed by Askew (1988), El-Kassaby *et al.* (1984), Matziris (1994) and others. The effect of pollen dispersal should sufficiently be compensated by a proper seed orchard design. However, under a situation, when 15% to 35% of ramets are mislabelled and planted on wrong positions, clone dislocation can significantly affect the mating balance.

The differences between individual seed orchards in effective population size are partially associated with age. In the youngest seed orchard Kolkáreň, flowering is most irregular, what results in reduced status number as well as inbreeding effective number as compared with the older ones. Similar observation was reported by Kjær & Wellendorf (1998) in an old Danish Scots pine seed orchard, where the relative status number increased within a 20-year period from 29% to 81%.

The crucial problem of the investigated seed orchards is the identity of alien ramets. All they were established at a time, when almost the whole production of material for seed orchards was centralized in one state forest enterprise. Forest estates sent scions to this center and they received back the grafts without having any control about the whole process. There are quite many possibilities of errors during the whole procedure, starting from collecting and labelling the scions, over the manipulation during the transport, grafting, handling the material in the nursery, up to planting. Most frequently, the scion probably aborted (in the nursery or after planting in the seed orchard) and the rootstock survived. In this case, alien ramets represent genotypes of unknown (mostly average or even inferior) genetic quality. In the seed orchard Háj, groups of identical genotypes occur among alien ramets, so that here probably grafts were erroneously labelled in the central nursery. Alien ramets represent thus clonal descendance of plus trees (i.e. genotypes of putatively superior quality). However, these plus trees might have been selected in different seed zones or for different breeding objectives, so that the presence of any alien material must be considered an internal contamination of the seed orchard gene pool.

The presented estimations of effective population numbers are based on the assumption that clones in first-generation seed orchards are unrelated and non-inbred. In fact, plus-trees represented in the investigated seed orchards were selected on 10 localities. Some of the represented clones may thus be relatives or descendances from mating between relatives, even if it is not very probable. Effective population number estimates may therefore be biased upwards, but the bias is probably small.

Pollen contamination from outside sources may be another factor distorting the effective population size estimation. However, in these three particular seed orchards, it probably would not be a serious problem. All the investigated seed orchards are well-isolated, they are situated in regions where broadleaves (beech and oak) absolutely predominate and the share of Scots pine is quite low. The nearest large and continuous occurrence of pine is situated 60–100 km eastwards (Spiš region), another one 120–160 km to the southwest (Záhorie lowland), both are separated by mountain ridges, so that they can contribute to the pollination in the investigated seed orchards only under exceptional meteorological situations. Nevertheless, it is necessary to consider also the external contamination, even if the contamination rates cannot be expected to be as high as, for example, in Scandinavia.

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Appendix I. Composition of a crop of an ideal (upper line) and real (symbols with apostrophs, lower line) seed orchard.

Male clone (♂)	Female clone (♀)			
	1	2	...	N
1	a (I) a'_{11}	a a'_{12}	...	a a'_{1N}
2	a a'_{12}	a (I) a'_{22}	...	a a'_{2N}
...	...	...	...	...
N	a a'_{N1}	a a'_{N2}	...	a (I) a'_{NN}

Progenies on the diagonal (designated I) are inbred, they originate from selfing of seed orchard clones. The total crop size in an ideal seed orchard is N^2a, so that the total number of coancestry relationships is $(N^2a)^2$.

In an ideal seed orchard, the following coancestries must be considered:

Self-coancestry of inbred individuals (coancestry $♂_i♀_i \times ♂_i♀_i$):
There are N inbred progenies, each represented by a individuals which can mate with themselves, therefore the number of coancestries of this type is Na, their frequency is $Na/(N^2a)^2 = 1/N^3a$.

Full-sib coancestry between inbred individuals (coancestry $♂_i♀_i \times ♂_i♀_i$):
Within N inbred progenies, each represented by a members, there are a^2-a possibilities of mating of different individuals (including reciprocal matings), *i.e.* the number of coancestries is $N(a^2-a)$, their frequency is $N(a^2-a)/(N^2a)^2 = (a-1)/N^3a$.

Half-sib coancestry, one of the individuals inbred (coancestry $♂_i♀_i \times ♂_i♀_j$ or $♂_i♀_i \times ♂_j♀_i$, $i \neq j$):
Each of N inbred progenies can mate with $N-1$ progenies with common male parent and $N-1$ progenies with common female parent, so that the number of progeny combinations is $2N(N-1)$. If a progeny contains a individuals, the number of possible matings between individuals of different progenies including reciprocal matings is $2a^2$. The number of coancestries is then $4N(N-1)a^2$, their frequency is $4N(N-1)a^2/(N^2a)^2 = 4(N-1)/N^3$.

Self-coancestry of non-inbred individuals (coancestry $♂_i♀_j \times ♂_i♀_j$, $i \neq j$):
There are in total N^2-N non-inbred progenies, each represented by a individuals, the number of self-coancestries is then $(N^2-N)a$, the frequency is $(N^2-N)a/(N^2a)^2 = (N-1)/N^3a$.

Full-sib coancestry between non-inbred individuals:
There are N^2-N non-inbred progenies, and a^2-a possibilities of mating of different individuals within a progeny (including reciprocal matings) (coancestry $♂_i♀_j \times ♂_i♀_j$, $i \neq j$). In addition, there are N^2-N pairs of non-inbred progenies originating from reciprocal matings of maternal clones (coancestry $♂_i♀_j \times ♂_j♀_i$, $i \neq j$, which must also be considered a full-sib one) – because these are different progenies, the number of possible matings is a^2. The total number of coancestries is then $(N^2-N)(a^2-a+a^2) = (N^2-N)(2a^2-a)$, the frequency is $(N^2-N)(2a^2-a)/(N^2a)^2 = (N-1)(2a-1)/N^3a$.

Half-sib coancestry, both individuals non-inbred:
For each of N seed orchard clones, there are $\binom{N-1}{2} = (N-1)(N-2)/2$ non-inbred progeny pairs with common male parent (coancestry $♂_i♀_j \times ♂_i♀_k$, $i \neq j \neq k$), the same number of progeny pairs refers to the case of common female parent (coancestry $♂_i♀_j \times ♂_k♀_j$, $i \neq j \neq k$), cases when the common parent is male in the first progeny and female in the second one (coancestry $♂_i♀_j \times ♂_k♀_i$, $i \neq j \neq k$), and progeny pairs where the situation is opposite (coancestry $♂_i♀_j \times ♂_j♀_k$, $i \neq j \neq k$), so that the number of progeny combinations is $4N(N-1)(N-2)/2$. Progenies are different, so that the number of possible matings between their members is $2a^2$. The number of coancestries is then $4N(N-1)(N-2)a^2$, the frequency is $4N(N-1)(N-2)a^2/(N^2a)^2 = 4(N-1)(N-2)/N^3$.