

**Factors affecting effective population size estimation in a seed orchard: a case study of  
*Pinus sylvestris***

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**ABSTRACT**

Effective population size as a parameter closely correlating with the genetic and genotypic diversity of the seed orchard output is an important indicator of seed orchard functioning. It is determined by the variation of male and female gametic contributions of parental genotypes (including those outside the seed orchard), influenced by the variation in male and female gamete production, reproductive phenology, pollen dispersal within seed orchard and other factors. The assessment of fertility and phenological variation requires labor and finances. We tested empirically what is the relative importance of these factors for effective population size (status number) estimation by gradual adding one-by-one in the order of labor requirements (female contribution, male contribution, reproductive phenology, pollen dispersal) in three Scots pine (*Pinus sylvestris* L.) seed orchards in central Slovakia. The study has shown that in old, fully fructifying seed orchards, effective number of clones is a satisfactory estimator of the effective population size, but the inclusion of female and male fertility variation improves the estimate of status number. On the other hand, phenological variation and spatially-dependent pollen dispersal do not considerably affect  $N_S$  estimates and need not be assessed for practical purposes. In contrast, a young seed orchard proved to be unbalanced and phenologically not synchronized; consequently, effective number of clones was a poor estimator of the effective size and status number was affected by all factors (male + female fertility, phenology, spatial design)

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

## INTRODUCTION

In contrast to seed stands, clonal seed orchards generally contain a limited number of genotypes. However, the seeds they produce are used for reforestation of large areas. In Slovakia, forest reproductive material of European larch and partially Scots pine is almost completely limited to seed orchard seeds. Great attention must therefore be paid to the genetic composition of seed orchard crops.

Panmixis is frequently taken as a reference of functioning of seed orchards. An ideal seed orchard should function as a randomly mating Mendelian population, i.e. probability of mating for any pair of the population members should be independent of their genotypes (no mating preferences, equal fertility) and equal viability of all produced offspring zygotes. These conditions are known to be frequently violated in seed orchards, mainly in clonal seed orchards. Clones can be represented by different numbers of ramets with different fecundity, what produces variation in the numbers of produced male and female gametes. Differences in floral phenology may cause unequal probabilities of mating between particular parental pairs. Genetic incompatibilities (including self-incompatibility) result in different viability of embryos, e.g., in Scots pine, viability of selfed embryos was observed to be only approx. 30% as compared to non-inbred ones (Yazdani & Lindgren 1991).

Genetic marker studies performed during the past 30 years have considerably contributed to our understanding of mating processes in seed orchards. They generally showed only small deviations of genotypic frequencies from the Hardy-Weinberg expectations and low levels of self-fertilization in conifer seed orchards (citations). On the other hand, significant shifts in allele frequencies between seed orchard and its offspring, proving deviations from panmixis, have also been reported (Gömöry & Paule 1993). Nevertheless, marker studies are too laborious and too expensive to be operationally performed in every seed orchard to provide information on the genetic diversity in seed orchard outputs for management decisions. Various types of effective population sizes (based, however, on *a priori* assumptions about the mating process) are most frequently used for this purpose.

The concept of effective population size is characterized by two principal features: a concept of an ideal population, which generally is a panmictic population, and a characteristic variable, such as inbreeding coefficient, coancestry, or variance of gene frequencies (Gregorius 1991). Kjær & Wellendorf (1997) provided a good overview of effective population numbers and their specific meanings in case of clonal seed orchard crops. Lindgren *et al.* (1996) developed status (effective) number, which refers to the concept of

group coancestry ( $\Theta$ ), 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 ( $N_S = 0.5/\Theta$ ). It measures the number of equally fertile clones in an ideal seed orchard that – following random mating in the seed orchard progeny – gives rise to the same amount of inbreeding as will be experienced following random mating in the present seed orchard progeny.

The simplest estimator of the effective population size of a clonal seed orchard is the effective number of clones, reflecting the variation in the numbers of ramets representing individual clones in the seed orchard. It is based on the (unspoken) assumption that all ramets contribute equally to the offspring, or, more exactly, that the contribution is not clone-dependent. As this assumption is not realistic, the potential parental contributions of individual clones have frequently been estimated in seed orchard studies. The most easy-to-assess parameter is the female fertility, mostly estimated based on cone crop (number or weight of cones), eventually seed crop. Male fertility based on male strobili scores (what is, however, more labor-demanding) has also been frequently assessed (El-Kassaby & Cook 1994, Muona & Harju 1989, Kjær 1996, Kjær & Wellendorf 1997, Reynolds & El-Kassaby 1990). Less often, the role of reproductive phenology was recognized (Askew 1988, O'Reilly *et al.* 1982, Xie *et al.* 1994). Phenology scoring requires several visits of seed orchard during the flowering period. The effects of the localization of individual clones on the genetic composition of seed orchard crop have been generally neglected. Their assessment requires previous studies on the shape and parameters of the pollen dispersal function. The effects of other factors, such as anisotropies in the pollen dispersal due to prevailing wind direction during the pollination season or genetic incompatibilities are difficult to assess and their incorporation in the modeling of the mating process is hardly possible.

The assessment of fertility and phenological variation requires labor and finances. From the point of view of practical management decision-making in seed orchards, it is necessary to know which factors affect considerably effective population size, in order to decide which are thus worth of being assessed. We tested empirically what is the relative importance of these factors for status number estimation by gradual adding one-by-one in the order of labor requirements (female contribution, male contribution, reproductive phenology, distance-dependent pollen dispersal) in three Scots pine (*Pinus sylvestris* L.) seed orchards in central Slovakia.

Table 1 Description of the investigated Scots pine seed orchards

Characteristic	Seed orchard		
	Kolkáreň	Háj	Sýkorová
Geographical coordinates	48E46'N 19E16'E	48E15'N	48E29'N 18E42'E
Altitude	450 m	19E19'E	475 m
Year of establishment	1982/1987	350 m	1979
Census number of clones	43	1979	35
Coefficient of variation of clone representation <sup>1)</sup>	40.8%	30	59.4%
Number of ramets	589	43.0%	372
Number of wrongly planted ramets	117	574	56
Number of alien genotypes	160	199	45
Number of alien ramets	160	63	45
Spacing	5 m × 7 m	81	7 m × 8 m
Average cone yield per ramet 1995–1999 (fresh weights)	0.47 kg	8 m × 8 m	2.80 kg
		2.38 kg	

<sup>1)</sup> Without alien genotypes

## MATERIALS AND METHODS

A detailed description of the studied seed orchards is given in Table 1. Clone identity was verified using 7 polymorphic allozyme loci (for details on allozyme analysis, see Gömöry et al. 2003). In all seed orchards, a considerable proportion of alien material (overgrown rootstocks, non-registered ramets) was found and several ramets were placed on wrong positions. In all three seed orchards, cone crops of individual ramets were scored in five consecutive years (1995 to 1999). Numbers and sizes of male strobili were assessed in 1997 and 1998. Assessment of floral phenology essentially followed Jonsson *et al.* (1976). Phenological phases were scored on three to five days in each seed orchard in 1997 and 1998 (Table 2). A complete dataset (male strobili, cones, phenology) was available only for the year 1997, which was a year of full fructification.

Table 2 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		

To describe the dependence of mating success from the distance between mates, we used the model according to Adams & Birkes (1991):

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

where  $\varphi_{ij}$  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  $\beta$  is the distance parameter.

On the basis of the data published by Müller(-Starck) (1977), the value of  $\beta$  was set to 0.04. For the self-pollination within the crown of one ramet ( $i = j$ ), we estimated  $d_{ii}$  as the average distance of two randomly chosen points on a globular surface, which 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. Under the consideration of all factors, relative gamete contribution of the pair of  $i$ th male ramet and  $j$ th female ramet was estimated as:

$$GC_{ij} = \frac{\sum_k \varphi_{ij} P_i p_{ik} O_j o_{jk}}{\sum_i \sum_j \sum_k \varphi_{ij} P_i p_{ik} O_j o_{jk}}$$

where  $P_i$  is the pollen gamete contribution of the  $i$ th ramet,  $O_j$  is the ovule contribution of the  $j$ th ramet,  $p_{ik}$  is the relative pollen shedding rate of the  $i$ th ramet at date  $k$ ,  $o_{jk}$  is the relative receptivity of female flowers of the  $j$ th ramet at date  $k$  ( $p_{ik}$  and  $o_{jk}$  depend from the phenological phase of male and female strobili, respectively),  $\varphi_{ij}$  is the distance-dependent mating success of the  $i$ th male ramet with the  $j$ th female one. Gametic contributions were subsequently summed according to clones. When a particular factor was not considered (female fertility, male fertility, flowering phenology, distance), the respective parameter was set to 1.

It is only male (pollen) gametes, which compete in the fertilization. Unless pollen density is low (e.g., at the beginning or end of the pollination season), all available ovules have almost equal chance to be fertilized. Although this assumption is not absolutely realistic, the assessment of clonal gamete contribution was based on it, as it would be difficult to quantify the differences between clones in the proportions of fertilized ovules. Therefore, the total proportion of female gametes contributed by  $j$ th clone was estimated based on the produced ovules:  $f_j = O_j / \sum_k O_k$ , whereas the total proportion of male gametes contributed by the  $i$ th clone was estimated as  $m_i = \sum_j GC_{ij}$  (whereby  $\sum_i m_i = \sum_j f_j = 1$ ).

Effective number of clones was calculated as:

$$N_e = 1 / \sum_i p_i^2$$

where  $p_i$  is the relative number of ramets of the  $i$ th clone. Group coancestry and status number of seed crop were estimated according to Gömöry *et al.* (2000).

## RESULTS AND DISCUSSION

A complete dataset (cone weights, male flower numbers, phenology) was available only for the year 1997 (i.e. cone collection in the spring 1999), which was a year of relatively rich flowering in all seed orchards under study.

In all three seed orchards, there is a considerable proportion of alien genotypes. Therefore, we considered three situations: (a) alien ramets were removed (census number of clones is equal to the planned number), (b) alien ramets were not removed, but no cones were 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 were collected from all ramets (census number is equal to the total number of genotypes).

Male as well as female contributions of alien ramets exceeded 10% in all three seed orchards. Harvesting cones from alien ramets (c), and even leaving alien ramets in the seed orchard (b) lead thus to a substantial contamination of the gene pool of the seed crop by genes of parents of unknown phenotype. It is questionable, if this disadvantage is balanced by increased genetic diversity in the crops, since effective numbers increase negligibly as compared with the situation (a).

In both older and fully fructifying seed orchards (Háj and Sýkorová), effective number of clones seems to be a relatively satisfactory descriptor of the genetic diversity of seed orchard crops, especially when seed orchards do not contain alien ramets. Effective number of clones is generally substantially lower than the population census (table 3a, b). Status number considering the variation in female fertility substantially is not substantially lower. This, naturally, does not mean that there is no among-clone variation in female gamete production, but the differences among clones are not huge and actually compensate the differences in the ramet numbers per clone.

On the other hand, variation in male fertility (which is bigger in both seed orchards) decreased status number. Flowering phenology and distance-dependent pollen dispersal did not affect parental imbalance. In Sýkorová, the inclusion of the phenological variation into the estimation even increased slightly status number. Apparently, there is a kind of compensation effect, when some of the abundantly flowering clones are not sufficiently synchronized with the major part of seed orchard clones either in pollen shedding or in female strobile receptivity.

The situation is different in the younger seed orchard Kolkáreň. The estimated effective population sizes decrease steadily with the inclusion of each considered factor, whereby status number under each model is substantially lower than the effective number of clones.

Table 3 Effective number of clones and status number under consideration of different sources of parental imbalance in three Scots pine seed orchards

	Aliens removed		Aliens not harvested		All harvested	
	$\Theta$	$N$	$\Theta$	$N$	$\Theta$	$N$
Háj						
Census		30		93		93
$N_e$		25.5		34.1		34.1
$N_S$ : F	0.0198	25.2	0.0171	29.2	0.0150	33.3
FM	0.0236	21.1	0.0207	24.1	0.0186	26.9
FMP	0.0241	20.8	0.0207	24.1	0.0186	26.9
	0.0248	20.1	0.0213	23.5	0.0190	26.3
FMPD						
Sýkorová						
Census		35		80		80
$N_e$		26.1		33.5		33.5
$N_S$ : F	0.0205	24.4	0.0181	27.6	0.0159	31.5
FM	0.0237	21.1	0.0199	25.1	0.0177	28.3
FMP	0.0234	21.4	0.0198	25.3	0.0176	28.4
	0.0236	21.4	0.0201	24.9	0.0178	28.0
FMPD						
Kolkáreň						
Census		43		203		203
$N_e$		36.9		68.0		68.0
$N_S$ : F	0.0159	31.4	0.0122	40.9	0.0095	52.4
FM	0.0214	23.4	0.0162	30.9	0.0123	40.7
FMP	0.0241	20.7	0.0177	28.2	0.0148	33.8
	0.0263	19.0	0.0197	25.4	0.0165	30.4
FMPD						

This means that the effective number of clones does not properly reflect parental imbalance in a young seed orchard.



There is no unanimity about the significance of phenology for reproductive success of clones in a seed orchard: Askew (1988), El-Kassaby et al. (1984), Matziris (1994), Nikkanen (2001) or Codesido et al. (2005) underlined the effects of reproductive phenology on the genetic composition on seed orchard crops, but O'Reilly et al. (1982) consider these effects unimportant. Our results support rather the latter opinion, at least in old seed orchards. The effect of pollen dispersal seems to be more or less compensated by clone dislocation in the case of random seed orchard design. Only in Kolkáreň with more than one third of mislabelled or wrongly placed ramets, this effect was more pronounced. However, spatial pollen dispersal can be more important under clonal-row design, as indicated by the study of El-Kassaby et al. (2007).

The presented effective population number estimates (44%–85% of the seed orchard census) are to what has been reported from clonal seed orchards of conifers. In a large-scale study of 255 conifer seed orchards in Korea, Sweden and Finland, effective number of clones ranged between 20% and 100% of the census number with an average of 74% (Kang et al. 2001). This study considers only the variation in the ramet numbers per clone. Effective population size estimated considering fertility variation ranged in similar, even broader intervals: 60% to 93% in Scots pine (Muona & Harju 1989), 22% to 91% in East Asian pine species (Kang & Lindgren 1998, Choi et al. 2004), 54% to 73% in Norway spruce (Kjær 1996, Nikkanen & Ruotsalainen 2000) from Finland. In these studies, the effective number was estimated based on male and female gamete contributions of clones derived from the counts and/or weights of male strobiles and cones, without consideration of phenological variation and spatial pollen dispersal.

A different behaviour of the youngest seed orchard Kolkáreň is associated with age. Flowering is most irregular, therefore, relative status number much lower than in Háj and Sýkorová. Kjær & Wellendorf (1998) also observed a gradual change of the relative status number from 29% to 81% in a Danish Scots pine seed orchard within a period of 20-years.

The current model of mating process does not count with pollen contamination. Actually, no specific study of background pollination rates has been performed in either of the investigated seed orchards. However, all of them are situated in broadleaved areas, far from large complexes of Scots pine stands, which are situated approximately 150 km westwards in the Zahorie lowland and 80 km eastwards in the Spis region and are separated from southern Slovakia by mountain ridges. Scots pine is by far less represented in Slovak forests than in, e.g., Scandinavia, so that much lower pollen contamination rates are to be expected. Under this situation, genetic diversity of the seed orchard crop depends primarily on the pollination

within the orchard. The study of Gömöry et al. (2000) has shown that in mature seed orchards, the among-clone variation in male gamete production is three to four times higher than for female gametes, and relatively stable over years (non-significant clone-by-year interaction). Our results indicate that male fertility is the key parameter determining effective population size in mature Scots pine seed orchards in Central Europe.

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