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Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard

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Summary – Clonal variation in flowering characteristics and cone production was investigated in a Scots pine (*Pinus sylvestris* L) clonal seed orchard consisting of 32 clones. At the time of observations, the orchard was 17–19 years old. It was found that on average, within a clone, female flowers were receptive about 1 day before the beginning of pollen shedding and there was a significant correlation between the ranks of clones according to their onset of flowering in 2 consecutive years. Male and female flowering periods were synchronized among the majority of clones and the index of phenological overlap was over 0.41. Significant variations among clones were found for male and female cone production as well as for some selected pollen-related characteristics. On average, individual clones in the orchard produced in total from 0.4 to 4.5 kg of pollen and from 900 to 6500 cones a year. It was found that 25% and 50% of clones produced 46% and 72% of pollen, respectively. Analogous numbers for cone production were 35% and 63%. Some patterns of sexual asymmetry among clones were detected; however, genetic correlations between pollen and cone productions were positive. Effective population sizes were generally high, but the estimate was lower for pollen (75.9%) than for cone production (95.9%). The expected outcrossing rate, based on effective population size calculated using both male and female contribution and background pollination, was high (0.977). The efficiency of the orchard and its potential use for reforestation purposes is discussed.

***Pinus sylvestris* / seed orchard / phenology / pollen and cone production / sexual asymmetry / mating patterns**

Résumé – Variabilité de la floraison et de la fructification et son effet sur l'équilibre entre parents dans un verger à graines de clones de pin sylvestre. Les variations clonales de caractéristiques de floraison et de fructification ont été étudiées dans un verger à graines de clones de pin sylvestre (*Pinus sylvestris* L) comportant 32 clones. Le verger est situé dans le district forestier de

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Gniewkowo, en Pologne. Au moment des observations, ce verger était âgé de 17 à 19 ans. Les caractéristiques de floraison ont été observées sur quatre ramets de chacun des 32 clones pendant 2 (fructification et phénologie de la floraison) ou 3 (floraison mâle) années consécutives. On a montré qu'en moyenne à l'intérieur d'un même clone les cônes femelles sont réceptifs environ 1 jour avant le début de la libéralisation du pollen (fig 1). Il existe une corrélation significative dans le classement des clones pour leur mise à fleur entre les 2 années consécutives mais les dates de début de floraison sont décalées de 3 semaines entre ces 2 années. Les périodes de floraison mâle et femelle sont synchronisées pour la majorité des clones et l'index de recouvrement phénologique est de 0,41. Les indices de recouvrement phénologique sont corrélés pour les paires de clones s'intercroisant et les clones intervenant comme mâles, mais pas pour les clones intervenant comme femelles. Des variations significatives entre clones sont observées pour la production de cônes mâles et femelles (tableaux I et II), de même que pour certaines caractéristiques liées au pollen (tableau II, fig 2). Le nombre de pousses portant des cônes mâles, et le nombre de ces cônes varie selon les années et selon les secteurs de la couronne. Quelques interactions apparaissent statistiquement significatives. En moyenne, chaque clone du verger produit entre 0,4 et 4,5 kg de pollen par an à partir de 900 à 6500 cônes (figs 3 et 4). On a trouvé que 25 et 50 % des clones ont produit respectivement 46 et 72 % du pollen. Les estimations correspondantes pour la floraison femelle sont respectivement de 35 et 63 % (figs 3 et 4). Des asymétries sexuelles ont été détectées chez certains clones (fig 5) mais les corrélations génétiques entre production de pollen et de cônes femelles sont positives (tableau IV). Une contribution clonale combinée à la descendance du verger a été estimée en prenant en compte à la fois les effets mâles et femelles (fig 6). Les effets phénologiques semblent expliquer la légère modification de classement des contributions clonales d'une année à l'autre. La taille effective de population estimée pour ce verger est généralement élevée, mais les estimations sont plus faibles pour la production de pollen (75,9%) que pour la production de cônes femelles (95,9%). Les taux d'allogamie attendus, en se basant sur la taille effective de population calculée en utilisant à la fois les contributions mâles et femelles, et la pollution pollinique sont élevés (0,977). La productivité de ce verger et les possibilités de l'utiliser pour les reboisements sont discutées.

***Pinus sylvestris* / verger à graines / phénologie / production pollen et cônes / asymétrie sexuelle / lois de croisements**

INTRODUCTION

Clonal seed orchards are expected to provide a large amount of seeds of high genetic value. The potential value of seed is primarily determined at the stage of orchard establishment when particular clones are selected to build the orchard. However, reproductive processes including unequal production of male and female strobili, different compatibilities and lack of male and female flowering synchronization among clones, as well as significant amounts of self-fertilizations and influence of external sources of undesirable pollen (background pollination), has usually for a result that not the whole potential of an orchard is realized in the filial generations.

There are generally two approaches for studying reproductive processes in plant populations: the first – before fertilization, by studying flowering characteristics, and making hypotheses on mating patterns; the second – after fertilization, by investigating progeny (including embryos) using such techniques as isozymes and estimating the mating patterns on the basis of paternity and other mating system models (Adams and Birkes, 1991). However, only investigations on both the potential and effective mating patterns give a profound insight into the mating behaviour of a population (Gregorius, 1989).

Generally, it was found that a large variation among clones in flowering characteristics usually causes unbalanced male and

female contributions to the progeny (Jonsson et al, 1976; O'Reilly et al, 1982; Schmidting, 1983; Boes et al, 1991; Chaisurisri and El-Kassaby, 1993). This may reduce the effective population size, and consequently decrease the genetic variability of the orchard progeny.

In Central Europe and Scandinavia, Scots pine (*Pinus sylvestris* L) is one of the most important forest tree species used for reforestation and a large number of seed orchards of this species was created (Mikkola, 1991). Thus, for tree improvement it is important to investigate the extent of flowering variation in Scots pine seed orchards and to make predictions about the genetic composition of seed. It is also important for the understanding of eventual differences between expected and realized genetic gains.

In this paper we present the results on flowering and cone production of a Scots pine clonal seed orchard that reached its full production stage (17–19 years old) and make conclusions about its potential reproductive patterns.

MATERIALS AND METHODS

Observations of flowering and cone production were carried out on a Scots pine clonal seed orchard located near Gniewkowo, Poland. It was established in 1972 by the Toruń Regional State Forest Administration. The orchard consists of 32 clones representing trees growing in three forest districts of the Tuchola Forests (about 150 km north of the orchard). Grafts were outplanted in three blocks with 5 × 5 m spacing. Each block contained all the clones but with different numbers of ramets per clone and different randomization of clone positions (Burczyk, 1990). The majority of clones ($n = 22$) were represented by 30 to 44 ramets, six clones had 21–25 ramets, and only three clones were represented by less than 20 ramets (clone 211 = 8; 222 = 19; and 227 = 18). The exact numbers of ramets per clone are given in figure 3. At the time of analyses there was a total of 1056 trees, about 8–10 m high with a mean diameter of about 15 cm

(DBH). The orchard was not subjected to any flowering induction treatment.

Four ramets per clone were chosen randomly for the observations, avoiding trees growing at edges of blocks. Although ramets were located in all three blocks, most of them originated from blocks I and II. Block effect had no impact on the development and flowering of trees, and it was not included in the analysis of variance (ANOVA) models (see later). Observations of flowering and cone production were made on the same trees each year.

Flowering phenology was investigated every day in the spring of 1990 and every other day in 1991 until all pollen was released and seed cones were no longer receptive. Development of male and female strobili was examined on all the selected grafts on three to five branches on the trees' south side (at 2–3 m from the ground for male strobili, and 5 m for female strobili). The maturity of male strobili was based on their ability for pollen release and that of female strobili when their scales were half-opened. Female strobili considered as receptive corresponded to the development stages shown on figures 7–9 in the work of Jonsson et al (1976). An index of phenological overlap was calculated following Askew and Blush (1990). The index was estimated for each possible pair of mating clones, for individual clones acting as males and/or females, and finally for the entire orchard. For both male and female phenology, the intensity of flowering of a graft was expressed as a percentage of flowering strobili according to a four-degree scale: 0, 25, 50 and 100%. We counted all the strobili which flowered during the observation or were already out of bloom (no longer flowering) (Askew and Blush [1990] used only a proportion of flowering strobili). Because of this specific data collection the phenological indices were overestimated; however, they were still useful for investigating interclonal variation.

Intensity of male flowering was studied during the spring of the year 1989 and 1990. In 1989 it was possible to take also into account the flowering which occurred in 1988, due to the evidence of the male strobili (twig scars) from 1988 existing on branches. Because of the difficult access to upper crown levels, and the distribution of the majority of male strobili in the lower crown parts, we decided to continue observations only up to 3 m from the ground, thus male shoots numbers and pollen amount per tree should be considered underestimated. The trunk

Table I. Mixed analysis of variance of male flowering (number of male strobili bearing shoots).

Source of variation	Degrees of freedom	Mean square	Expected mean square	Pseudo-F-test
Sectors (S)	2	8209277.50	$\sigma_W^2 + 3\sigma_{SR(C)}^2 + 12\sigma_{SC}^2 + 384\Phi_S$	59.186***
Years (Y)	2	525853.31	$\sigma_W^2 + 3\sigma_{YR(C)}^2 + 12\sigma_{YC}^2 + 384\Phi_Y$	24.960***
Clone (C)	31	841691.50	$\sigma_W^2 + 3\sigma_{YR(C)}^2 + 3\sigma_{SR(C)}^2 + 9\sigma_{R(C)}^2 + 36\sigma_C^2$	2.658***
Ramet(Clone) R(C)	96	316691.53	$\sigma_W^2 + 3\sigma_{YR(C)}^2 + 3\sigma_{SR(C)}^2 + 9\sigma_{R(C)}^2$	2.531***
S * Y	4	102394.59	$\sigma_W^2 + 4\sigma_{CSY}^2 + 128\Phi_{SY}$	7.398***
S * C	62	138702.30	$\sigma_W^2 + 3\sigma_{SR(C)}^2 + 12\sigma_{SC}^2$	1.249 ^{ns}
S * R(C)	192	111086.01	$\sigma_W^2 + 3\sigma_{SR(C)}^2$	8.494***
Y * C	62	21067.89	$\sigma_W^2 + 3\sigma_{YR(C)}^2 + 12\sigma_{YC}^2$	1.098 ^{ns}
Y * R(C)	192	19192.01	$\sigma_W^2 + 3\sigma_{YR(C)}^2$	1.467***
S * Y * C	124	13841.71	$\sigma_W^2 + 4\sigma_{SYC}^2$	1.058 ^{ns}
Residual	384	13078.68	σ_W^2	

*** Test significant at the level $P = 0.001$; ns: test not significant at the level $P > 0.05$; clone mean basis heritability $h_C^2 = 0.610$; individual tree basis heritability $h_S^2 = 0.167$.

of a graft was divided into three 1 m sectors (0–1, 1–2, 2–3 m) and all shoots with male strobili were counted from a randomly chosen branch within each sector (more or less southern orientation). In order to calculate the total number of shoots with male strobili per graft, the number of strobili per branch was multiplied by the number of all branches in a sector and the value was summed across the three levels (Muona and Harju, 1989; Savolainen et al, 1993). The variation of male flowering (male strobili bearing shoot number) was studied using a three-way ANOVA (table I). The three main sources of variation were: clones (random effect), crown levels and years (fixed effects). Ramets within clones were considered random.

In the spring of 1990, 50 male strobili bearing shoots from each of four ramets of eight randomly chosen clones were sampled for detailed analysis of pollen production. The shoots were collected 1 to 3 days before pollen shedding and dried for several days under low constant humidity. The pollen was then extracted and weighed. The amounts of pollen per one male strobili bearing shoot, per 1 cm of that shoot, and per one

male strobilus were estimated. However, because of the rough method of extraction used, the amounts of pollen should be considered underestimated. Variation of these characteristics among clones was studied by a one-way ANOVA, but the precision of clonal variance estimation and heritability is low due to the low number of clones (eight) studied. Clonal variation of the length of male strobili bearing shoot and the number of pollen strobili per shoot was also investigated using a hierarchical model of ANOVA, assuming all effects to be random (table II). Additionally, the length of 100 male strobili bearing shoots (25 per ramet) was measured for 32 clones during 3 consecutive years in order to obtain clonal averages. The data was used to estimate pollen production; however, because of the underestimated amounts of pollen in this study, we assumed that 1 cm of shoot bearing male strobili produces on average 0.028 g of pollen (Koski, 1975; Bhumibhamon, 1978; Muona and Harju, 1989).

Intensity of seed cone production was examined in the autumn of 1989 and 1990. All cones of each sampled graft were counted carefully

Table II. Analysis of variance of the length of male strobili bearing shoots and the number of pollen strobili per one shoot among eight randomly chosen clones.

Source of variation	Degrees of freedom	Expected mean square	Length of male strobili bearing shoots			No of pollen strobili		
			Mean square	F-test	Variance components (%)	Mean square	F-test	Variance components (%)
Clone	7	$\sigma_W^2 + 50\sigma_{R(C)}^2 + 200\sigma_C^2$	619.88	2.93*	10.14	4698.13	8.24**	23.43
Ramet(Clone)	24	$\sigma_W^2 + 50\sigma_{R(C)}^2$	211.46	14.95**	19.60	570.37	9.97**	11.65
Residual	1568	σ_W^2	14.14			57.19		

* Parameter significant at the level $P = 0.05$; ** parameter significant at the level $P = 0.001$.

Table III. Analysis of variance of seed cone production.

Source of variation	Degrees of freedom	Mean square	Expected mean square	F-test
Clone (C)	31	4511.97	$\sigma_W^2 + 2\sigma_{R(C)}^2 + 8\sigma_C^2$	2.706***
Years (Y)	1	27204.50	$\sigma_W^2 + 4\sigma_{CY}^2 + 128\Phi_Y$	10.830**
C * Y	31	2511.85	$\sigma_W^2 + 4\sigma_{CY}^2$	1.674*
Ramet(Clone)	96	1667.18	$\sigma_W^2 + 2\sigma_{R(C)}^2$	1.111 ^{ns}
Residual	96	1500.85	σ_W^2	

* Parameter significant at the level $P = 0.05$; ** parameter significant at the level $P = 0.01$; *** parameter significant at the level $P = 0.001$; clone mean basis heritability $h_C^2 = 0.631$; individual tree basis heritability $h_S^2 = 0.183$.

from the ground by three observers, and the estimate for a tree was the average of the three observations to avoid possible error from the observer. In order to analyze the extent of variation of cone production among clones, a two-way ANOVA model was used (table III). Clones and ramets within clones were assumed to be random, whereas the years were fixed.

Data on pollen and seed cone production was used to estimate expected male and female contribution into the progeny produced by the orchard. Contribution of a clone was expressed as its proportion of the total pollen or seed cone production. General contribution of a clone in the relative production of both male and female gametes was estimated by the formula:

$$W_i = \sqrt{p_i \cdot c_i} \quad (1)$$

where p_i is the proportion of pollen produced by the i -th clone, and c_i is the analogous proportion of seed cone production. The male contributions were further recalculated using additional information on flowering synchronization among mating clones based on formulas:

$$p_i^j = \frac{p_i \cdot PO_i}{\sum_{j=1}^N p_j \cdot PO_j} \quad (2)$$

where PO_i is an index of phenological overlap of i -th clone acting as male (Askew and Blush,

1990). Since in this study we used the number of seed cones as a measure of female fecundity, we did not modify them, because the number of cones represented the final female reproductive output of a clone.

Inbreeding effective population number of the orchard was calculated, based on male flowering and cone production intensity, according to Crow and Kimura (1970) using the formula: $N_e = 1 / \sum(p_i c_i)$. Effective numbers of male and female parents were calculated as: $N_{e(m)} = 1 / \sum(p_i^2)$, and $N_{e(f)} = 1 / \sum(c_i^2)$, respectively.

Sexual asymmetry of clonal contribution was investigated using a maleness index proposed by Lloyd (1979): $M_i = p_i / (c_i E + p_i)$, where $E = \sum p_i / \sum c_i$. The index was calculated on pollen and seed cone production averaged over the years of observation. It was also calculated on pollen production in 1988 and seed cone yield in 1989 and also on pollen and seed cone productions in 1989 and 1990 respectively. Maturation of seed

cones takes place during the second winter following pollination; thus, the seed cone yield of a specific year must be related to pollen production of the preceding year. We also calculated phenotypic, genetic and residual correlations (including environmental, rootstock and error effects) in order to investigate trade-offs between male and female allocation. This was done following partitioning variance and covariance components obtained from a one-way ANOVA and multivariate analysis of variance (MANOVA) of pollen and seed cone production (Żuk, 1989).

RESULTS

Phenology

The phenograms of male and female flowering in 1990 and 1991 are presented in figure 1. In 1990 flowering started about 3

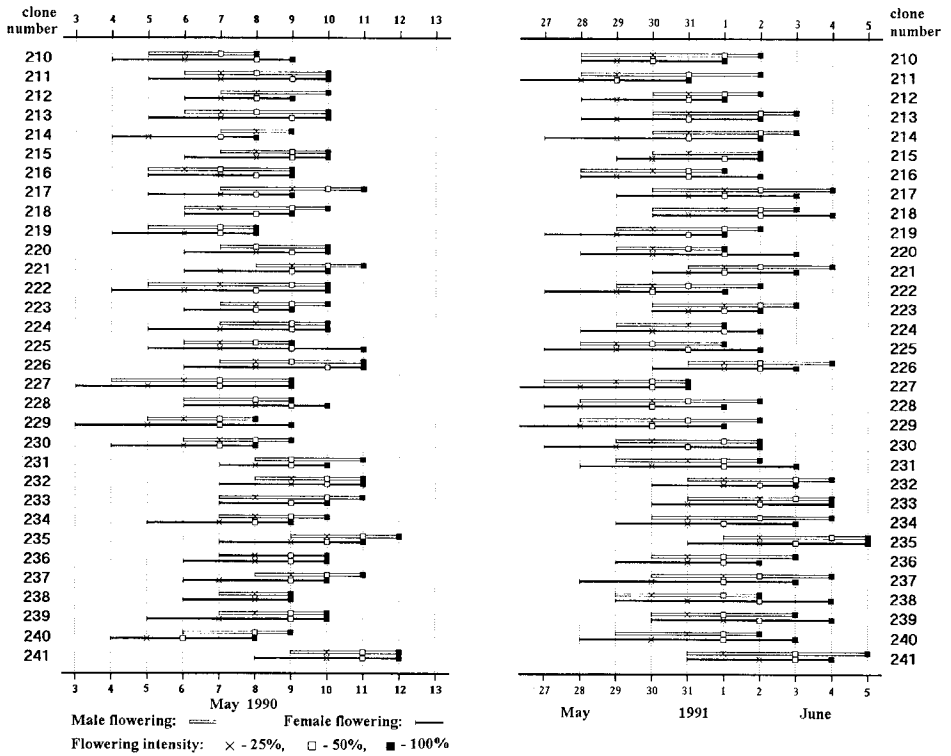


Fig 1. Phenograms of male and female flowering for 32 clones observed in 1989 and 1990.

weeks earlier than in 1991. Since the typical flowering period for Scots pine in Poland is on the turn of the first decade of May (Wesoly, 1982), the flowering in 1991 should be considered rather late, probably due to a cold spring with many late frosts. On average, female flowering started 1.25 days earlier than male flowering in 1990, and 1.09 days earlier in 1991. In 1990 female flowers of clones 227 and 229 were receptive earliest, and 1 year later, besides the two mentioned clones, clone 211 also flowered early. The latest female flowering clone appeared to be 241, and in 1991 so did clone 235. In the year 1990 maximum flowering (100% of receptive flowers) of all clones was achieved after 10 days, and in 1991 after 11 days from the beginning of the flowering period. Maximum flowering was already observed after 6 days for four clones in 1990, and for two clones in 1991. In both years pollen shedding was initiated by clone 227. Clones 235 and 241 were the latest to achieve a maximum of male as well as female flowering. Top male flowering was observed the fifth day from the beginning of pollen shedding for three clones in 1990 and for one clone in 1991. The ranks of clones according to their flowering beginning in 1990 and 1991 appeared to be highly correlated for both male and female flowering ($r = 0.764$ and $r = 0.719$, respectively, both $P < 0.001$), based on Spearman's rank correlation method.

The index of phenological overlap, calculated for any possible pair of mating clones, varied greatly for both years ranging between 0 and 1 with averages of 0.409 (standard deviation [SD] = 0.282) and 0.401 (SD = 0.257) during the 2 years. An index equal to 0 indicates that the periods of pollen release and female flower receptivity of respective clones do not overlap at all, while a value of 1 means complete overlap (Askew and Blush, 1990). Mean values for the 2 years ranged between 0.010 for the clone pair 235→227 and 0.986 for the pair 234→233 (an arrow indicates a clone func-

tioning as female). On average, the best overlapping male flowering clone appeared to be 211 (0.551) and the least 235 (0.134). The estimates calculated for individual clones inform about the general synchronization of a clone with other clones existing in an orchard (Askew and Blush, 1990). The range of variation of the index calculated for female flowering was narrower and ranged between 0.246 (clone 227) and 0.531 (clone 215). The index of phenological overlap estimated for the entire orchard was similar in both years (0.423 and 0.414).

Significant, though low correlation (Spearman rank correlation) was found between 1990 and 1991 for the indices of phenological overlap of individual pairs of mating clones ($r = 0.293$, $P < 0.001$) and for the indices of individual clones acting as pollen parents ($r = 0.360$, $P < 0.043$). The correlation was not statistically significant for the indices of clones functioning as females ($r = 0.331$, $P < 0.064$). Generally, considering male flowering, clones that started flowering earlier had higher overlap indices, while for female flowering the highest overlap indices were observed for intermediate flowering clones.

Male flowering and pollen production

Results of ANOVA for the production of male strobili are presented in table I. Significant variations among clones, crown sectors and years were found. The number of shoots with male strobili produced by a single ramet in the 3 consecutive years was on average 1110 (standard error [SE] = 67.1), 1046 (SE = 62.2) and 894 (SE = 50.2), respectively. The average number of male shoots within crown sectors was: 220 (SE = 12.6) (0–1 m), 502 (SE = 20.4) (1–2 m) and 293 (SE = 10.2) (2–3 m). Clone 237 appeared to be the most productive, producing on average 2178 male shoots per graft a year. The least fruitful was clone 215, with an average production of 413

shoots. The differences were even more distinct when individual grafts were compared, since the worst graft of clone 223 had only 45, while the best flowering graft of clone 240 had 3 157 male strobili bearing shoots. The ANOVA demonstrated also significant interaction between crown sectors and years, indicating that the flowering in different crown levels changed in consecutive years, which could be due to competition effect between crowns.

The eight clones selected for detailed analysis of pollen production varied significantly ($P < 0.001$) with respect to the three studied characteristics: weight of pollen per one male strobili bearing shoot, per 1 cm of that shoot, and per one pollen strobilus (fig 2). The ANOVA also demonstrated that the eight studied clones varied significantly in length of shoot bearing male strobili, as well as in the number of pollen strobili per one shoot; however, the variation among grafts within clones was also significant (table II).

Based on the number of male strobili bearing shoots, their average length, and the number of grafts of respective clones, the total pollen production of clones was esti-

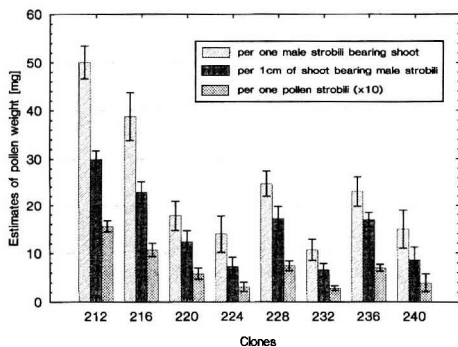


Fig 2. Weight of pollen (in milligrams) produced by one male strobili bearing shoot, 1 cm of shoot bearing male strobili, and one pollen strobili observed in 1990 among eight randomly chosen clones.

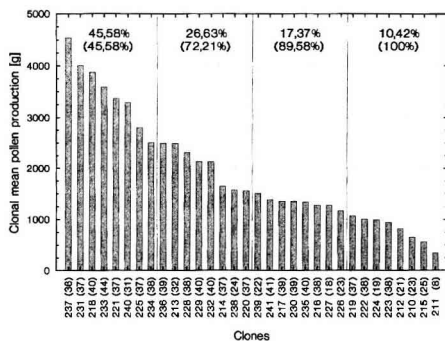


Fig 3. The contribution of individual clones in total pollen production of the orchard averaged over 3 years of observations. Numbers of ramets of respective clones are given in parentheses.

ated, using the fact that 1 cm of shoots bearing male strobili produces on average 0.028 g of pollen (Koski, 1975). The most productive appeared to be clone 237 (> 4.5 kg of pollen) and the least productive clone 211 (< 0.4 kg) was, however, represented only by eight ramets. It was found that the best 25% of clones in the orchard provided about 46% of pollen whereas the best 50% of clones were the producers of over 72% of pollen (fig 3). The entire pollen production of the orchard over the 3 consecutive years (1988, 1989, 1990) was calculated to be, respectively, 22.3, 19.3 and 14.7 kg/ha, with a mean of 18.6 kg/ha. Considering pollen production on a tree basis, the mean pollen production was 57.58 g per tree (SE = 4.80). The most productive was clone 237 (126.25 g), and the least productive clone 215 (22.71 g).

Seed cone production

Seed cone production varied significantly among clones and among the 2 years of observations (1989 and 1990) (table III). On average the clones produced from 71 (clone 219) to 184 (clone 238) cones per graft. However, from four to 316 cones were

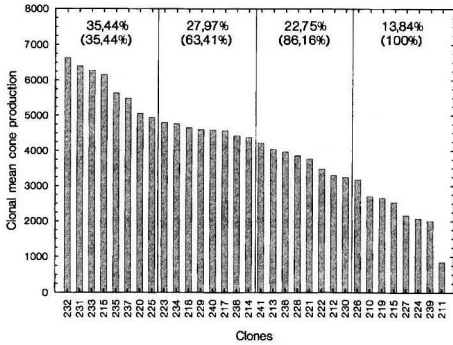


Fig 4. The contribution of individual clones in total seed cone production of the orchard averaged over 2 years of observations.

observed on individual grafts in different years. In 1989, cone production was smaller and averaged 110 cones per graft while it increased to 138 the year after. Considering both the number of grafts of respective clones and their cone production the total cone crop of individual clones was estimated (fig 4). Clone 232 produced on average over 6500 cones a year while clone 211 produced only 900. The 25% of the most productive clones provided 35% of cones and the analogous percentage for 50% of clones was 63%. Cone production on a tree basis ranged between 72 (clone 219) and 185 (clone 238) cones per tree. The total average production in the orchard was about 40000 seed cones (about 5.6 kg of seeds) per ha per year.

Sexual asymmetry

The maleness indices, indicating the degree of sexual asymmetry of clones, are presented in figure 5. The higher maleness of a clone indicates that relative clonal contribution of pollen production is higher than in cone production as compared to other clones existing in an orchard. The highest maleness was detected for clone 218 (0.684) and the low-

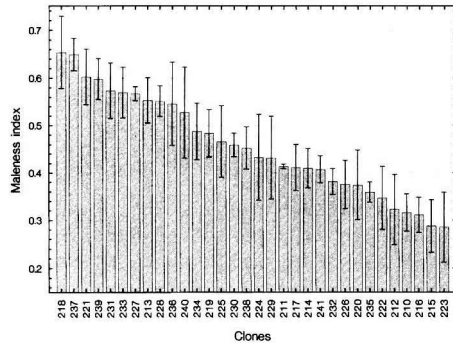


Fig 5. Maleness indices calculated for individual clones. Higher maleness index of a clone indicates that its proportional male contribution was greater than the female one, as compared to other clones.

est for clone 215 (0.289) (fig 5). Maleness indices which were calculated for clones based on pollen production in 1988 and seed cone yield in 1989 and the indices of analogous productions in 1989 and 1990 were significantly correlated ($r = 0.810$; $P < 0.001$). The variation calculated for individual ramets in different years (see Materials and Methods) was even greater and the index ranged between 0.032 and 0.968 in 1988/1989 and between 0.017 and 0.824 in 1989/1990. The correlation coefficient of maleness indices calculated for ramets between the two pollination seasons was $r = 0.708$ ($P < 0.001$). The significant correlations indicate that the sexual asymmetry for individual clones remained similar between the two pollination periods. One-way ANOVA of maleness indices indicated strong clonal variation ($F = 2.29$; $P = 0.001$; clonal mean basis $h^2_C = 0.56$). However, distribution of maleness indices calculated for clones and ramets did not deviate significantly from normal distribution.

Genetic and phenotypic correlations between pollen and seed cone production were all positive and appeared to be significant for the averaged and 1989/1990 data

Table IV. Genetic (r_g), phenotypic (r_p), and environmental (r_e) correlations between pollen and seed cone production.

Correlation	r_g	SD	r_p	r_e
Averaged pollen/seed cone	0.239*	0.095	0.257*	-0.142
Pollen 88/seed cones 89	0.124	0.104	0.142	0.016
Pollen 89/seed cones 90	0.371*	0.093	0.235*	-0.513*

* Parameter significant at the level $P < 0.05$. SD: standard deviation of r_g .

(table IV). Residual correlations for the averaged and 1989/1990 data were negative; however, only the latter one was statistically significant. None of the correlations of 1988/1989 data were significant.

Hypothesis on mating patterns

Assuming that the pollen pool is homogeneous in the orchard, the expected proportions of progeny of all possible individual mating pairs of clones were calculated on the basis of the intensity of pollen and seed cone production. The proportions varied widely from 0.006% for mating pair 211→215 to 0.373% for the pair 232→237 (an arrow indicates female). The proportions were also recalculated including indices of phenological overlap (Eq 2). Then, the differences were even greater, and ranged from 0.002% for several pairs to 0.520% for the pair 217→237; however, these proportions could be overestimated because of overestimation of the overlap index (see Materials and Methods). Since the years of observations of phenology, male flowering and cone production do not correspond among themselves in our study, we used only estimates averaged over all years. Thus, the obtained results should be considered only as general approximations.

The combined clonal contribution into the progeny of the orchard, assuming both male and female effect (Eq 1), is presented in figure 6. Clone 231 appeared to be the

best one. When phenology was also involved, the rank of clonal contribution changed slightly, which was most evident for clones 222 and 235, of which the first one improved and the second one worsened its rank.

Based on clonal variation in pollen and seed cone production and the variation in the number of ramets per clone, the effective population size was calculated to be 28.8 individuals, ie, 90.0% of the total number of clones. Effective numbers of male and female parents were calculated to be 24.3 and 30.7 (75.9% and 95.9%), respectively. Including phenology effect, the estimate increased for the whole population 29.5

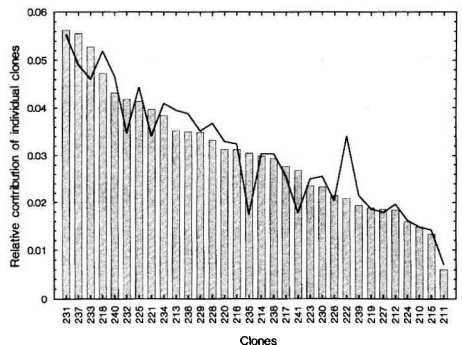


Fig 6. Expected relative contribution of individual clones in total reproductive output of the orchard: bars: estimation not corrected by the phenological overlap index; line: estimation corrected by the phenological overlap index.

(92.2%) while decreasing for males 23.8 (74.4%). Assuming that there is no background pollination, the expected proportion of selfed progeny, which resulted from fertilization between grafts of the same clone, should equal $0.034 (1/N_e)$. However, the proportion of detectable contaminants (minimum estimator of background pollination) in the studied orchard was found to be 15.3% on average (Bucznyk, 1992). Thus the decrease in outcrossing estimate ($t = 1-s$) in the orchard (see Muona and Harju, 1989) should be only 2.3% and finally the outcrossing rate should be equal to 0.977.

DISCUSSION

Genetic diversity of clones included in seed orchards is essential for tree improvement; however, large variation in flowering characteristics (intensity and phenology) may lead to unbalanced contribution of clones into the progeny and finally it may reduce the genetic diversity of filial generations (Boes et al, 1991). Reproductive phenology was often studied in Scots pine (Laura, 1973; Jonsson et al, 1976; Bhumibhamon, 1978; Chung, 1981; Wesoly, 1982; Boes et al, 1991; Pulkkinen, 1994). Although in many species male and female flowering phenology starts at about the same time (Blush et al, 1993), female flowering of Scots pine starts usually 1 or a few days earlier than the male one (Sarvas, 1962; Jonsson et al, 1976; Bhumibhamon, 1978). The time difference between female and male flowering may vary from year to year (Pulkkinen, 1994). However, departures from these assumptions were observed (Boes et al, 1991). It was also found that the beginning of flowering of a clone as compared to other clones in an orchard is constant and the rank of clones from the earliest to the latest flowering is repeated every year (Chung, 1981). Both these phenomena were generally observed in the studied seed orchard; however, correlation between the 2 years was

stronger for male flowering phenology than for the female one. The rank of flowering clones remaining similar from year to year may indicate that based solely on phenology the probability of pollination between particular clones may be similar in different years. Some inconsistency observed here for female flowering or the reported variation between the start of male and female flowering may probably be attributed not only to the daily sum temperature but also to weather conditions. There may also be some effect of photoperiod which was found to influence male and female flowering to a different degree (Giertych, 1967).

In the studied orchard there were no clones the phenology of which departed remarkably from the majority of clones in the orchard, and generally high coincidence between male and female flowering was observed. This could result from the fact that all clones originated from three forest districts located near each other in the Tuchola Forests.

The ANOVA of male strobili production demonstrated significant variation among clones, years and crown sectors but also significant interaction between crown sectors and years, indicating that flowering at different crown levels changed during consecutive years. This is probably due to the relatively high density of the orchard (spacing 5×5 m) and specific age of the grafts when they attained crown contact. This could effectively shadow lower branches, and in fact, the number of male strobili bearing shoots decreased at levels 0–1 and 1–2 m while it increased at level 2–3 m. The average number of male shoots decreased in the consecutive years. These results may indicate a need to thin the orchard.

Many authors frequently observed large variations in flowering intensity and cone production among clones (Chalupka, 1978, 1980, 1981, 1984; Wesoly, 1980; Nikkamen and Velling, 1987). It appears that most strobili are produced by a relatively small

number of clones and usually only one quarter of the best flowering clones produce more than half of the male and female strobili and about 50% of the best producing clones produce 80–90% of flowers of both sexes (Jonsson et al, 1976; Wesoly et al, 1984; Danusevicius, 1987; Chalupka, 1991). A similar pattern of flowering distribution among clones was observed in this study. While the best 25% male flowering clones produced 45% of total pollen, the best 25% seed cone producers provided only 35% of the seed orchard cones. Analogous numbers for half of the superior clones were 72% and 63%. The distribution of these proportions is more uniform than on other seed orchards studied to date (Jonsson et al. 1976; Wesoly et al, 1984). However, those orchards were usually younger (10–14 years old). Differences in flowering intensity between clones were noted to be greater for male than for female flowering (Chalupka, 1985), which is in line with our findings. However, after many years of observations, clonal variation in flowering and cone production intensity is decreasing with the age of grafts (Bhumibhamon, 1978; Van't Leven, 1979; Wesoly et al, 1984), which probably depends on the attainment of generative maturity by most clones in an orchard.

In this study we found that variations among ramets within clones and their interactions with years and crown sectors were also significant (table I). This could be due to microenvironment variation or even to rootstock or topophysis effects (Van Haverbeke, 1986). Variation among grafts of the same clone was found to be correlated with some growth characteristics such as height, diameter at breast height (DBH) and crown size (Andersson and Hattermer, 1975, 1978; Bhumibhamon, 1978; Nikkanen and Velling, 1987). Such a correlation is especially visible for grafts around 3–6 m high and with DBH over 6 cm (Koski, 1975).

A Scots pine seed orchard which consisted of grafts over 7 m high with a DBH of 16 cm is able to produce more than 20 kg pollen/ha (Koski, 1975). However, average pollen production in a 100-year-old Scots pine stand in southern Finland was estimated at 34.5 kg/ha (Sarvas, 1962), while Chalupka and Fober (1977) observed in a 67-year-old stand in Poland 35.9 kg pollen/ha. However, the amount of pollen may vary widely from year to year (Koski, 1991) and in years of heavy flowering, the figure may rise up to 80–120 kg/ha (Koski and Tallqvist, 1978).

Koski (1975) found that the amount of pollen produced by 1 cm of shoot bearing male strobili ranged from 0.013 to 0.072 g among 27 Scots pine clones. The results obtained in this study were much lower (0.007–0.030 g, fig 2). However in this paper, we examined the pollen related characteristics only in order to study the general extent of interclonal variation. The analysis of implicit values of pollen production would require the application of more precise methods. Studying clonal variation in the length of male strobili bearing shoots and the number of pollen strobili per one shoot, it appears that the variance components for the number of pollen strobili were greater at the clonal level while the variance components for the length of shoot bearing male strobili were greater at the graft level (table II). This may indicate that genetic control is stronger for the number of pollen strobili per one shoot than for the length of male strobili bearing shoot.

Extensive studies of male and female flowering intensity in Scots pine were made by Bhumibhamon (1978). He observed 16-year-old grafts that produced on average 1 889 male shoots and about 100 g of pollen each and the number of female strobili per graft was almost 700. However, the study was made on grafts transferred from northern to southern Finnish locations, thus flowering more intensively after climate change. Jonsson et al (1976) observed in the 3 con-

secutive years from 100 to 500 male shoots per graft, and from 75 to 150 seed cones on a Scots pine seed orchard at the age of 11 years. On the other hand, Wesoly (1980) noted that grafts of another Polish Scots pine seed orchard produced on average 800 male shoots (at the age of 14), and 200 seed cones (at the age of 13).

Data presented in this study, although underestimated (see Materials and Methods), indicate a relatively high production of male shoots (about 1017/graft). However, the number of seed cones does not seem to be high (124/graft). Year 1989 was preceded by 3 years of relatively high cone crops when about 2432 to 2680 kg of seed cones were collected from the orchard (Gniewkowo Forest District, unpublished data). The seed cone crops in 1989 and 1990 were 1205 and 1554 kg, respectively. Thus, it may be expected that in high crop years there may be up to 250 seed cones per graft.

Jonsson et al (1976) found correlations of flowering among consecutive years for particular clones. Comparable results obtained by Andersson and Hattemer (1978) and Bhumibhamon (1978) suggest that the observed differences among clones and correlations between years may have a genetic basis. In this study the correlations were found for male flowering intensity. There was no correlation for seed cone production while, on the contrary, interaction between clones and years was detected. Ruotsalainen and Nikkanen (1988) found in Norway spruce that there was no correlation for female flowering among the years of low flowering intensity while the correlations were still significant for male flowering. Probably a similar pattern of variation may exist in Scots pine.

In this study we found significant interaction between clones and years for seed cone production. Matziris (1993) observed in a black pine (*Pinus nigra* Arnold) seed orchard, that correlation coefficients were non-significant between consecutive years,

while it was highly significant between biennial years, indicating that this could be due to a carryover effect. The short period of observations in our study does not allow comparisons over many years.

Sexual asymmetry is common among monoecious plants (Ross, 1990) and it was often observed in Scots pine. Based on flowering data, Ross (1984) observed that maleness of individual Scots pine clones ranged from 0.17 to 0.93. Savolainen et al (1993) found negative genetic correlations between pollen and seed cone production. On the other hand, positive correlations among male and female flowering were often observed in Scots pine (Stern and Gregorius, 1972; Ross, 1984; Nikkanen and Velling, 1987) and in other species (O'Reilly et al, 1982; Schmidting, 1983; Schoen et al, 1986; Schmidting and Greenwood, 1993). Generally the latter phenomenon was observed in our study (table IV). However, it is possible that the relationships may change with the age of an orchard (Savolainen et al, 1993). Negative environmental correlations could possibly appear for many reasons. Besides the environmental factors, topophysis and competition effects may also be possible explanations. Collection of scions from different parts of a crown (with male vs female flowers) of a plus tree may increase maleness variation among ramets (within clones) and finally may cause negative environmental correlations. On the other hand, if scions are collected from one plus tree from the male flowering crown part and from another one from the female flowering part, this may increase interclonal variation causing negative genetic correlations. Although topophysis effects were found to be clear in Norway spruce (Dormling, 1970), they could not be ignored in pines. Unfortunately, we do not have any information on scion collections.

Positive correlations question the importance of sexual asymmetry or at least reduce its existence to several clones only. In fact,

maleness indices did not deviate from normal distribution. In the studied orchard there were no clones, or even grafts, which produced exclusively male or female strobili; however, it appeared that maleness was under high genetic control (clonal mean basis $h^2_c = 0.56$), probably as a consequence of variation in pollen and seed cone production among clones (tables I and III). The presence of sexual asymmetry may be considered advantageous for the reduction of probability of self-fertilization.

Using isozyme methods it was found that the contribution of male and female gametes into the pool of seeds produced may vary among clones and reproductive success may have changed in consecutive years (Müller-Starck et al., 1983; Müller-Starck and Ziehe, 1984; Müller-Starck, 1985). However, Muona et al (1987) demonstrated that for many clones of Scots pine the gender type remained similar for 3 consecutive years. This was also found in this study where maleness indices between different pollination periods were correlated.

It is rather difficult to make exact predictions about mating patterns based on observations of flowering and there were relatively few studies investigating this problem (Jonsson et al., 1976; Bhumibhamon, 1978; O'Reilly et al., 1982; Schoen et al., 1986). One of the parameters of a mating system is effective population size which is often calculated from flowering or cone production data. The ratio of effective to actual population sizes estimated for two Finnish seed orchards of Scots pine were 66% and 93% (Muona and Harju, 1989). The estimates calculated for Scots pine plantations in Germany were 46%, 55% and 61% in 3 consecutive years (Stern and Gregorius, 1972). The female effective population size in Sitka spruce (*Picea sitchensis* (Bong) Carr) calculated, based on cone production, was 45% and 70% in 2 years of observation (Chaisurisri and El-Kassaby, 1993). The results obtained in the present study

indicate that most clones could participate in the orchard reproduction. The effective numbers were very high and reached 75.9% and 95.9% for pollen and seed cone production, respectively. Including both the effect of male and female contribution the estimate was calculated to be 90.0%. Interestingly, when phenology was included with male contribution estimates, the effective number calculated for pollen production decreased (74.4%) while the estimate obtained for both sexes increased slightly (92.2%). This change, although not significant, may suggest that phenology could compensate differences in male and female contributions among clones. Relatively high effective population sizes observed in different seed orchards may suggest that seed orchard structure permits the creation of an ideal population (in terms of reproductive patterns) in a better way than natural populations.

Estimated outcrossing rate, based on the effective population size and the effect of background pollination, is high (0.977). This estimate is very similar to the outcrossing rate (0.987; SE = 0.02) calculated previously for this orchard based on isozyme analyses of seed embryos (Burczyk, 1991), especially when considering that the background pollination estimate was a minimum one.

It is generally expected that the studied orchard may be an interesting source of seeds used for reforestation. Small phenology differences, relatively high parental balance for both male and female contribution, including high effective numbers and high outcrossing rates, are positive symptoms for predicting general patterns of reproduction of the orchard. Although some aspects of the mating system may be deduced, based on flowering data, there may exist a substantial difference between reproductive investment and reproductive gain and the relationship between these is still an open question (Savolainen et al., 1993). In a future

study, we shall report results on detailed mating patterns revealed by isozyme analyses of progeny populations.

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