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STUDIES ON THE EFFECT OF UNEQUAL FLOWERING ON THE EFFECTIVE POPULATION NUMBER IN DANISH SEED ORCHARD CROPS.

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ABSTRACT
Number of male and female strobili, cones and/or seed have been observed in five Danish clonal seed orchard in order to investigate the differences between the clones in their relative contribution to the seed crop (gene pool of the offspring population). The effects have been quantified in terms of effective population numbers. Large variation was found between seed orchards of the same species, and between years of seed collection. The lowest levels of effective population number were found in young seed orchards (based on observation from two seed orchards), and in years with poor flowering (based on observations from one seed orchard). The so-called status number, which reflect the general build up of coancestry in the seed orchard progeny, varied from 20% to 80% of the actual number of clones in the seed orchard. Effects of unequal flowering should therefore be taken into consideration in seed orchard management decisions.

INTRODUCTION
Several studies have shown that large variation often can be found between the clones in coniferous clonal seed orchards in terms of flowering intensity and phenology (VARNELL et al. 1967, ERIKSSON et al. 1973, JONSSON et al. 1976, GRIFFIN 1982, O'REILLY et al. 1982, SCHMIDTLING 1983, MÜLLER-STARCZ & ZIEHE 1984, BRYAM et al. 1986, SCHEIN et al. 1986, EL-KASSABY et al. 1989, REYNOLDS & EL-KASSABY 1990, EL-KASSABY & REYNOLDS 1990, CHAISURISRI & EL-KASSABY 1993, SAVOLAINEN et al. 1993, EL-KASSABY & COOK 1994, KJÆR 1996, SIEGISMUND et al. 1996, BURCZYK & CHALUPKA 1997, KJÆR & WELLENDORF 1997). Gametes from abundantly flowering genotypes are over represented in the seed orchard progeny, and this reduces the effective population number, i.e. genetic drift and increase in inbreeding coefficient take place more rapidly in successive generations than would be predicted from the actual number (census number, $N_c$) of clones used in the orchard. The differences in gamete contribution between the trees are often found to include a strong genetic component, which increase the implications for domestication - especially if the reproductive energy is genetically correlated to other important traits. This has been discussed by e.g. SEDGLEY & GRIFFIN (1989).

The number of clones left to remain in a given clonal seed orchard after genetic thinning is largely determined as a trade off between using only clones with superior breeding values - and maintaining a certain level of genetic diversity in order to reduce inbreeding (LINDGREN 1974, 1987, 1993), and maintain some level of genetic variation. The effect of unequal gamete contribution should therefore be taken into account in management decisions, and a number of studies on reproductive behavior have therefore been initiated in Danish seed orchards.

CALCULATION OF EFFECTIVE POPULATION NUMBER FROM OBSERVATIONS ON FLOWERING AND SEED YIELD
The effective population number quantify the importance of deviation from the idealised panmictic breeding situations that is assumed in the theoretical framework behind most studies on effects of small population sizes (WRIGHT, 1931). The concepts has been developed to cover to number of
specific situations (see e.g. CROW & DENNISTON, 1988), including the highly unnatural situations in clonal seed orchards (MOUNA & HARJU, 1989; LINDGREN & MULLIN, 1997; KJÆR 1996; KJÆR & WELLENDORF, 1997). Three types of effective population numbers will be discussed below:

1. The inbreeding effective population number \( N_e^{(i)} \) that relates to the increase in the inbreeding coefficient \( F \). Inbreeding is only generated by selfing in the clonal seed orchard situation where clones are unrelated. \( N_e^{(i)} \) therefore merely reflects the expected amount of selfings in the seed orchard offspring (MOUNA & HARJU, 1989).

2. The variance effective population number \( N_e^{(v)} \) relates to the genetic drift from the population of seed orchard clones to their progeny (KJÆR, 1996). This measure is therefore sensitive to the number of progenies collected per clone (i.e. sample size).

3. The status number \( N_s \) (LINDGREN & MULLIN, 1997) assess the more general build up of coancestry in the seed orchard progeny.

An example of interpretation of the different kinds of effective population numbers in relation to the clonal seed orchard situation is presented in table 1. Please note that the effective population number always refer to seed orchard progeny, i.e. the seed crop, rather than to the actual seed orchard as such.

Table 1. Effective population numbers in a clonal seed orchard crop.

<table>
<thead>
<tr>
<th>Type</th>
<th>General meaning</th>
<th>Example of specific interpretation in clonal seed orchard crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbreeding effective population number,</td>
<td>The size of an ideal Mendelian population which give the same increase in</td>
<td>The number of equally fertile clones in an ideal, random mating clonal seed orchard that is expected to produce the same amount of selfed progenies - as is experienced in the present seed orchard crop.</td>
</tr>
<tr>
<td>( N_e^{(i)} )</td>
<td>inbreeding coefficient as experienced in the observed population</td>
<td></td>
</tr>
<tr>
<td>Variance effective population number,</td>
<td>The size of an ideal Mendelian population which give the same expected change</td>
<td>The number of equally fertile clones in an ideal, random mating clonal seed orchard that is expected to give the same change in gene frequencies between the seed orchard clones and the progeny due to genetic drift - as is experienced between the present seed orchard clones and the seed orchard progeny due to unequal contribution of gamets</td>
</tr>
<tr>
<td>( N_e^{(v)} (infinite) )</td>
<td>(random drift) in gene frequencies as experienced in the observed population</td>
<td></td>
</tr>
<tr>
<td>Status number, ( N_s )</td>
<td>The number of non-inbred and unrelated genotypes which including self-coancestry</td>
<td>The number of equally fertile clones in an ideal, random mating clonal 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</td>
</tr>
</tbody>
</table>

The inbreeding effective population number $N_e^{(i)}$ has been calculated following the idea of MUNA & HARJU (1989), as

$$N_e^{(i)} = 1/P,$$

where $P$ is the expected amount of selfings in the seed orchard offspring. $P$ was calculated below according to KJÆR & WELLENDORF (1997), who modified the formula MUNA & HARJU (1989) by assuming selection against inbred and selfing due to mixed mating systems:

$$P = (1-t) + w t \sum(f_i p_i).$$

where $f_i$ and $p_i$ are the relative female and male contribution of clone $i$ in the given seed orchard; $t$ is the expected outcrossing rates ($t$ different from zero indicated that the species mated according to mixed mating system); and $w$ is the relative fitness of selfed zygotes compared to outcrossed (see KJÆR & WELLENDORF (1997) for further explanation). Effects of pollen from sources outside the seed orchard was not included. Specific estimates of $t$ and $w$ were not available (except in one case where $t$ was estimated from allogeneic segregation in clonal progenies), and these critical parameters therefore had to be assumed based on other studies.

The variance effective population number $N_e^{(v)}$ relates to the genetic drift from the population of seed orchard clones to their progeny. This will depend on sample size, and $N_e^{(v)}$ will therefore be influenced by the number of progenies collected per clone. KJÆR (1996) argue that a large number of seeds are collected from each clone during seed harvest in a clonal seed orchard. The variance effective population number $N_e^{(v)}$ was therefore calculated below as,

$$N_e^{(v)\text{ (infinite)}} = N_c - \frac{1/2}{N_c \sum r_i^2 - 1}$$

which corresponds to very large (infinite) number of progenies per clone (KJÆR, 1996). $N_c$ is the number of clones in the given seed orchard, and $r_i$ is the average of male ($p_i$) and female ($f_i$) contribution ($r_i = \frac{1}{2}(f_i + p_i)$) from clone $i$. The relative variance effective population number (the effective population number relative to the number of clones in the seed orchard, $N_e^{(v)}/N_c$) was calculated in order to be able to compare the seed orchards.

The status number ($N_s$) was estimated below as,

$$N_s = 1/\sum r_i^2, \text{ for } i=1...N_c$$

following LINDGREN & MULLIN (1997). The relative status number ($N_s/N_c$) was also calculated in order to be able to compare the seed orchards.
OBSERVATIONS FROM DANISH SEED ORCHARDS

The differences in gamete contribution from the individual clones were estimated in five different Danish seed orchard based on assessment of flowering and/or seed production. The number of female and male strobili were scored in classes on a logarithmic scale following WELLENDORF (unpublished), because counting all single strobili would be very time consuming on abundantly flowering clones.

The abundance of male and female strobili were easily assessed. However, female reproductive success in terms of seed yield was also be estimated in some studies (listed below) on different subsequent levels (volume of cones per clone, weight of seeds per clone, number of filled seed per clone (derived from seed yield, seed per 100g and cutting percent), and number of germinating seedlings per clone (derived from seed yield, seed per 100g and germination percent).

Results from five Danish seed orchards are presented in present paper. The clonal seed orchard FP209, Søro, is a Picea abies clonal seed orchard consisting of 24 clones. It is only partly balanced due to mortality of the ramets. The clones were selected in Danish stands, which are expected to be second or third generation in Denmark after German origin. The ramets are spaced at 7 x 7 m and replicated in 10 blocks. Male and female strobili was observed in four of these blocks in 1990 (a very good seed year) and 1992 (a year with scattered flowering). All blocks were assessed in 1989 (a year with very poor flowering). Details are given in Kjaer (1996).

The seed orchard FP 240, Snævert, is a Picea abies clonal seed orchard consisting of 100 clones. The ramets are almost balanced with 36 - 42 ramets per clone, but the clonal average height of the ramets varied from 1.1 m to 5.7 m. Ninety-five of the clones originate from Danish stands, which are expected to be first to third generation in Denmark of west continental origin (probably imports from Germany). The remaining five clones were selected in southern Sweden in stands of west continental origin. Number of male and female strobili were estimated on 737 ramets in four unbalanced blocks in the seed orchard in May 1993 (a very good seed year). Cones were kept separate by clone for all ramets during the following harvest and processing. The volume of cones (hl/clone), seed yield (kg/clone), the 1000 seed weight and germination percent (after 4 weeks) was recorded for each of the 100 clones. Details are given in Kjaer & Wellendorf (1997).

The seed orchard FP238, Nødebo, is a Picea sitchensis clonal seed orchard consisting of 113 clones selected in Danish stands, which are expected to be first or later generation in Denmark (probably mainly of Washington State, USA, origin). It is only partly balanced due to mortality of the ramets, and initial problems with propagation. Cones were collected in 1993 (a year with fairly good seed yield) and kept separately by clones during the following harvest and processing. The volume of cones (hl/clone), seed yield (kg/clone), the percent of filled seed and the 1000 seed weight were recorded for each clone. Germination percent were recorded for 20 of the clones. The seed collection was the first commercial collection in this young seed orchard. Details are given in Kjaer et al. (1995).

The seed orchard FP623, C.E. Flensborg Plantage, is an Abies procera clonal seed orchard consisting of 100 clones. Number of ramets planted per clone ranges from 7 to 19. The ramets varies in height. Some ramets suffer from plagiotropic growth, but non-plagiotropic were on the average 6.5 m tall at the time of observation. Number of male and female strobili were registered on the 100 clones in the flowering season 1993 (a year with abundant flowering). In total 854 ramets (4-12 ramets per clone) were registered. Details are given in Siegismund et al. (1996).

The seed orchard FP 227 is an old Pinus sylvestris clonal seed orchard with only 9 clones. The clones were selected in a Danish stand of Scottish origin. Cones have been collected separately by clones during a 20 years period from first seed collection to several years after full production was initiated. Details are given in KJÆR & BARNER (1998).
An extract of the results from the observations in the five Danish seed orchards are presented in Table 2 and Table 3. It is seen that the inbreeding effective population numbers are difficult to estimate, because the sizes of the outcrossing rate ($t$) and relative fitness of selfing zygotes ($w$) are crucial to the result. However, the estimates of the variance effective population numbers (table 3) show that important variation between species, seed orchards - and also between different seed collection years within the a given seed orchards, - can be found. In one seed orchard, FP 209 (Picea abies), the variance effective population number was thus found to vary from 37% of census number in the year with poor flowering to 130% in the year with abundant flowering. The corresponding status number varied from 28% (poor seed year) to 57% (good seed year).

**Tabel 2. Inbreeding effective population number ($N_e(i)$) and relative status number ($N_s/N_c$) in Danish seed orchards**

<table>
<thead>
<tr>
<th>Species</th>
<th>FP-no</th>
<th>Observ. $\Sigma p_f/i$</th>
<th>Assumed fitness of selfed ($w$)</th>
<th>Assumed “outcross. rate” ($t$)</th>
<th>$N_e$</th>
<th>$N_e(i)$</th>
<th>$N_e(i)_{max}$</th>
<th>$N_s/N_c$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picea abies</td>
<td>FP209</td>
<td>0.0775</td>
<td>0.33</td>
<td>0.95</td>
<td>24</td>
<td>13.2</td>
<td>20</td>
<td>28%</td>
<td>Mod. after Kjær (1996)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>FP209</td>
<td>0.0754</td>
<td>0.33</td>
<td>0.95</td>
<td>24</td>
<td>13.3</td>
<td>20</td>
<td>57%</td>
<td>Mod. after Kjær (1996)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>FP209</td>
<td>0.0571</td>
<td>0.33</td>
<td>0.95</td>
<td>24</td>
<td>14.5</td>
<td>20</td>
<td>48%</td>
<td>Mod. after Kjær (1996)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>FP240</td>
<td>0.0133</td>
<td>0.33</td>
<td>0.95</td>
<td>100</td>
<td>18.1</td>
<td>20</td>
<td>76%</td>
<td>Kjær &amp; Wellendorf (1997)</td>
</tr>
<tr>
<td>Abies procera</td>
<td>FP623</td>
<td>0.0154</td>
<td>0.90</td>
<td>0.98</td>
<td>100</td>
<td>30.0</td>
<td>50</td>
<td>55%</td>
<td>Mod. after Siegismund et al (1996)</td>
</tr>
</tbody>
</table>

Source: Here after Kjær (unpublished). $N_e(i)_{max}$ is the inbreeding effective population number given that selfing only occur due to selfpollination within the crowns of the individual trees, i.e. no selfing is caused from pollination between ramets of the same clone ( $\Sigma p_f/i = 0$). See text for further explanation.

Higher effective population numbers were found in the study of FP 240 (Picea abies) based on observations in the very good seed year. Here, the relative variance effective population number of the seed crop was estimated to be above 300%, corresponding to a relative status number of 76% of the number of clones in the clonal seed orchard. Correlations between number of female strobili, cone yield and seed yield could be calculated in this study. It was found that number of female strobili could only partly predict the seed yield ($r = 0.72$), but good correlation was found between the cone yield and the seed yield ($r = 0.91$) of individual clones. However, the effective population number was not much different based on strobili compared to estimates based on seed.

The study of the young clonal seed orchard FP238 (Picea sitchensis) revealed a lower level of relative variance effective population number. 47% (cones) to 27% (filled seed), corresponding relative status numbers of 27% (cones) to 19% (seed). These estimated do not take the effects of pollen into account.

The result from the study of FP 623 (Abies procera) in a good seed year is quite similar to the observations from the good flowering year in FP209 (Picea abies), i.e. the relative variance effective population numbers were found to be lower than in FP240 (Picea abies), with a status number of approximately half the number of clones in the seed orchard.

The data from FP227 (Pinus sylvestris) show that the effective population number can vary substantially over time, from a low level during the first flowering to a much higher level when the seed orchard enter a more productive stage. This is shown in figure 1.
Table 3. Relative variance effective population number \( \left( \frac{N_e(\infty)}{N_c} \right) \) and relative status number \( \left( \frac{N_s}{N_c} \right) \) in Danish seed orchards

<table>
<thead>
<tr>
<th>Species</th>
<th>FP-no</th>
<th>Age</th>
<th>Overall seed crop</th>
<th>Reproductive structure assessed</th>
<th>( N_c )</th>
<th>( \frac{N_e(\infty)}{N_c} )</th>
<th>( \frac{N_s}{N_c} )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picea abies</td>
<td>FP209</td>
<td>Old</td>
<td>Very small</td>
<td>Male+fem. strobili</td>
<td>24</td>
<td>37%</td>
<td>28%</td>
<td>Kjær (1996)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>FP209</td>
<td>Old</td>
<td>Small-average</td>
<td>Male+fem. strobili</td>
<td>24</td>
<td>90%</td>
<td>48%</td>
<td>Kjær (1996)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>FP209</td>
<td>Old</td>
<td>Large</td>
<td>Male+fem. strobili</td>
<td>24</td>
<td>130%</td>
<td>57%</td>
<td>Kjær (1996)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>FP240</td>
<td>Intermed</td>
<td>Large</td>
<td>Male+fem. strobili</td>
<td>100</td>
<td>315%</td>
<td>76%</td>
<td>Kjær &amp; Wellendorf (1997)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>FP240</td>
<td>Intermed</td>
<td>Large</td>
<td>Male strobili + germinating seed</td>
<td>100</td>
<td>236%</td>
<td>70%</td>
<td>Kjær &amp; Wellendorf (1997)</td>
</tr>
<tr>
<td>Abies procera</td>
<td>FP623</td>
<td>Old</td>
<td>Large</td>
<td>Male + female strobili</td>
<td>100</td>
<td>121%</td>
<td>55%</td>
<td>Siegismund et al (1996)</td>
</tr>
<tr>
<td>Picea sitchensis</td>
<td>FP238</td>
<td>Young</td>
<td>average</td>
<td>Cones</td>
<td>113</td>
<td>46%</td>
<td>29%</td>
<td>Kjær et al. (1995)</td>
</tr>
<tr>
<td>Picea sitchensis</td>
<td>FP238</td>
<td>Young</td>
<td>average</td>
<td>Filled seed</td>
<td>113</td>
<td>27%</td>
<td>20%</td>
<td>Kjær et al. (1995)</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>FP227</td>
<td>Young</td>
<td>Small average</td>
<td>Seed</td>
<td>10</td>
<td>48%</td>
<td>29%</td>
<td>Kjær &amp; Barner (1998)</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>FP227</td>
<td>Intermed</td>
<td>average</td>
<td>Cones</td>
<td>10</td>
<td>151%</td>
<td>56%</td>
<td>Kjær &amp; Barner (1998)</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>FP227</td>
<td>Old</td>
<td>Large</td>
<td>Cones</td>
<td>9</td>
<td>443%</td>
<td>81%</td>
<td>Kjær &amp; Barner (1998)</td>
</tr>
</tbody>
</table>

Source: Here after Kjær (unpublished). Se text for further explanation.

DISCUSSION

Flower assessment can be a cheap way to estimate the gamete contribution of a large number of trees. The obtainable estimates are subject to a number of assumptions and limitations (see e.g. Kjær & Wellendorf, 1997), but seem to be a valuable way to obtain useful estimates of variance effective population numbers in seed orchards. However, marker based studies on the correlation between observed number of male strobili and actual contribution of offspring to the progeny is still required in order to verify the effectiveness on the male side. Also, effects of pollination from sources outside the seed orchards should be considered.

The simple flower assessments (observations of two persons in one day per seed orchard) - and especially observation on cone yield - have given useful estimates of the imbalance in terms of seed yield, i.e. imbalance on the female side. This suggests that variance effective population number and status number based on cone yield and number of male strobili in general give useful estimates of the impact of unbalanced seed production on genetic diversity.

The levels of effective population numbers found in the Danish clonal seed orchard indicate that unequal contribution of flowers should always be expected, and status number must be expected to be as low as 50% of the number of clones - even in years with good flowering. The level can be much lower in year in poor flowering - or during the first years of seed production. Gain trials or genetic tests should therefore in general not be based on simple collection of open pollinated progenies in young seed orchards. Especially not on bulk collections. This also have implications for \textit{ex situ} gene resource conservation. It will always as a minimum be recommendable to collect comparable amounts of cones from the selected seed trees. Of course, the correlation between cone yield and seed yield may vary substantially between species and populations.
Figure 1. Relative contribution of seed and corresponding development in status number over a 20 year period in a clonal *Pinus sylvestris* seed orchard

Source: Based on Kjær & Barner (1998), here after Kjær (unpublished). The seed orchard originally consisted of ten clones, but one clone was removed in 1972.
ACKNOWLEDGEMENTS

Several persons have been involved in the studies referred above. Hans Siegismund and Dag Lindgren are especially thanked for their valuable comments regarding use of effective populations numbers suitable for the clonal seed orchard situation.

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