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Genetic worth and diversity across 18 years in a Nordmann fir clonal seed orchard

Ulrik B. Nielsen · Ole K. Hansen

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Abstract

• **Context** Many tree breeding programmes rely entirely on clonal seed orchards as the link between breeding activities and forestry, and are thereby also exposed to yearly fluctuations in the orchard crops.
• **Aims** The main objective was to study the impact of year and clone fluctuations in strobili production on genetic worth, as well as on genetic diversity, of 18 consecutive commercial seed orchard crops.
• **Methods** In a mature *Abies nordmanniana* clonal seed orchard, a yearly visual assessment of female and male strobili was carried out on a total of 18 clones (260 ramets), and coupled with clone breeding values.
• **Results** Despite large annual and clone variation, and year-by-clone interaction in strobili production, the orchard's genetic worth was somewhat uniform when considering four evaluated traits in the 11 years of superior or medium crop size. These years accounted for 95% of the accumulated cone crop over the whole period (18 years). Noteworthy deviations were only seen in the seven poorest seed years, comprising the remaining 5% of the total cone crop, where genetic diversity measures were also low.

• **Conclusion** Avoiding small crop years seems to be a reasonable way to avoid fluctuations in the commercial seed orchard product.

Keywords Clonal seed orchard · Variation in flowering · Genetic worth · Strobili scoring · Genetic diversity

1 Introduction

Clonal seed orchards transfer results from tree breeding to the industry, and are currently the only commercially available technique for mass production of improved material in Nordmann fir (*Abies nordmanniana*), a species used for Christmas tree production in several European countries and North America. As clonal seed orchards (CSOs) are also the main vehicles for breeding of numerous other tree species around the world, the study of their functionality is a constant and relevant issue. Studies performed to date in conifer CSOs have established that there can be a large variation in fertility between the constituent clones, often following the 20–80 rule (Anonymous 1976), i.e. that 20% of the clones produce 80% of the gametes, a pattern also reported for Nordmann fir (Hansen and McKinney 2010). It is also well known among managers of seed orchards that there can be a large year-to-year variation in fertility, a fact which has been scientifically documented for a large number of species since the 1960s. As stated by Kroon et al. (2009), rather few studies have followed clone differences in flowering abundance over longer periods of time (e.g. Eriksson et al. 1973 (6 years); El-Kassaby et al. 1989 (8 years)) and, to the authors' knowledge, none for the genus *Abies*, and in general very few time series include both male and female strobili registrations (Schmidtling 1983; Nikkanen and Ruotsalainen 2000; Choi et al. 2004).

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Contribution of the co-authors Ulrik B. Nielsen: initiating the sample series of yearly ocular evaluations of clonal strobili contributions, partly data sampling, supervising field work, data management and analyses, main writer (60%) of the manuscript and literature search.
Ole K. Hansen: true partner on data analyses discussing models and statistical approach, focusing objective of the time series study, writing the manuscript (40%) including discussion and conclusion, linking experiment to molecular literature.

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Both within year, as well as between years, fertility variation among clones may have an impact of the genetic value of the seed crop, since the different clones possess different breeding values. These clonal breeding values are included in the genetic worth of the yearly crops as weighted proportions of each clone's gamete contribution. Due to the long production time of CSOs and the substantial clonal and yearly variation in fertility, it is relevant to study these fluctuating patterns and how they impact the genetic value of the seed crop, the ultimate output of tree breeding.

The straightforward approach for studies of variation in fertility in conifer CSOs is an assessment of the amount of strobili (e.g. Eriksson et al. 1973). Seeds are the harvested output from seed orchards, so estimates of the differential female gamete contribution from the clones have been relatively easy to obtain. In the last decade, the more cryptic differential male gamete contribution, i.e. the clonal variation in pollination success, has been investigated through highly polymorphic microsatellite markers (e.g. Moriguchi et al. 2004; Slavov et al. 2005; Hansen and Kjær 2006; Hansen 2008; Funda et al. 2008). Recently, whole parental analyses on bulk seed crops from CSOs have been performed (Hansen and McKinney 2010; El-Kassaby et al. 2010).

While microsatellite markers may be the most precise and reliable tool for investigating variation in parentage in seed crops from CSOs, their application involves substantial effort and cost. In combination with the relatively recent appearance of microsatellite markers, longer time series with annual analyses of the seed crop from CSOs do not exist. However, Hansen and Nielsen (2010) found that visual evaluation of the amount of male strobili gave a satisfactory estimate of the actual paternity of the seed orchard crop of Nordmann fir. Furthermore, female strobili on *Abies* are relatively easy to count, being mostly situated in the upper part of the crown, and just before budburst, they are very distinct both in form and size and are characterised by a light green colour. Recently, Funda et al. (2011) demonstrated congruence between phenotypic assessment methods of both male and female strobili and the parentage success determined by DNA fingerprinting for three different conifer species.

In the late 1960s, a grafted clonal Nordmann fir seed orchard (FP.620) was established, and this orchard, as well as its harvested progenies, has been used in several studies (Nielsen and Hansen 2000; Nielsen and Chastagner 2005). Reliable breeding values have been estimated from both open-pollinated offspring (Nielsen and Hansen, in preparation) and also from a set of full-sibs (Hansen and Nielsen 2010). At the same time, annual visual evaluations of male and female strobili numbers in a considerable subsample of the ramets in the CSO has been carried out each year since 1992. The combination of these data—the estimated clonal breeding values as well as annual clonal variation in fertility—allows us to estimate the total aggregated breeding value of all clones

and ramets in the orchard (designated the genetic gain or quality (Griffin 1982) or the genetic worth (GW) of the CSO (Stoehr et al. 2004)) in each of the 18 years where flower assessment has been undertaken.

The objective of this study was, based on an 18-year time series, to quantify the year-to-year variation in the genetic worth of a CSO, as well as describing the genetic diversity of the seed crop. More specifically, we aimed to describe the variation in male and female strobili production between clones and years, calculate the impact of the fertility variation on the GW of the CSO and estimate genetic diversity measures and to compare them with the estimates of the orchard's GW for a variety of characters important for the Christmas tree industry. Practical impact of the results is discussed.

2 Material and methods

2.1 Clonal seed orchard FP.620

The initial 23 clones in FP.620 were selected at the beginning of the 1960s from older approved Danish stands. The selection criterion was bough quality. The orchard was established in the years 1966–1968 and covers 3.0 ha. A total of 854 ramets were planted at approximately 6×6-m intervals: each clone was represented at various frequencies ranging from 9 to 77 ramets. In 1992, when the strobili counts were initiated (see below), the orchard had 701 remaining grafts, but before the registration of strobili in 2001, the orchard was thinned and ramets of clones FV5, FV8, FV19, FV22 and FV23 were removed, leaving 18 clones in the orchard and a total of 576 ramets.

2.2 Visual strobili counts

The quantity of male and female strobili in the seed orchard was assessed by visual scoring, using a logarithmic scale from 1 to 8 (Sirikul et al. 1991), where the scores are: 1=0 strobili, 2=1–3, 3=4–15, 4=16–60, 5=61–250, 6=251–1,000, 7=1,001–4,000 and 8=4,001–16,000 strobili. For each score, a mid-range value was assigned to estimate the production of strobili in actual numbers for each score: 1=0, 2=2, 3=10, 4=38, 5=155, 6=625, 7=2,500 and 8=10,000 strobili. Scoring was carried out on a subset of 340 ramets in the seed orchard. Due to thinning in 2001 and wind throw, only 260 ramets were measured in all 18 years. Counts were made from the ground using binoculars. Observations were made by a scientific field worker (single individual) in 13 of the years, the main author did the observations in four of the remaining years, and observations in one single year were carried out by a third person. Both the scientific field worker and the third person were carefully instructed in methodology.

2.3 Statistical analysis of strobili scores

All statistical analyses were carried out using the SAS® 9.2 software. The following model [1] was applied to examine clone differences within year for female and male strobili production respectively in actual numbers (based on the mid-range value of each score).

$$Y_{ij} = \mu + c_i + e_{ij} \quad (1)$$

where Y_{ij} is strobili production of the single ramet, μ is the grand mean, c_i is the effect of clone i and e_{ij} is the residual. All random effects were independent, but showed some deviations from a normal distribution.

Analysis of variance across all 18 years for the 18 clones represented in the CSO for the whole period was carried out for male and female strobili production using the following model (Eq. 2).

$$Y_{ijk} = \mu + c_i + a_j + i_{ij} + r_{k(i)} + e_{ijk} \quad (2)$$

where Y_{ijk} is the strobili production of the single ramet, μ is the grand mean, c_i is the effect of clone i , a_j is the effect of year j and i_{ij} is the interaction between clone and year, $r_{k(i)}$ is the effect of ramet within clone and e_{ijk} is the residual. All effects were random and independent, but did show some deviation from a normal distribution.

Model 2 is identical to a repeated measurements analysis using a compound symmetry option (CS option), i.e. assuming an identical correlation across years despite differences in time. In the analysis of numbers, deviations in normality and homogeneity were found to be due to the substantial strobili production of a few single ramets in each year. Omitting these outliers did not change the main results; neither did scaling the numbers per ramet with the standard deviation of each year's observations.

To allow for a statistical evaluation of different models for the error correlation in a repeated measurements analysis, it was necessary to omit the year 1999, in which there was almost no flowering, thereby making sure that the models converged. Based on the 17 years used in analysis, the compound symmetry option was compared to Gaussian spatial correlation as well as to a model with an autoregressive correlation with the previous year. The two latter models did not improve the analysis (results not shown), and hardly influenced the estimated components of variance.

Model assumptions were evaluated testing normality of errors and by plotting errors versus predicted values. Some deviations from normality and variance homogeneity were seen, especially for small crop years. Using the actual scored values for each ramet improved the analysis due to the logarithmic nature, and resulted in a

generally higher genetic component: both types of data have been used (see text).

Simple means of scores of strobili were calculated for each clone for each year, and Spearman rank correlations were used for correlations between years for female and male strobili counts, respectively. Based on the mean of numbers of strobili for each clone, Pearson correlations were also calculated between female and male strobili numbers within each year.

2.4 Heritabilities

For each year, broad-sense heritability for actual numbers of strobili was calculated assuming the grafts were true clones $H^2_{bs} = V_{clone} / (V_{clone} + V_{error})$, where V_{clone} was the variation among clones and V_{error} the residual variance.

Broad-sense heritability across years was calculated as $H^2_{bs} = V_{clone} / (V_{clone} + V_{year} + V_{clone \times year} + V_{ramet(clone)} + V_{error})$, variances specified as above, and further V_{year} was the variation among years, $V_{clone \times year}$ was the clone by year interaction and $V_{ramet(clone)}$ the ramet within clone variation. Analyses were made on both strobili numbers and strobili scores.

2.5 Genetic diversity—effective population numbers

Effective population numbers were calculated for the seed orchard crop in each year, assuming an equal number of fertile clones in an ideal random mating CSO that is expected to produce (1) the same change in frequencies between the seed orchard clones and the progeny due to genetic drift and (2) gives rise to the same amount of inbreeding as expected following random mating in the present seed orchard progeny (Kjær and Wellendorf 1997):

1. Variance effective population number: $N_e^{(v)}(\text{infinite}) = (N-0.5) / (N \sum r_i^2 - 1)$ (Kjær 1996) where N is the number of clones in the parent generation, c_i and p_i are clone i 's relative contribution of male and female gametes, respectively, $r_i = (c_i + p_i) / 2$.
2. Status number (Lindgren and Mullin 1998): $N_s = 1 / \sum r_i^2$

2.6 Cumulative gametic contribution

For each year, the percentage of clones producing 80% of the cumulative gametic contribution for male as well as female strobili was calculated using linear interpolation according to Griffin (1982). The clones' relative contributions of male and female gametes, respectively, were described by a ratio for each clone as c_i/p_i . Furthermore, this sexual asymmetry was quantified using the differences in relative frequency between sexes: the absolute value of

$(c_i - p_i)/2$ and the accumulated asymmetry for the orchard clones was estimated as $A_s^{(x)} = 0.5 \sum |c_i - p_i|$ summed across all clones, according to Choi et al. (2004), with the larger values representing the more pronounced differences between the respective male and female strobili production.

2.7 Genetic worth across years

Assuming no inbreeding and no outside pollen contamination, and that the scores of female and male strobili are reliable measures of the male and female gamete contribution, respectively, it is possible to calculate the genetic worth (GW) of the orchard in the different years for female (GW_f), male (GW_m) and an aggregated value (GW) with an expected value of zero for a fully panmictic population with even numbers of grafts per clone. The genetic worth of the CSO was estimated as:

$$GW = (GW_f + GW_m)/2 = \left(\sum c_i \times BV_i + \sum p_i \times BV_i \right) / 2 \quad i = 1..18$$

where c_i and p_i are the proportions of female and male strobili, respectively, for clone i in the actual year, and BV_i is the breeding value for clone i (according to Griffin (1982) and Stoehr et al. (2004)). Breeding values for a set of Christmas tree traits were used to predict the GW, GW_f and GW_m for single years. Breeding values for budburst were taken from Hansen and Nielsen (2010) based on a full pedigree model. Because half-sib and full-sib breeding values of the clones in FP.620 were almost identical (Hansen and Nielsen 2010), breeding values based on open-pollinated offspring were used to estimate 3-year height (in centimetre) and Christmas tree quality (scoring scale), where the offspring from FP.620 were tested on nine sites, using approximately 40 trees per site in single tree plots (Nielsen and Chastagner 2005). Christmas tree quality was evaluated on a scale from 1 to 9, and the proportion of high-quality trees with a score of 6–9, according to Nielsen et al. (2010), quantifies the number of sold quality trees over a full rotation. Post-harvest needle retention was evaluated according to the methods used by Nielsen and

Chastagner (2005), using the proportion of trees shedding 2% or more of their needles after indoor display for 10 days.

2.8 Realised commercial seed harvest

In each of the years 2005, 2006 and 2008, commercial seed was harvested from all ramets of clones FV1, FV3, FV9 and FV18 (FV3 and FV9 only in 2 years). For each seed lot, the number of filled seeds, weight of 1,000 seeds and number of filled viable seeds per kilogramme were recorded, according to commercial standards.

3 Results

A linear random model analysis with repeated measurements showed significant effects ($p < 0.001$) of clone, year and year-by-clone interactions for female as well as male strobili production (Table 1). Clones accounted for 4–6% of the random variation in strobili production and clonal interaction with year, for approximately 11–19%. Year was the factor with the largest impact on the number of strobili, accounting for approximately 19% and 42% of the random variation for female and male strobili, respectively (Table 1). Analysis of the scored values gave a more consistent result between the two sexes: clones 7%, variation due to year 50%, and 10–12% for clone-by-year interaction (data not shown).

3.1 Year-to-year variation in strobili quantity

A large variation between years in the average number of female and male strobili was observed, ranging from 0 to 188 female strobili and 0 to nearly 7,000 male strobili, on average per ramet (Table 2). During the 18-year period, from 1992 to 2009, all clones had female strobili in 11 years on at least one ramet, and correspondingly all clones had male strobili in 8 years (Table 2). There were 5 years with a superior female strobili production, six intermediate years and 7 years with little production. The five superior years

Table 1 Random variation in female and male strobili production across 18 years for 18 clones: variation in strobili numbers as percentage of total random variance and estimates of heritability

	Female numbers variation	Percent (%)	Male numbers variation	Percent (%)
Clone	1,186	5.7	512,325	4.2
Year	3,822	18.5	4,947,736	40.4
Clone*year	3,899	18.8	1,276,933	10.4
Ramet(clone)	461	2.2	794,354	6.5
Residual	11,343	54.8	4,701,223	38.4
Total	20,710	100.0	12,232,571	100.0
H ² bs		0.06		0.04

Table 2 Yearly variation in female and male strobili counts. For both females and males: overall mean value of all clones, lowest and highest clone mean and percentage of clones with strobili

Year	Clones	Female strobili				Male strobili				Genetic diversity		Clonal heritability		
		Mean	Min	Max	% Clones contributing	Mean	Min	Max	% Clones contributing	Ns	Nev	Female H ² bs	Male H ² bs	Corr f×m
1992	23	102	0	329	100	4,864	295	10,000	100	11	21	0.31	0.29	0.58
1993	23	52	0	240	100	1,013	0	4,498	91	9	15	0.26	0.23	0.70
1994	23	3	0	18	61	138	0	1,958	26	5	6	0.12	0.22	0.57
1995	23	38	3	86	100	937	115	3,172	100	12	24	0.14	0.08	0.61
1996	23	8	0	65	65	84	0	534	35	5	7	0.26	0.08	0.77
1997	23	15	0	104	78	406	0	2,851	43	6	7	0.12	0.14	0.58
1998	23	181	27	425	100	6,839	3,137	10,000	100	13	30	0.29	0.21	0.13
1999	23	0	0	0	4	0	0	0	0			0.01	0.00	
2000	23	188	14	781	100	5,648	1,313	10,000	100	13	29	0.26	0.19	0.25
2001	18	4	0	29	61	130	0	1,517	56	4	4	0.18	0.15	0.57
2002	18	39	7	77	100	2,743	52	7,239	100	11	25	0.14	0.27	-0.07
2003	18	67	0	312	100	988	0	3,008	83	7	11	0.33	0.16	0.49
2004	18	26	0	87	100	967	0	7,500	83	7	11	0.21	0.67	0.74
2005	18	48	4	155	100	1,229	23	5,688	100	7	12	0.35	0.36	0.14
2006	18	127	18	436	100	3,152	241	6,447	100	10	23	0.39	0.20	-0.16
2007	18	5	0	30	72	67	0	855	61	3	4	0.31	0.43	0.51
2008	18	20	0	113	89	186	0	1,476	72	6	9	0.24	0.09	0.60
2009	18	144	32	390	100	5,215	1,068	10,000	100	12	39	0.34	0.16	0.24
	Average	59			85	1,922			75			0.24	0.22	0.43
	Min	0			4	0			0			0.01	0.00	-0.16
	Max	188			100	6,839			100			0.39	0.67	0.77

The genetic diversity is expressed as status number (N_s) and variance effective population size ($N_e^{(v)}$) and N_c is the number of clones. Clonal heritability is shown for female and male strobili numbers within each year and Pearson correlations between clonal male and female strobili numbers within each year (based on clone means)

produced 70% of the aggregated cone crop during the 18-year period, and including the six intermediate years, these 11 years accounted for 95% of the total.

There was a strong correlation between overall male and female flowering across years, $r=0.95$ ($p<0.001$), meaning that years with high female strobili production were in general accompanied by large quantities of male strobili and vice versa. Within each year, Pearson correlations between mean clone male and female strobili numbers varied from -0.16 to 0.77 with an average of 0.43 (Table 2).

3.2 Clone variation in strobili production

On average across the 18 years, 65 female strobili were produced per year per ramet, but this ranged from as low as 22 (clone FV16) to a peak at 168 female strobili (clone FV10), which, when calculated as the relative proportion of total seed crop across years, was equal to

1.9% and 14.3%, respectively (Table 3). Male strobili were more abundant (total average=1,906/year/ramet), but varied less (from 892 to 3,383 strobili). Given as a relative proportion of total male strobili production, the range was from 2.6% (clone FV21) to 9.9% (clone FV15) (Table 3). The overall average clonal contribution of female and male strobili varied substantially, and no significant correlation could be found between female and male strobili production based on 18 clone means across the 18 years, $r=0.07$ ($p=0.793$). The tendency for each clone to be more female than male was estimated as the ratio between relative contributions of female and male strobili (Table 3). The clonal range for this ratio was from 0.33 (predominantly male strobili producing), to 2.83 (largely producing female strobili). However, all clones were capable of producing both male and female strobili, so it appeared to be a matter of sexual skewness. The accumulated sexual asymmetry, $A_s^{(x)}$, for the 18 clones was 0.218, based on the relative contributions.

Table 3 Clone means of female and male strobili for the 18 clones across 18 consecutive years; the relative proportion of the accumulated male and female strobili production and a ratio between female and male proportion describing the sexual asymmetry

Clone	Ramets evaluated No.	Average no. strobili		Proportion of strobili		Ratio of proportion Female/male
		Female No.	Male No.	Female %	Male %	
FV1	8	81	2,403	6.9	7.0	0.99
FV2	20	82	1,760	7.0	5.1	1.37
FV3	20	36	1,549	3.1	4.5	0.68
FV4	27	80	2,101	6.9	6.1	1.12
FV6	3	69	1,859	5.9	5.4	1.09
FV7	2	63	2,450	5.4	7.1	0.76
FV9	3	86	3,368	7.3	9.8	0.75
FV10	21	168	1,738	14.3	5.1	2.83
FV11	21	98	1,546	8.3	4.5	1.85
FV12	23	31	1,108	2.6	3.2	0.82
FV13	18	96	1,214	8.2	3.5	2.31
FV14	13	29	2,120	2.5	6.2	0.41
FV15	12	41	3,383	3.5	9.9	0.35
FV16	10	22	1,984	1.9	5.8	0.33
FV17	15	39	1,409	3.3	4.1	0.80
FV18	17	42	1,496	3.6	4.4	0.83
FV20	13	68	1,922	5.8	5.6	1.03
FV21	14	39	892	3.3	2.6	1.28
Mean	14.4	65	1,906			

3.3 Correlation among years

The vast majority of year-to-year correlations within sexes for both female and male strobili production were positive. The average Spearman rank correlation across years was 0.35 for female strobili and 0.41 for male strobili, based on a yearly clone mean of strobili production in actual numbers (data not shown). The average correlation between two consecutive years was very similar to the overall correlation between years, indicating a rather uniform correlation pattern across years for both sexes.

3.4 Broad-sense heritability of strobili quantities

Average female and male strobili production had a heritability of 0.24 and 0.22, respectively, but both showed large year-to-year variations: 0.01 to 0.39 and 0.00 to 0.67, respectively (Table 2). Analysis based on scored values (approximate logarithmic transformation) revealed heritabilities that were almost doubled: 0.40 and 0.33, respectively. Part of the variation in heritability for female strobili production was related to crop size; the more strobili, the higher the estimates of heritability ($r=0.55$, $p=0.018$). For male strobili production, no such correlation

was seen ($p=0.252$). Heritability for accumulated strobili production across 18 years for the 18 clones was 0.55 for female and 0.32 for male. However, the large impact of yearly variation, and the interaction of clone and year, gave a rather low estimate of broad-sense heritability for the repeated measurement analyses: 0.04 and 0.06 for female and male strobili counts, respectively (Table 1).

3.5 Cumulative gametic contribution

The percentage of clones producing 80% of the cumulative yearly gametic contribution was calculated for female and male strobili for the 18 years of observations. Large differences between years were observed. The percentage of clones that produced 80% of the gametes was as low as 10% for female strobili, and 20% for male strobili. Yearly results were plotted against the overall average cone production per ramet, used as a crop index indicating the size of the potential harvest, and two thirds of the total variation could be described as a logarithmic relationship with the crop index for both sexes (Fig. 1). Except for the seven poorest years, the clones which accounted for a cumulative female strobili production of 80% ranged from 35% to 59%, and for male strobili from 30% to 68%.

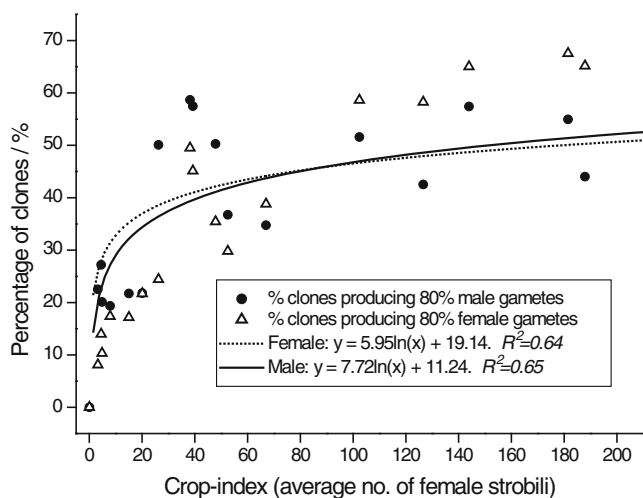


Fig. 1 Percentage of the clones producing 80% of the cumulative gametic contribution: female and male strobili, respectively. For each year, the results are plotted against the average female strobili production used as a crop index. Based on Lorenz curves for each year according to Griffin (1982)

3.6 Variation in genetic worth of CSO across years

For a set of four different characters included in the Danish Christmas tree breeding programme, the yearly genetic worth (GW), as well as the female and male GW respectively, was estimated, depending on which clones contributed the most gametes to the seed crop (Fig. 2). For height, the GW varied from -0.8 to $+1.8$ cm across years, or by -1.9% to 4.5% (average height = 40.7 cm) (Fig. 2a). Average score of budburst ranged from -0.21 to 0.63 , corresponding to a change in the average day of budburst from 1.5 days earlier to 4.5 days later than average budburst, assuming an equal contribution (a difference in one scale unit normally corresponds to 1 week, unpublished data) (Fig. 2b). Post-harvest quality was measured as the percentage of trees within each progeny losing more than 2% of the needles when allowed to dry under indoor conditions. The GW deviated from -3% units to nearly $+4\%$ units, from the overall average level of 9% trees in which

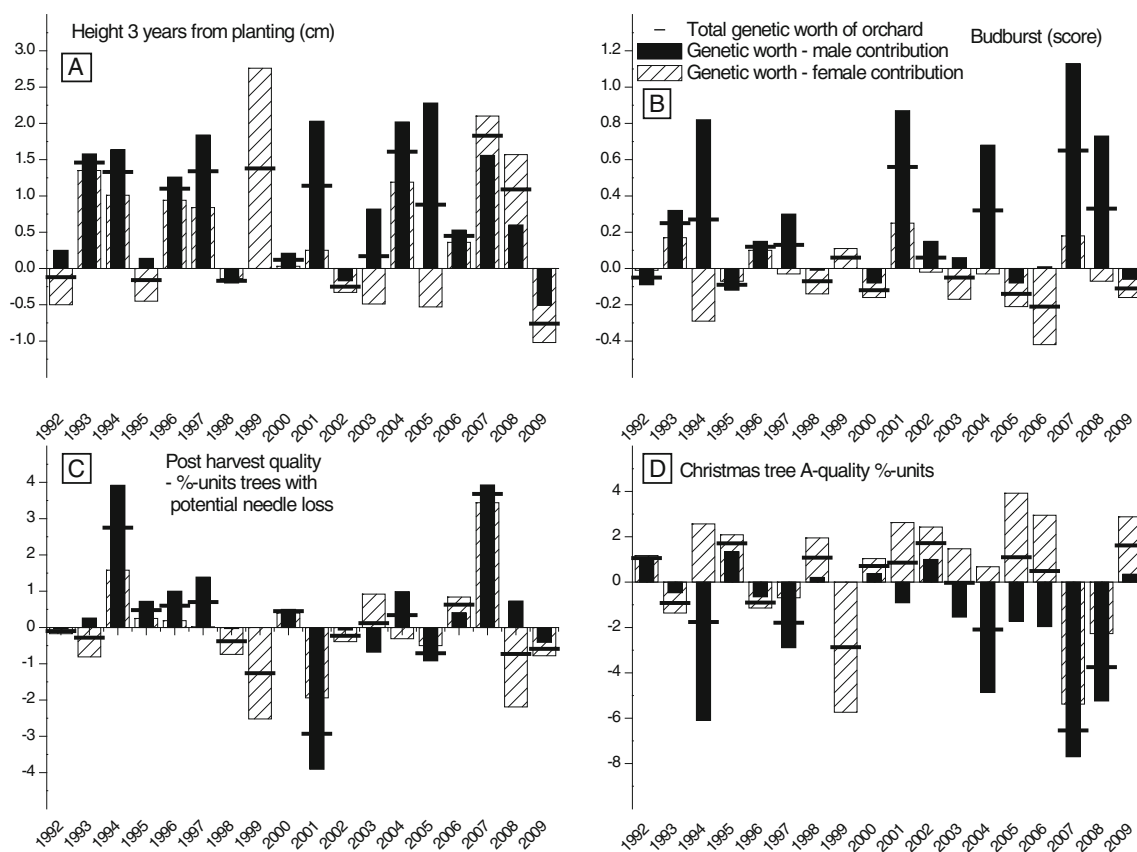


Fig. 2 Genetic worth (GW) of the seed orchard estimated for the years 1992 to 2009, based on the variation in gamete contributions (18 clones), presented as deviations from the genetic worth with an even gametic contribution to the orchard progeny. GW is the average genetic worth of the orchard (bold line), calculated as the average of

the genetic worth of the female gametic contribution (GW_f —light bars), and the genetic worth of the male gametic contribution (GW_m —dark bars). **a** height 3 years from planting (in centimetres); **b** budburst (score); **c** post-harvest quality (percent units); and **d** saleable high-quality Christmas trees (percent units)

needle loss was observed (Fig. 2c). The number of saleable premium quality Christmas trees ranged from -6.4 to +1.7% units, expecting a clonal mean average of 45% (Fig. 2d). Depending on year and trait, rather large differences between female and male GW were observed, although in most cases, female and male GW deviated in the same direction.

3.7 Genetic diversity of the seed crops

Across years, corresponding results were observed, $r=0.95$, ($p<0.001$) for the status number (N_s) and variance effective population number ($N_e^{(v)}$) (Table 2). Estimates for N_s and $N_e^{(v)}$ across years were very variable, reflecting the large yearly variation in strobili production, and both showed a nearly logarithmic relationship to crop index, but for some years, rather large deviations from the general pattern were seen (Fig. 3).

The variation in GW, used as a proxy the genetic drift for the four traits of interest, was compared to the variance effective population size (Fig. 4). For height and budburst, there was a clear tendency for low variance effective numbers to be associated with larger deviations in GW than large diversity numbers. No such relationship was seen for Christmas tree production or needle loss. Visually, only numbers below 10 for variance effective population size appeared to be related to deviations of GW of over 5%.

3.8 Cone counts and seed yield

A linear relationship between female strobili production and the realised yield of filled seed was established (Fig. 5). Average clonal female strobili counts, multiplied

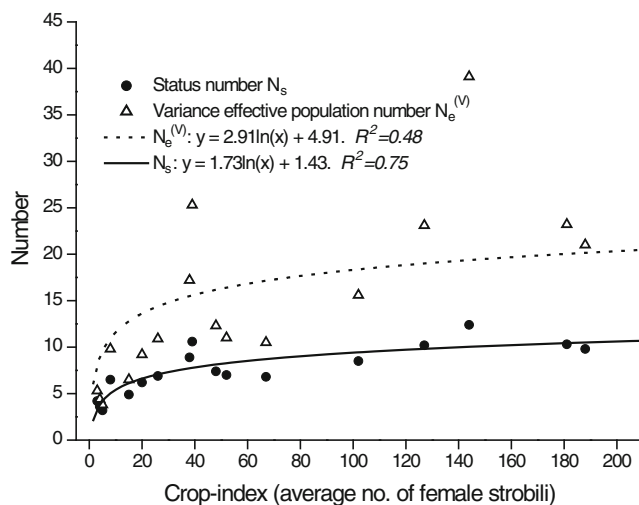


Fig. 3 Genetic diversity measured as the variance effective population number for an infinite offspring population ($N_e^{(v)}$) and status number (N_s). Each year, the diversity measures are plotted against average female strobili number per ramet, indicating poor or good crop years (used as a crop index)

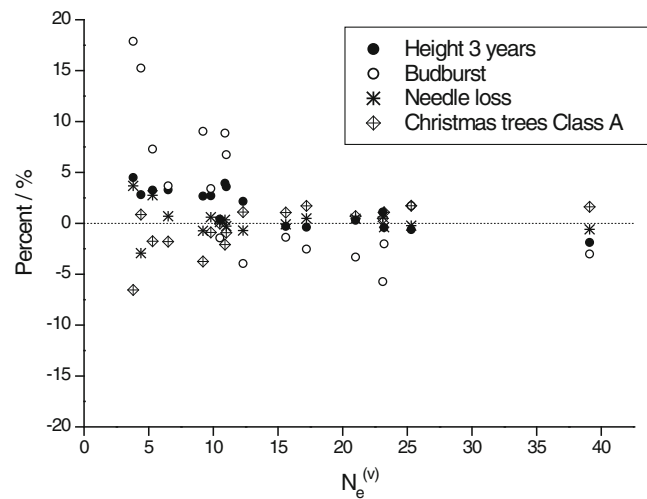


Fig. 4 Yearly estimates of the orchard genetic worth for four traits shown as a response of the variance effective population number ($N_e^{(v)}$). Results for 18 clones are shown for all 18 years. Shown are height and budburst percent deviation from the mean, and needle loss and saleable high-quality Christmas trees in percent unit deviation from the mean

by number of ramets, could account for 67% ($r=0.82$) of the variation in filled viable seeds (Fig. 5). Clonal germination rates were only available for 1 year, and ranged from 78% to 85%.

4 Discussion

The Nordmann fir, which is an exotic species in Denmark, has been shown to be a suitable species for evaluating the population genetics of seed orchards. Based on the same seed orchard as in our study, Hansen and Nielsen (2010)

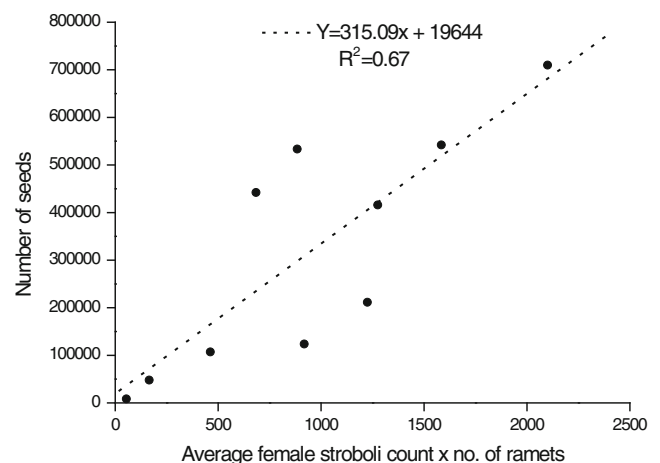


Fig. 5 Female strobili count used as a predictor of realised filled seed yield, based on data from a subset of four clones in the years 2005, 2006 and 2008

demonstrated, by use of microsatellite markers, that visual evaluation of male strobili numbers had a good correlation ($r=0.87$) to the resulting paternity—based on the seed year 1992—and also that the assumption of true half-sibs after open pollination is correct. Furthermore, studies show that the selfing rate is low, and pollen contamination from outside sources is of either no or minor importance in Danish Nordmann fir orchards (Hansen and Kjær 2006; Hansen and McKinney 2010; Hansen and Nielsen 2010). Moreover, female strobili are easy to identify visually, and are situated upright in the upper part of the crown. Although only based on a subsample of clones and years, the female strobili count in our study did correlate well with the number of filled seed, $r=0.82$. Kjær and Wellendorf (1997) used exactly the same scoring method, and found a reasonably good correlation between female strobili scorings and seed yield, $r=0.72$ in *Picea abies*, and a similar correlation of $r=0.61$ in *Picea sitchensis*, with no change when correlated with actual germinated seed (Kjær et al. 1995). Based on data for Douglas-fir from El-Kassaby and Thomson (1996), correlation between cone counts and actual number of emergents (i.e. germinated seeds) was $r=0.67$ and $r=0.62$ for filled seeds. Nielsen and Hansen (2010) found, in Nordmann fir, an average germination rate of 94% for filled seed, for outcrosses as well as selfings, which further indicates that filled seed is a reliable measure of gametic contribution when cones and seed are handled properly. Some of the possible year-to-year variations in cone size and general pollination success due to fluctuating climate conditions, as well as potential pest problems, may alter the reliability of female strobili scores as an estimate of seed produced per year. However, it is unlikely that the main results of our study—the relative proportions of seed and pollination success of the different clones in specific years—would be seriously impacted due to environmental issues. Furthermore, there were apparently no differences in timing between the clones (data not shown). Verified by DNA methods, the robustness and reliability of phenotypic strobili assessment methods as a proxy of parentage success have been demonstrated for three conifer species (Funda et al. 2011). Although there were some uncertainties, the strobili counts seemed to be a reliable proxy of the relative gametic contribution for both male and female.

4.1 The time series

The orchard was grafted in 1966–1968 using scion material already capable of flowering. The actual time series evaluated comprised the years 1992–2009, thereby starting 24–26 years after grafting. The obtained results therefore purely relate to mature seed orchards, following the definition of Kroon et al. (2009), which used a threshold of 20 years for maturity.

4.2 Clone and year variation

An overall analysis of variance, based on scored values of strobili production, revealed a very high influence of year as a factor, approximating 50% of the variation in strobili production, and the interaction between clone and year, approximating about 10–12%, leaving 7% of the total variation being due to clones.

Large clonal differences in female and male strobili production were seen within the single years, and for accumulated production across years, as observed in earlier studies for a variety of species and orchards (Nikkanen and Ruotsalainen 2000; Kroon et al. 2009). A phenotypic correlation between clonal means of accumulated female and male strobili production was absent, leaving room for some sexual asymmetry, as found for pines (Kang and Lindgren 1998), but in contradiction to findings for *Picea abies* (Kjær 1996; Kjær and Wellendorf 1997). Choi et al. (2004) estimated the sexual asymmetry for *Pinus densiflora* across 8 years to be 0.176, a little lower than the 0.218 we found. In our study, broad-sense heritability for male and female strobili production averaged 0.22 and 0.24, respectively, based on actual numbers. Based on scores, the heritability was 0.40, and of the same magnitude as the 0.40 found by Kroon et al. (2009) for a mature Scots pine orchard. A fairly similar heritability estimate of around 0.55 for accumulated strobili production was seen in both studies. Analogous heritability for single years was found by Nikkanen and Ruotsalainen (2000) for female as well as male strobili production (0.37–0.38). Spearman rank correlations between years were predominantly positive, and we did not see any differences that supported an alternate nature of the clone strobili production, as reported in some studies (see e.g. Nikkanen and Ruotsalainen 2000). Both our correlation study and the analyses of the 17 years (1 year omitted due to a lack of flowering) of longitudinal data in a repeated measurements model seemed to confirm a rather uniform correlation between years. In a comparable study of cone production in *Pinus sylvestris*, Kroon et al. (2009) found a stronger correlation between clone seed production years with a short time distance, and correspondingly a best fit for an autoregressive model describing a higher autocorrelation between years close in time.

4.3 Accumulated cone crop

Ninety-two percent of the accumulated cone crop was produced in 10 out of 18 years (~56%), which was less than that reported by Choi et al. (2004), who found that 6 of 8 years (~75%) produced 90% of the cone crop in *P. densiflora*. Our findings were very similar to the results from orchard studies including early harvest years: Kroon et al. (2009) found, based on years with seed, that 10 of the 15

seed harvest years (~67%) produced 90% of the seed crop in *P. sylvestris*, but when the years with no seed were included, this implied that only 10 out of 30 years (~33%) gave 90% of the total cone crop. Nikkanen and Ruotsalainen (2000) found that 6 out of 13 years (~46%) produced 93% of the cone crop in *P. abies*, and Kjær and Barner (1998) showed that 7 of 14 years (~50%) produced 92% of the seed in *P. sylvestris*.

4.4 Genetic diversity

This approximate 50/90 relationship between seed years and accumulated cone crop is noteworthy when evaluating the genetic diversity measures. Our study demonstrated a rather strong relationship between a crop index based on average female strobili production, the genetic diversity measured as status number and variance effective number based on estimates of female as well as male strobili contributions. These findings were comparable with Nikkanen and Ruotsalainen (2000), who found that the more abundant the flowering, the higher the genetic diversity. Kjær and Wellendorf (1998) came to a similar conclusion based on several different seed orchards. In the case of Nordmann fir, there was a nearly logarithmic relationship between diversity measures and cone crop, strongly indicating that low diversity measures are related to small crop years. The often cited rule of thumb—the “20/80” rule (Anonymous 1976)—stating that 20% of the clones constitute 80% of the gametic contribution, holds only for poor seed years.

Although genetic diversity is not particularly important in Christmas tree orchards (Nielsen and Hansen 2010), the isolated and almost non-contaminated Nordmann fir orchards are good predictors for the influence of variability in flowering on genetic diversity: this is knowledge which could be useful for other conifer species, where the balance between genetic gain and diversity is more critical.

4.5 Genetic worth of the seed orchard

As already stated, strobili counts appear to be a reliable proxy for the relative gametic contribution, and the large variation in annual and clonal strobili production can potentially cause large variation in the genetic worth of the orchard. Each individual year is, to a large extent, unique in its gametic composition, due to the large variation in strobili production and, as stated by Funda et al. (2009), crops should be treated on a yearly basis. Griffin (1982) estimated the genetic gain (= genetic worth) for an orchard by summing of clone estimates of gametic contribution multiplied by breeding values of the same clones. Recently, Funda et al. (2009) found a fairly small variation in the GW of the orchard over 3 years when harvesting at a level of

100% in western larch (*Larix occidentalis*); other studies only estimate the impact of uneven flowering in single years (e.g. Askew 1988; Lindgren et al. 2004). Stoeckl et al. (2004) describe a basic protocol based on similar principles for estimating the GW and diversity, but do not report on actual data. For the majority of years, rather small differences in GW of the orchard were seen for the chosen four characteristics of interest for Christmas tree production. The orchard produces a reliable and, in general, reproducible product across years, despite the rather large variations in strobili production. For the number of saleable high-quality Christmas trees, the superior and medium-sized crop years deviated with a maximum of 2% units. Our estimates across years were based on equal number of ramets per clone but, if the technical imbalances in seed orchards are included, e.g. different numbers of ramets due to variation in grafting success and deployment of clones, larger and more significant differences in GW may occur, but can also, on some occasions, even out deviations. The smaller the census number, the larger will the expected impact of single deviating clones be.

We did not include the impact of selfing in our study, but it is expected to be low. Hansen and Kjær (2006) found a selfing rate of 6.0% in a Nordmann fir clonal seed orchard for $N_s=4.2$ and a census number of 13, but selfing was fairly unevenly distributed among clones. In our study, N_s was estimated to be 11 in 1992 (Table 2), and a selfing rate of 2%, based on seedlings from the same seed lot, was estimated in an earlier study (Nielsen and Hansen 2010). Furthermore, the tendency to sexual skewness among clones might lower the selfing problem.

4.6 Genetic worth and diversity—management tools

We considered the relationship between genetic diversity, expressed as the variance effective population number ($N_e^{(v)}$), and the variation in the GW of the seed crops, the latter expressed as yearly deviations across the whole period. It was found that nearly all “outliers” with large deviations in the orchards GW (Fig. 4), related to the five to seven poorest seed years, with little flowering and low genetic diversity. Furthermore, these deviations only related to 5% of the accumulated cone crop across years. Commercially, these years of low seed production are of minor interest, because of the expected large harvesting costs for a small outcome. For a steady seed supply to the industry, effective seed storage might therefore be a better option than using fresh seed from small crop years, which in any case might be insufficient in quantity.

Evaluating the impact of N_s and $N_e^{(v)}$ by simple averaging across years overestimates the actual impact on the diversity of the established forest or Christmas tree stands, because the seed amount related to these diverging seed years is rather sparse. Planning the number of clones

in new orchards, or thinning existing ones, with the intention of securing a particular minimum level of diversity in all years (measured with e.g. N_s) might be associated with a penalty in gain. Theoretically, one could take into account the possibility of avoiding harvesting seeds in years of low seed production. Higher gains could thereby be achieved in the major crop years, where the diversity criteria are far more easily met, and therefore a stronger clone selection can be applied.

In the case of our study, where visual evaluation appeared to give a quick and simple pre-harvest estimate of the GW, diversity and potential crop size, a yearly evaluation protocol for the orchard could be interesting as a management tool. Visual evaluation methods have earlier been suggested by Kjær and Wellendorf (1998) and Nikkanen and Ruotsalainen (2000), as well as by Stoeckl et al. (2004). Although costly, marker-based parentage analysis (El-Kassaby et al. 2010; Hansen and McKinney 2010; Hansen and Nielsen 2010; Funda et al. 2011) would be a relevant tool for seed crops with a high expected value, e.g. due to a large quantity of seed or high economic value. Thereby, one would be able to label the seed with actual information of worth and diversity for the growers of the crop. Such a procedure has been developed for hybrid larch (Achere et al. 2004) and is used for labelling the actual hybrid percentage in seed orchard crops. Christmas trees are a high-value crop and the economic return is very sensitive to the number of saleable trees (Nielsen et al. 2010). Seed years, with a GW of 5% units of lower saleable Christmas trees than average, will cost the Christmas tree grower approximately 300 Euros in lost yearly income per hectare (Nielsen and Hansen, in preparation).

5 Conclusion

Compared to marker-assisted studies, the main advantage of this time series of visual strobili counts is the long time span it covers. The series showed that the constituents of each seed crop are unique, and that there is an annual variation in clonal strobili production, as well as in the general quantity of strobili.

Despite this variation, the orchard's genetic worth was shown to be rather uniform for the four evaluated traits in the 11 superior or medium crop size years, and these years accounted for 95% of the accumulated seed crop over the whole period. Noteworthy deviations were only seen in the poorest seed years, where genetic diversity measures were also low. Avoiding small crop years based on a yearly visual evaluation of strobili production in the orchard appears to be a reasonable tool to avoid fluctuations in the commercial seed orchard product, and could be assisted by DNA marker-based parentage analysis for labelling high-value seed crops.

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