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Relative Female Reproductive Output of Grafted Scots Pine (Pinus sylvestris) Clones Planted on Different Places

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Content

Abstract

A necessary decision when a clonal seed orchard is established is the choice of the clones. The goal of a seed orchard is to produce many seeds of a high genetic quality. Almost all earlier studies have focused on selecting genotypes with good genetic constitution, and little attention has been focused on the options to affect cone yield by clone selection. This study investigates if the cone production of clones can be predicted by the cone set of the selected plus tree or the performance of its ramets in a clone bank.
Two main sources of data for predicting female fertility under seed orchard conditions are used – cone set in a clone bank at Sävar comprising most early plus tree selections in northern Sweden, 748 clones with 4 ramets per clone; and verbal evaluation of cone set from 69 plus tree. These data were compared with female fertility of the same clones growing as grafts on seven locations (seed orchards or clonal tests) No significant relationship was found. This strongly indicates that it is almost impossible to make meaningful predictions of future cone production in seed orchards from cone set information from plus trees or young clone banks. In order to utilize fully the collected data some more calculations and comparisons were made. They show that the clones planted in the clone bank at Sävar behave different due to their origin. The clones with more northern origin grow slower than the other ones and even with the same diameter they produce fewer cones. No relation was found between the origin of the clones and their survival ability.

Key words: clone female fertility, clone origin, ramet survival, *Pinus sylvestris.*

Introduction

Scots pine (Pinus sylvestris)

This coniferous evergreen tree has native area in almost whole Europe - from Great Britain trough Lapland up to eastern Siberia on north and from Spain to Caucasus Mountains on south. This species has also been widely planted in New Zealand and much of the colder regions of North America.

Scots pine is admirable with its large tolerance of soil types, precipitation requirements, climates variety and altitude occurrence. We can find it on the extreme soil basis as most ancient rocks and also on the most recent glacial deposits. Pine of course grows also on much better soils but because of its light demand it is mostly pushed out by other tree species.

It grows in areas with an annual precipitation exceeding 1780 mm and in areas with an annual precipitation as little as 200 mm. Scotch pine survives in the Verkhoyansk Mountains of eastern Siberia where winter temperatures have been recorded as low as -64° C. In some areas it grows where the subsoil is permanently frozen. Scots pine can also survive high temperatures, and it is found at middle altitudes in the Mediterranean region. In the north of its range, it occurs from see level up to 1000m, while in the south it reaches 2500m altitude (Darroll D. Skilling).

Using of the pine wood takes place mainly as a timber, in furniture production and paper production.

Sweden and its forests

Forest utilization

The Forests in Sweden have been utilized since the inland ice melted, mainly from hunters and later from farmers. The wood played very important role as firewood and timber for domestic use. Big quantities of wood were used up to build houses, for heating and cooking. It was a wood culture, which used many forest products. It is worth mentioning that the Swedish peasant farmer never took care of his forests, which had to withstand many attacks during the 18th and 19th centuries. In many parts the forests were rooted out, as poverty stricken peasant scoured the district in search of something to burn. By cultivating, cutting down trees for their own use, burn-beating and making charcoal, potash and saltpeter they almost brought the forest to its knees in the middle and southern parts of the country.

After years of political pressure the first real silvicultural law was created in 1903. Its requirements of economy and regeneration guided Swedish forestry into a reproductive cycle, which together with the import of fertilizer for agriculture and industrialization, has led to forestry's present prosperous state. The forests now show figures for growth which were not ever before expected (Leif Wastenson et al., 1990).

Scots pine - present numbers

Scots pine together with Norway spruce is the most common tree species in Sweden and it occurs all over the country. Its standing volume measured at 1998-2002 was 1195 million cubic meters. First data about the forest stock in Sweden were collected in 1920 and since that time standing volume increases (Figure 1). Most of the trees are on forest land – 1111 million cubic meters. Trough to regularly and increasing harvesting of pine the stock doesn't slacken, because the pine is growing faster due to silviculture and modern forestry treatments. Annual gross felling attains to more than 80 millions cubic meters per year and didn't decrease below 45 millions m^3 per year since 1955 (Figure 2). Of course big amount of reproductive material is necessary to keep present forest stock and even to continue with increasing trend. Needs of seedlings are shown in table 1.

Figure 1. Trend for total standing volume since 1920 in Sweden.

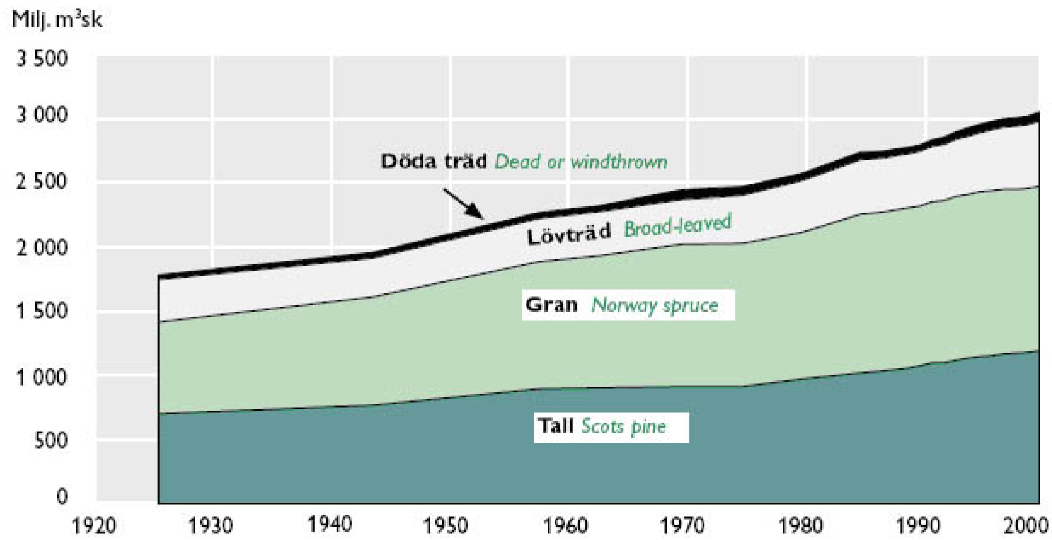


Figure 2. Annual gross fellings calculated by National Board of Forestry

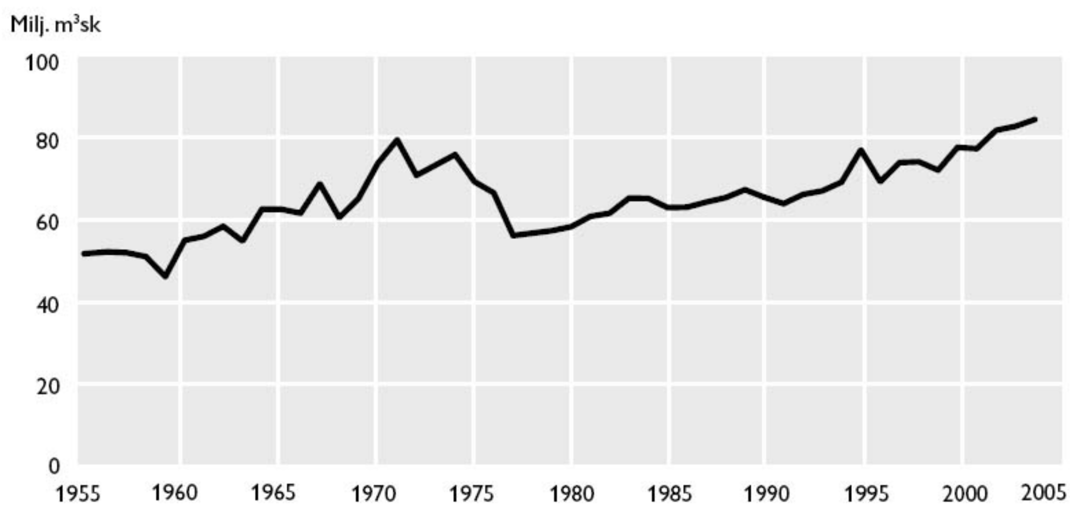


Table 1. Scots pine seedlings used in Sweden since 1998 to 2004

	mil. of plants
1998	139
1999	124
2000	125
2001	124
2002	115
2003	119
2004	126

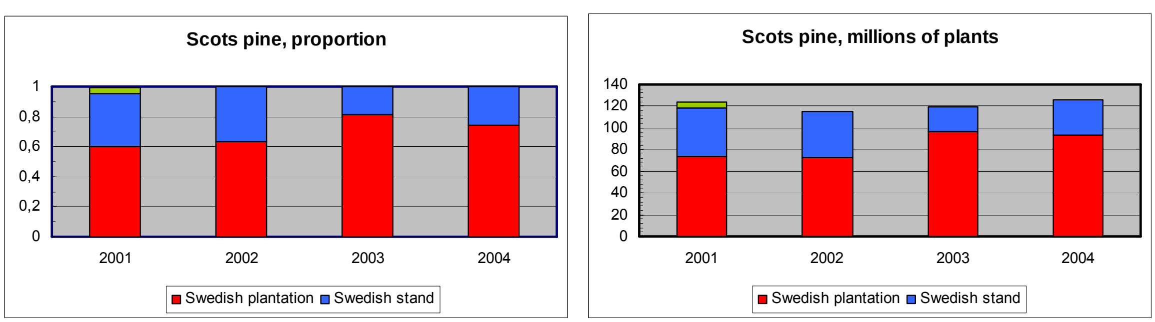
The high seedlings demand is usually covered by seeds with better genetic gain and better quality produced in natural or managed plantations – seed orchards.

The figures above (figure 1, 2 and table 1) were taken from Swedish Statistical Yearbook of Forestry 2005 - “Skogsstatistisk årsbok 2005”.

The thickest Swedish pine has a girth of 4.49 m and is growing at Strängsered in Ulricehamn. The oldest Swedish pine tree is growing in Muddus National Park. It is at least 711 years old. Researchers have found that the pine has survived forest fires in the years of 1413, 1507, 1596 and 1771. The oldest tree harvested in Sweden was 654 years old when it was felled in 1913 at Svärdsjö, Dalarna.

In comparing with Norway spruce demand for Scots pine seeds and seedlings in Sweden is covered mainly by domestic production and most of the reproductive material originates from plantation (seed orchards). It is obvious that seed orchards in Sweden are the most important source of reproductive material and of course for each country is economically and in protective point of view preferable not to import seeds from abroad. It means that scientific and economic efforts in developing and improving of the seed orchards are justified by the aim – to produce sufficient amount of improved seeds. This work is part of those efforts and it should help in the choice of ortets for the future clones in the seed orchards.

Figure 3, 4. Number of seedlings for use in Sweden by method of production and origin 2004.



Source: National Board of Forestry

Seed orchards

Needs of reforestation are actual all over the world mainly because there are no places bend out of large harvest programs or because of the catastrophic natural events as wind, wildlife, insects or diseases. The natural regeneration is not enough and there are other seed sources for forest regeneration as:

– Natural stands as good stands, seed production areas (seed stands), proven seed sources (Zobel and Talbert, 1984) or sometimes just from individually good phenotypes.

- Seed orchards - serve first of all as alternative way of reforestation than cloning is. The most important impact of seed orchard's crop is the fast growing and more productive forests established by them.

Since the establishing to producing sufficient seed crop in seed orchard several years are needed and that's why natural stands can be regarded as source for immediate seed needs.

Seed orchard is defined as a plantation of genetically superior trees isolated to reduce pollination from genetically inferior ones, and intensively managed to produce frequent, abundant, and easily harvested seeds.

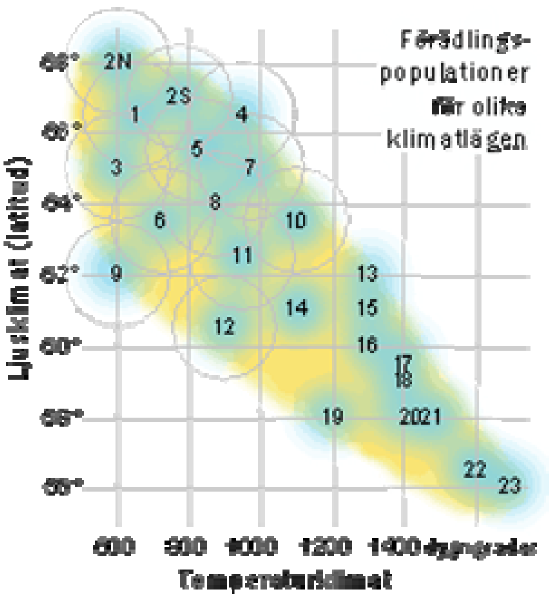
Usually seed orchards fulfill long term needs of reproductive material. There are two main types of seed orchards regarding to the way of establishing:

1. Seedlings seed orchards, which have been grown from seeds collected form plus trees. As advantages could be said that costs for establishing such a plantation are lower as the time to do it. In genetic point of view these orchards have large genetic base.
2. Clonal seed orchards, which have been grown either from grafts or from rooted cuttings. In graft propagation some incompatibilities could occur and consequently those trees die. Indeed grafts have the same physiological age as their parents (ortets) which allows flowering earlier (Kyu-Suk Kang, 2001).

A lot of work has been done in research of clonal propagation techniques and it still goes on – tissue culture, somatic embryogenesis and artificial seeds are under investigation, so in the future the role of the seed orchards may drastically change.

An important proposition is to locate the seed orchard in place where pollen contamination is minimized (i.e. enclosed with tree species which does not intercross with the species in the orchard). Indeed pine and other conifer species need long isolation distance, because the wind could transmit pollen for tens (even more) kilometers. Spacing, slope of terrain and placing of the clones (or seedlings) take an important place at establishing too (Faulkner, R. 1975).

Figure 5. The Swedish Scots pine breeding population is divided in 24 separate subpopulations with separate targets expressed described by latitude and temperature



In Sweden there are 24 Scots pine breeding subpopulations with different targets. A seed orchard has a specific target area and draws genotypes from several breeding populations. Thus breeding population targets can be visualized as slightly overlapping. For every one of them a seed orchard is created for regeneration uses. The trees in the orchards usually originate from more northern localities (this is valid only for Scots pine – the opposite is true for Norway spruce) and sometimes they are tested by their progeny before they are planted. Only 35% of the harvested trees each year come from "natural" regeneration (is not really natural, because the soil is scarified and only selected trees supply with seeds) and 80% of planted Scots pine trees are originate seed orchards.

Seed production in seed orchards

The modern history of seed production in seed orchards more or less starts with the Swedish Scots pine seed orchards. In Sweden the igniting event took place in 1936 when it was proven that an aspen with giant leaves was triploid. This was an evident and striking proof that genetics had the potential to increase tree growth. That inspired the setting up of a tree breeding institute at Ekebo 1938 and other similar activities. There were some early efforts and disorganized thoughts before the second word war in other countries but they were mainly crushed in the war, while Sweden did not take part in the war and kept its tree-breeding organization intact. After the war Europe should be rebuild and that meant that much money became available for activities improving the performance of Swedish forest. The thoughts about seed orchards and a way to get sufficient amounts of easily available genetically improved seeds were developed and presented to an international forum in the first forest genetic textbook by Bertil Lindquist (1946). It was translated to many languages (including Czech) and used as a template and inspiration for forest tree breeding in many programs including that in SE US (Zobel 1984).

The first real seed orchard in Sweden was established 1947 in Värmland. Since that time up to the mid-1970s, 574 ha of Scots pine seed orchards were established in Sweden. Presently the need of pine seeds is approximately 1,3tonnes (1,5 tonnes for direct seeding) (Redgörelse nr 1, 2000). Production of big amount of seeds is not the only aim of seed orchard but genetic quality of seed crop takes an important place too.

Clonal bank (clonal archive)

A clonal archive (or clone bank) is a plantation with selected trees which are used to conserve certain genetic characteristics, as ortets for producing clones and as ground for experiments and surveys. Similar like in the seed orchards as initiatory material can be used seedlings or clones, but second group is preferable.

Clone banks are constantly evolving. New selections are always being made in progeny and clonal tests for further breeding work prior to their being preserved in clone banks. As new and superior selections are made and placed in clone banks, the decision may be made to remove related but genetically inferior clones (www.forestresearch.gov.uk).

Prediction of cone set

To fulfill still expanding needs of wood products all over the world, the foresters and forest scientists have to develop different ways how to offer enough timber to the market and simultaneously how to maintain the forests for the next generations. One of the manners is to regenerate forests with high quality seeds and to increase wood production in this way. To ensure better cone yield need of prediction becomes very important. Before the seed orchard is established the main assumption is that we can influence cone production by convenient choice of used trees. Logic says that if a clone or plus tree has got extraordinary cone set; their ramets or seedlings will produce more cones too. That is the way in which it has worked in practice and there are no studies denying or confirming this seemingly obvious theory.

Only very few, if any, investigations were done to find how close relationship exists between female fertility of same clones growing on different places. This work compares cone production of two main tree sources - plus trees (ortets) as first and clones in a clone bank as second base for comparison with seven other plantations, where the same clones were planted. The aim is to find out if prediction of cone set is possible, when we look at the cone set of the identical clone growing somewhere else. One of the most important things when a seed orchard is established is to have idea how big cone yield can be expected.

But in the sphere of seed orchards many surveys were already done. The main interest is aimed on genetic gain, gene diversity, seed yield, breeding value and fertility variation in the seed orchards. Naturally all this researches are related with the purpose of every seed orchard – producing more seeds in better quality. Sometimes breeding value is more important than the amount of seeds, but when there is a big actual demand of reproductive material the opposite situation becomes true.

The genetic worth of seeds is a function of the breeding values of the parent genotypes, the distribution of maternal and parental gamets produced by the orchard trees and the amount of pollen contamination from outside the orchard (Stoehr et al. 2004).
Genetic improvement can be defined as a process whereby genetic value is improved while joint consideration is given to the gene diversity of deployed material (Rosvall 1999).

Variation in female fertility

Fertility is defined broadly as the ability of an individual to produce successful gamets, living offspring (Bila et al. 2001). As the data about the cone set used in this work were collected in different (mostly single) years and tree ages, variation among the years can strongly influence the results.
Flowering varies among the stands, individuals and among the years. This variation is bigger in the natural forest stands than in the managed forests. Anyway smaller doesn't mean unessential – an average coefficient of variation equal 101% (and median CV = 89%) among the clones was found in survey inclusive 30 conifers seed orchards (Bila et al. 2001). In many other studies the large female (and male) variation among the clones was confirmed. In two articles from the same authors (Kang, K.S. and Lindgren, D. 1998,1999) the variation in female fertility for 339 clones growing in three plantations (two seed orchards and one clone bank) was calculated for threes pine species as follows: CV(%) = 94, CV(%) = 36, CV(%) = 113. Some more calculations were made by Finnvid Prescher, which are still not published. For eleven plantations the average CV was calculated – CV among the clones was 45% (varied between 12% and 69%) and within the clones 52% (varied between 13% and 84%).
For more reliable results and to minimalize the effect of the fertility variation among the years information about the fertility from more years is required. Purportedly steadier cone production capacity of the clones was found by Ulfstand Wennstrom when data from several years were included.

Material and Methods

Used materials

The study uses overall one big source of data and eight another about Scots pine (*Pinus sylvestris*) grafts in a number of objects mentioned below (Table 2). The main object is a plantation in Sävar serves as clonal bank. Data about the breast height diameter, cone set and grafts survival were available there. The second source of consequence giving information about cone set isn't a plantation while plus trees spread in north Sweden – in all counties included in this study. The clonal bank cone set and cone class from the plus trees are set against cone set from the other localities – seven more plantations. Cone set have been compiled for a number of objects where different subsets of these clones have been inventoried and where it is possible to make a comparison with the inventory in the clonal archive. Cone set was inventoried in a clonal archive at Sävar with the purpose of avoiding selecting bad cone producers to seed orchards.

Some more analysis were done from the Sävar data set:

1) Survival rate and the average ramet diameters are compared for all counties (BD, AC, Z, Y).
2) Relationship between the diameter and the cone set is shown.
3) Female fertility (the cone set) is compared between the counties.
4) Analysis of clone variance is done.

Methods

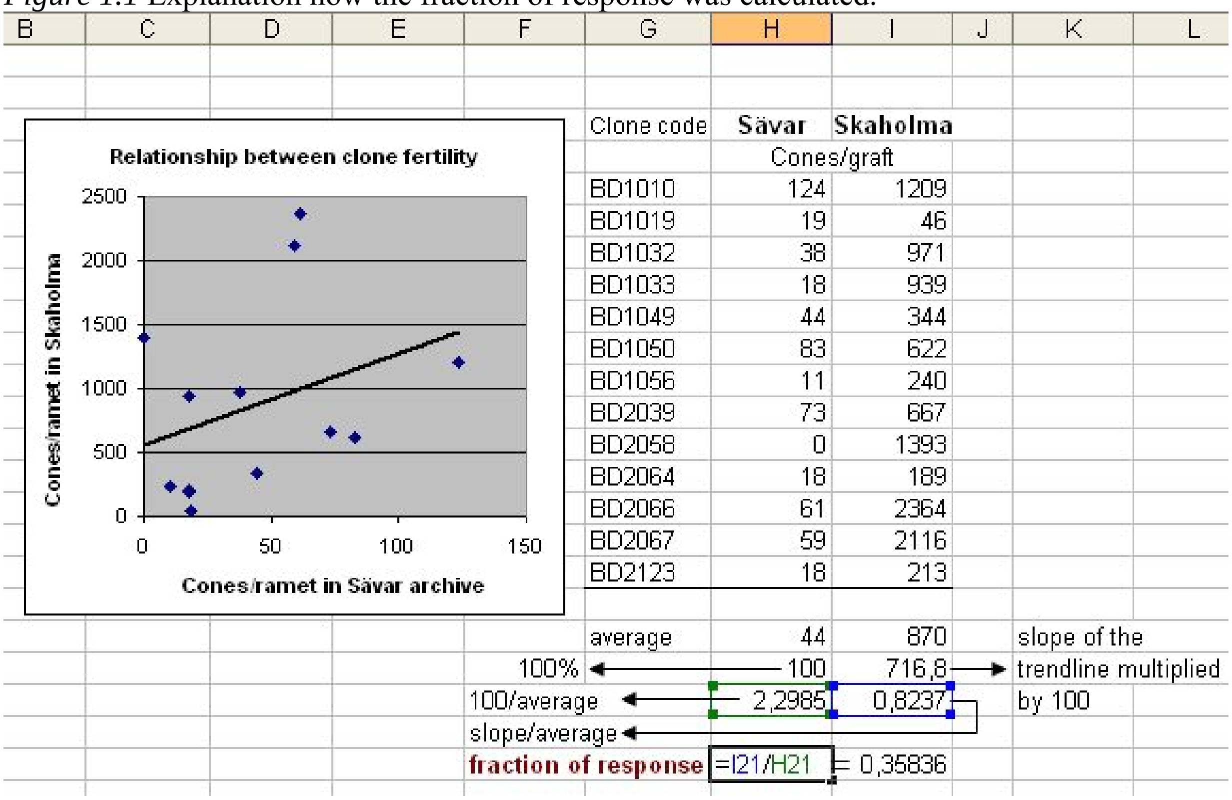
The main used method is regression function, looking for relationship between cones, seeds and cones or relative number of cones and real number of cones.

To obtain an expression indicating the potential gain in seed set, which can expected from a selection among the observed clones, a fraction of response was calculated (Equation 1). That's the value in percentage, which shows how much more cones we can obtain in average from the clones planted in some location, if we look at those clones in the initial location.

Equation 1. Fraction of response

$$\text{Fraction of response} = \frac{\dfrac{\text{The slope of the regression line in percentage}}{\text{Average number of cones per each locality}}}{\dfrac{100}{\text{Average number of cones per clone in Sävar (or average cone class for the plus trees)}}}$$

Figure 1.1 Explanation how the fraction of response was calculated.



Clone code	Sävar	Skaholma
	Cones/graft	
BD1010	124	1209
BD1019	19	46
BD1032	38	971
BD1033	18	939
BD1049	44	344
BD1050	83	622
BD1056	11	240
BD2039	73	667
BD2058	0	1393
BD2064	18	189
BD2066	61	2364
BD2067	59	2116
BD2123	18	213
average	44	870
100%	100	716,8
100/average	2,2985	0,8237
slope/average		
fraction of response	=I21/H21	0,35836

slope of the trendline multiplied by 100

The cone set form clonal archive and the cone class from the plus trees was compared with the other localities, where the same genotypes were planted. Counting of the cones was carried out in different years and subsequently different tree age. In two cases (Sävar seed orchard, Klocke) the cone production was estimated by statistical model (with SAS),

because different treatments (different spacing, soil and herbicide treatments, cutting) were used in that plantations.
Correlation was looked by regression function in Excel and some charts showing a trend line are attached.

Description of the plantations

The plus trees

The clones used in the study originated from plus trees selected half a century ago. The selection of plus trees were formally approved and a data card was made for each plus tree. The plus trees were assigned identifications, first a letter for the county where the plus tree have grown and then four digits. Information about cone set was usually documented on plus tree cards, the field registrations were usually made around 50 years ago. Cone occurrence was expressed verbally. This verbal classification was transferred to numeric categories from zero to ten. These digits may be seen as very rough quantitative numeric cone set registrations. Probably because of the differentiation between the plus tree ages the cone sets were counted and noted down in different years starting from 1950 up to 1969 and for the majority of the trees only one record was done. The cone set of some plus trees was counted twice even three times and the verbal expression was estimated as an average. No adjustments were made to omit annual variation in cone set.
The plus trees served originally as ortets for all clones used in this study (originating from the four north counties – BD, AC, Z, Y.)

The clonal bank (clonal archive) at Sävar

The clone bank was established in Sävar - Västerbotten county, approximately 20 km north-east from Umeå. Grafting was done 1965-69 (at Sundmo) and the grafts were planted 1969-70 in the experimental field outside the breeding station at Sävar (currently

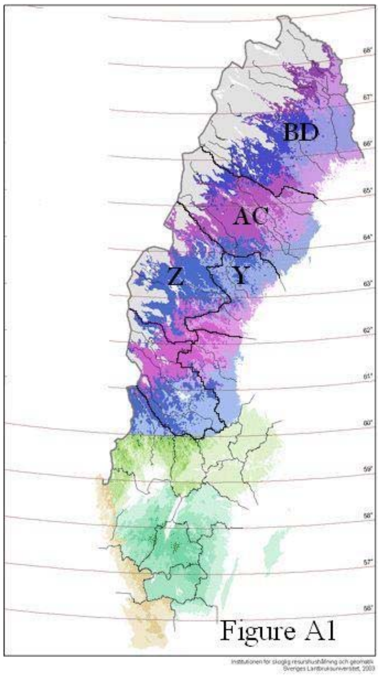


Figure A1

SkogForsk Umeå station). They originated from plus trees of Scots Pine (*P.sylvestris*) in the counties assigned AC, BD, Z and Y ("län"),(Figure A1). There were 4 grafts per clone planted in rows with a spacing of 555 grafts/ha. The number of cones per graft was estimated from the ground and written down in seven classes as follows:

3 = 1-5; **18** = 6-30; **53** = 31-75; **113** = 76-150; **251** = 151-350; **551** = 351-750

1151 = 751-1500. This means that graft, which had approximately between 31 and 75 cones was recorded in the same cone class as it had 53 cones. Diameter at breast height was measured for each alive graft. Dead, dying and trees with breast height diameter zero were noted down too. Observations were done in 1984 for 2992 ramets.
The data were obtained from Torgny Persson.

Picture A1. Illustrative picture – The grafts in the clone bank had approximately the same age and size when observations were made in this study.

Picture A2. The clone bank in 2006.

The seed orchard Klocke

The seed orchard called Klocke is a clonal seed orchard of *P.sylvestris* established 1969 near Nordingrå, Sollefteå, Västernorrlands Län. On the area of 16ha 60 clones were planted originating about 5 degrees of latitude north of the orchard. The number of ramets per clone was unbalanced and varies from 1-10 clones and the spacing was 5,6x5,6m (Eriksson & Wilhelmsson, 1991), (Eriksson & Palmer, 1991).

Average height of grafts was approximately 3,5m when in the autumn of 1990 all cones on some grafts were harvested. Number of cones, their volume, the numbers of filled and empty seeds and the 1000-grain weight (g) were separately determined for each graft. The seed production per clone for some clones was published in the PhD thesis by Eriksson (1996). Seed per clone values used in this study had a higher accuracy and was obtained by personal communication with Curt Almquist and Mats Eriksson 2006.

The seed orchard Skaholma

Grafted seed orchard of Scots pine (*Pinus sylvestris* L.) is situated in northern Sweden close to Umeå airport. The area of the seed orchard is 14 ha. Within some hundred meters from the seed orchard there are only a few sparsely dispersed individuals of Scots pine among Norway spruce stands. Initially the seed orchard was established with 34 clones. Losses of grafts occurred, mainly because of vole damage, and fill in of additional grafts of additional 8 clones was done of age 13-18 years old. The clones at Skaholma originates from plus trees selected in natural stands situated between latitudes 66^{o}30' and 67^{o}50' and altitudes 225m-460m. The average latitude of origin is 66.9 and altitude 360. The seed orchard has produced seed crops since 1974. Until 1994 994 kg of seeds have been collected here, on an average about 4 kg per year and hectare. The seed orchard has been thinned and pruned to control size of the grafts.

Cones were collected in autumn 2004. Three ramets of 12 clones respectively were assessed for number of cones/graft, number of cones/liter, number of filled seeds/cone and number of filled seeds/graft. Only the number of filled seeds per graft is used for this study (Prescher et al 2006 in preparation).

The seed orchard Robertsfors

Seed orchard in Robertsfors was established around 1962. Coordinates are written in the table 2. Cone set per ramet was registered. The data will be used as a part of the work Prescher et al 2006, they originate from Torbjörn Lestander (Lestander, 2006).

The seed orchard Sävar

Cone production in 1999 from clones from the experimental seed orchard at Skogforsk in Sävar, close to Umeå at latitude 63º54´ was used. The seed orchard with Scots pine grafts was established in 1969. It consists of 16 plots originally established for research on different treatments, concerning spacing, soil treatment, herbicide effects and cutting. The design is described by Andersson and Rosvall (1986) who also presented early results. The seed orchard can be regarded as being in full production. Statistical analyses were made with Proc glm in SAS (SAS 1999). Clones with less than three ramets were not taken into account. Clone effects as "LS-means" were predicted by the following model:

$$y_{ijk} = \mu + a_i + b_j + \gamma*(D85) + e_{ijk} \qquad (1)$$

where y_{ijk} is number of cones in plot i on individual ramets of clone j , μ is the intercept, a_i is a fixed effect of plot, b_j is a fixed effect of clone, γ is a linear regression coefficient, D85 is the tree diameter at breast height (covariate, assessed in 1985), e_{ijkl} is a random error term assumed ~N(0, σ^2) .The calculated values of cones per ramet for each clone used was given to me by Johan Kroon, personal communication 2006.

Clone experiments at Röskär, Västra Stenshult (Degeberga) and Sävar

A graft experiments with the same 10 northern clones has been established in north, middle and south (Sävar, Röskär, Degeberga) Sweden, (Figure A2). (Degeberga is a small not very known location that's why the experiment there was called by the nearest bigger place - Västra Stenshult). The initial intention with the experiment was to compare primary and secondary grafts, the experiment is described and the analyses for the initial purpose made by (Andersson and Hattemer 1975). All three sites were inventoried 1982. Correlations between the clonal averages, obtained on the different sites for a number of characters, including male and female strobili per ramet, were presented by Lindgren (1985). After that the Sävar site was inventoried 1993 and a rough cone inventory was made 2004, these results have not been presented or used before. Sävar was established later than the two

Figure A2

other sites, therefore the results 1993 at Sävar are more comparable to Röskär and Degeberga 1982 than the 1982 measurement at Sävar.

Table 2. Locations and types of all plantations included in the survey.

Name of the plantation	**Type**	**Age of the plantation (years)**	**Identification code**[1]	**Number as basic material**[2]	**Location**		
					Lat	**Long**	**Alt**
Skaholma	Seed orchard	43		FP-1	63°50'	20°15'	5m
Robertsfors	Seed orchard	42	S23FP1410	FP-886	64°	20°50'	40m
Röskär	Clone experiment	21	20711		59°25'	18°11'	25m
Va Stenshult (Degeberga)	Clone experiment	22	20811		55°47'	14°04'	120m
Sävar 8208 I.	Clone experiment	12	20611	FP-605	63°54'	20°33'	10m
Sävar 9309 II.		23					
Sävar 0410 III.		34					
Klocke	Seed orchard	21	123	FP-123	62°54'	18°16'	75m
Sävar SO 99	Seed orchard	30		FP-605	63°54'	20°33'	10m
Sävar CA	Clonal bank	14			63°54'	20°33'	10m

1) Experiments and objects get national identification numbers, currently first part of the code is the organization assigning the code (sometimes not given). Sometimes it is older identifications.

2) Basic materials registered for trade in the European Union get numbers in a National list (preceded by S for Sweden). If no number, the material can not be traded, but may perhaps still be used on own land.

Results

The main aim of this work was to find if a prediction of cone set is possible on clones, if we look at the same genotype at a different place. The correlations between clones cone set growing at Sävar CB and the cone sets from all 7 plantations are featured in separate charts. The correlation coefficient “r” and the slope of the regression line are attached and those results are introduced in the second part of the chapter.

With a view to utilize the main cluster of data from Sävar clone bank, some more calculations were done with the clones from all counties (BD, AC, Z, Y). The cone distribution and the coefficient of variation CV among the clones and within the clones (or among the ramets) were calculated. The survival rate, the average diameter of the ramets inclusive of distribution to the diameter classes, average diameters per county and relationship between the diameter and the cone set are mentioned below.

Calculations from Sävar clone bank’s data

All 748 clones inventoried at Sävar CB are included and the number of cones per clone is the average cone number from all living ramets of the clone. Only 8,5% of the clones had produced less than 25 or more than 1000 cones, what can be regarded as extreme values. A single clone represented by a single ramet had 1100 cones, double as high as the second ranking gives a high weight. Also many of the minimum values were obtained from clones represented by only one ramet what partly explains too low or high values. The other part (91,5%) had produced between 25 and 1000 cones per ramet (Figure B1).

Figure B1. Distribution of cones per ramet

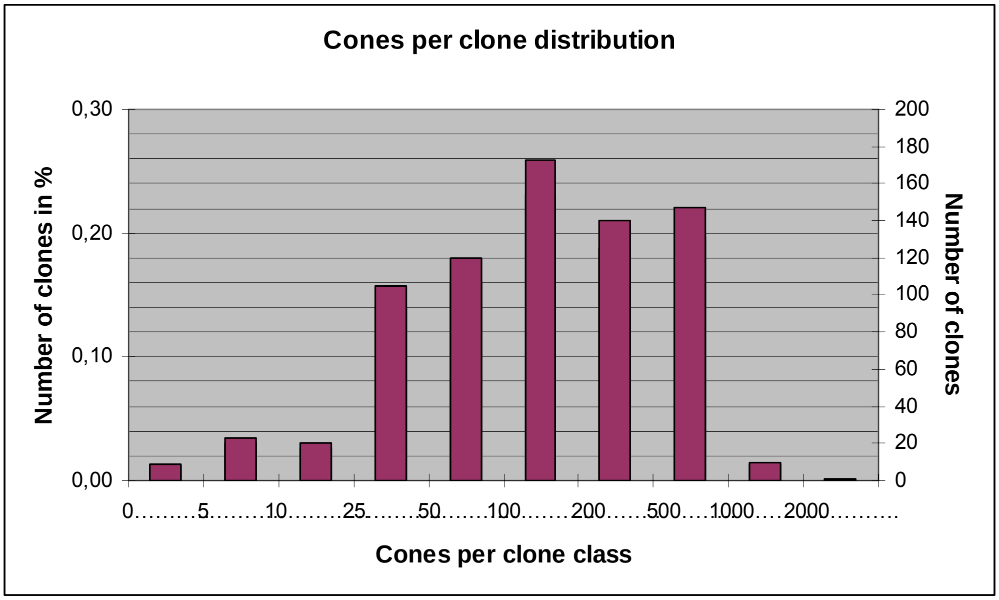


Table B1 . Mean number of cones per ramet in Sävar clone bank for clones originated from all four counties.

Mean number of cones	
All clones	119,5
BD-clones	68,1
AC-clones	151,0
Z-clones	115,6
Y-clones	130,6

Analysis of variance

Table B2. Analysis of variance

Variance cause	Square Sum	Degrees of freedom	Mean square	Estimate of	Coefficient of variation in percentage!
Among clones	25219969	746	33806	$\sigma_r^2 + 3.34\sigma_c^2$	CV_c=94
Within clones	7984701	1747	4571	σ_r^2	CV_r=68
Total	33204670	2493			

The factor in front of the variance component for clones in the estimate of mean square among clones is here approximated as the average number of ramets per clone, this is not the best possible approximation but for this purpose it was judged to be sufficient; σ_c^2 = 8753 (CV_C = 94), σ_r^2 = 4571 (CV_r = 68). The variance analyses was done for percentages and the variance components are here expressed as the standard deviation as a percentage of the mean percentage (100) rather than as a fraction. This operation was made by make it more comparable with other values on variation in female fertility. (Kang et al 2001), (Prescher et al in preparation). This means that the cone numbers among clones are somewhat more variable than ramets within a clone. The low cone set of the northern clones may contribute to this among clone variation. Because of the skewed distribution and the approximate observation in classes these components have limited application.

Table B3. Correlations between cone set in Sävar clone experiment for 10 clones.

	Sävar		
	aug-82	sep-93	okt-04
	Column 1	*Column 2*	*Column 3*
Column 1	1		
Column 2	0,080118	1	
Column 3	0,455644	0,1906941	1

That is the only plantation where data about the cone set of the clones were available for more than one year and the variation among the years was studied. No statistically significant relationship was found in any of the cases (p_{95}=0,549).

Figure B2. Dead trees included dying trees and trees with diameter 0 in breast-height.

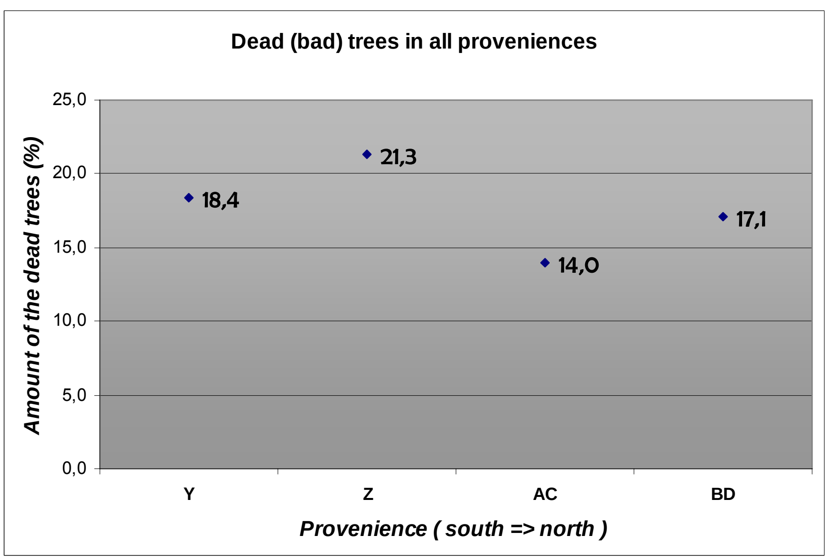


Figure B3. An average diameter of the BD, AC, Y, Z – ramets.

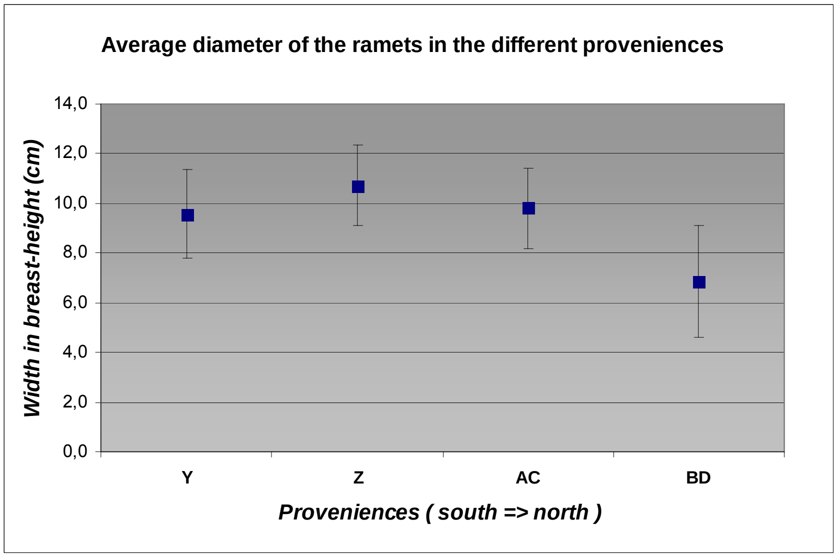


Figure B4. Distribution of the ramets in diameter classes.

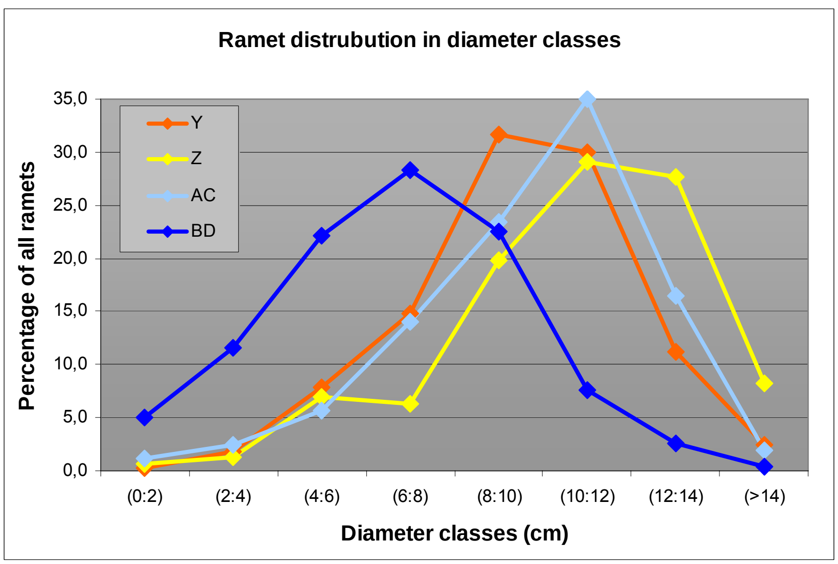
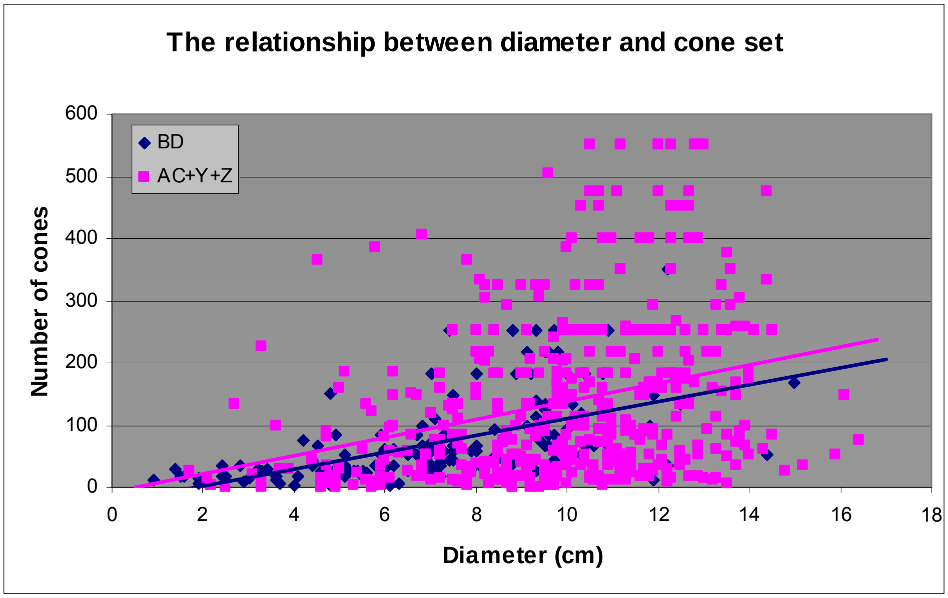


Figure B5. The relationship between diameter and cone set was plotted for ramets in "good shape" – means trees with diameter in breast-height bigger than zero.



Number of cones and diameter for healthy grafts are shown in the chart. The clones from the most northern county, Norrbotten (BD) are compared with the other three counties (AC, Y and Z). The regression line of cones on diameter was similar for the two groups. The Norrbotten clones had fewer cones than the more southern ones even if compared at the same diameter. There seems to be few abundantly cone setting grafts with northern origin.

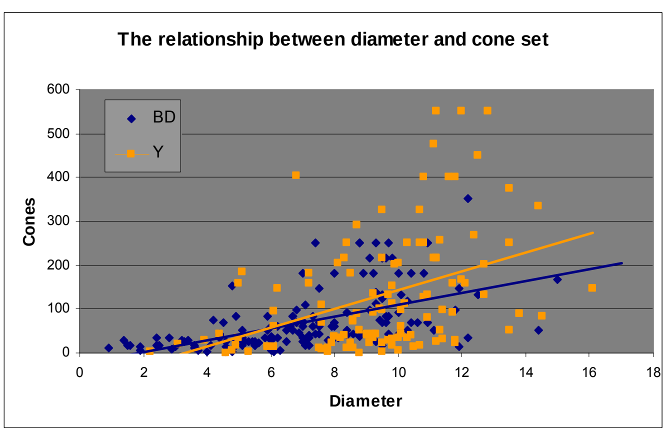


Figure B5a. The relationship between diameter and cone set for clones from most northern county, Norrbotten (BD) are compared with the clones from most southern county Y.

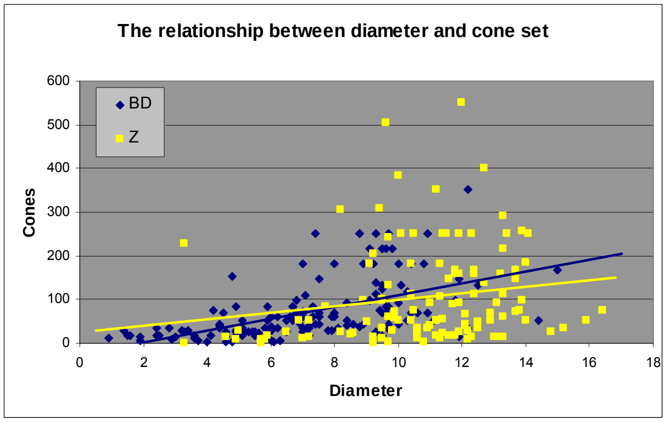


Figure B5b. The relationship between diameter and cone set for clones from most northern county, Norrbotten (BD) are compared with the clones from county Z.

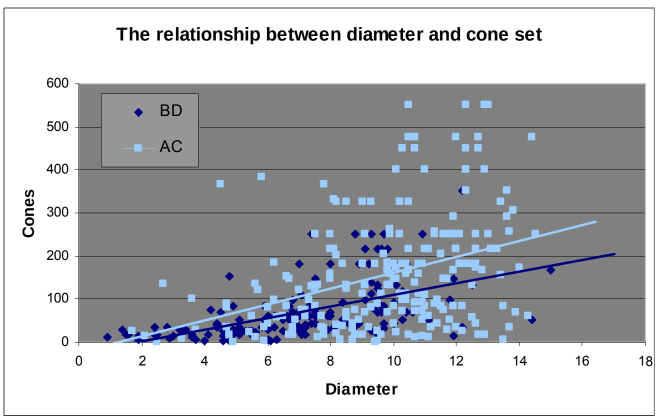


Figure B5c. The relationship between diameter and cone set for clones from most northern county, Norrbotten (BD) are compared with the clones from county AC.

Female fertility for different clones estimated in different places

In this paragraph two main sets of data were used to compare them with the data from 7 different localities. Information about the cone set or eventually seed set were available first from clonal bank in Sävar made in 1984 and second from the plus trees which had served as orteds for the other clones – measured approximately 50 years ago.

The data from Sävar clonal bank

Charts with regression line included cones per ramet at Röskär, Va Stenshult, Sävar (three counting made in 1982, 1993, 2004), Skaholma, Robertsfors and seeds per ramet in

Klocke, outspread to the number of cones per ramet at Sävar archive are placed below - figures B6 – B14.

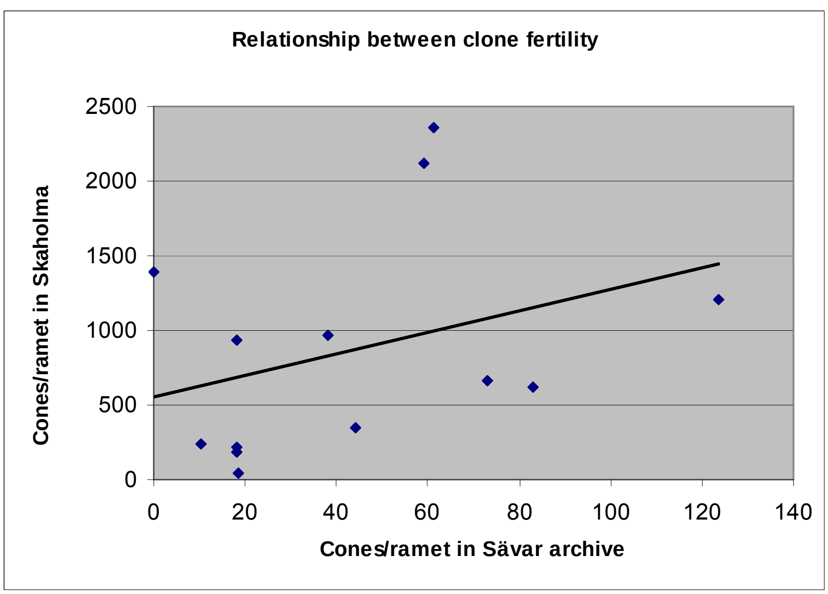
Two values are added to each chart to make them more clear and meaningful:

The value of coefficient of correlation r, gives the strength of a relationship, thus how scattered the points are around the regression line. Even if there is no relationship between values there may appear to be one by random fluctuations. Given the number of value pairs it can be evaluated if a correlation is high enough to make it improbable that there is no real relationship among the variables studied. The connection between the correlation and significance is evaluated in tables B4 and B5.

The slope of the regression line is also shown. That can be interpreted as the expected increase in the explained variable (Y-axes) by one units increase in the explaining variable (X-axes).

Sävar clone bank as base for comparison

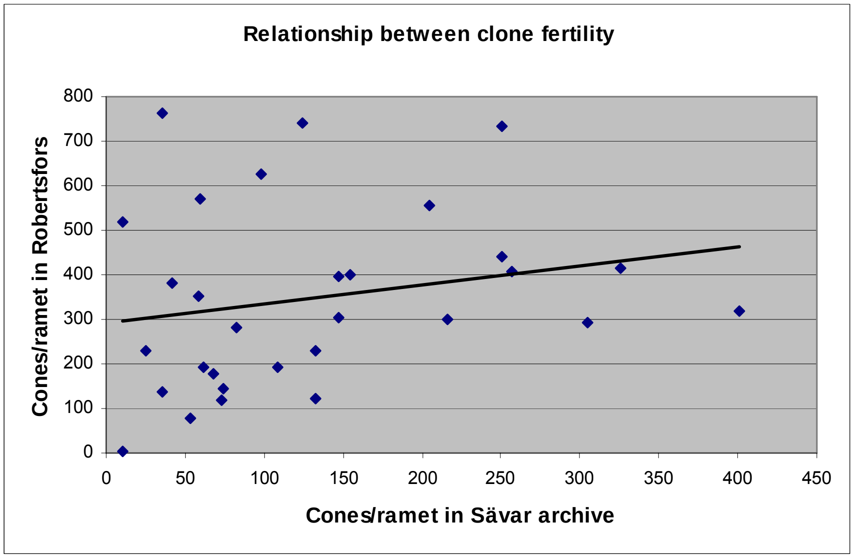
Figure B6. Regression function expressing the relationship between the number of cones per ramet in Skaholma seed orchard and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,344

Slope = 7,168

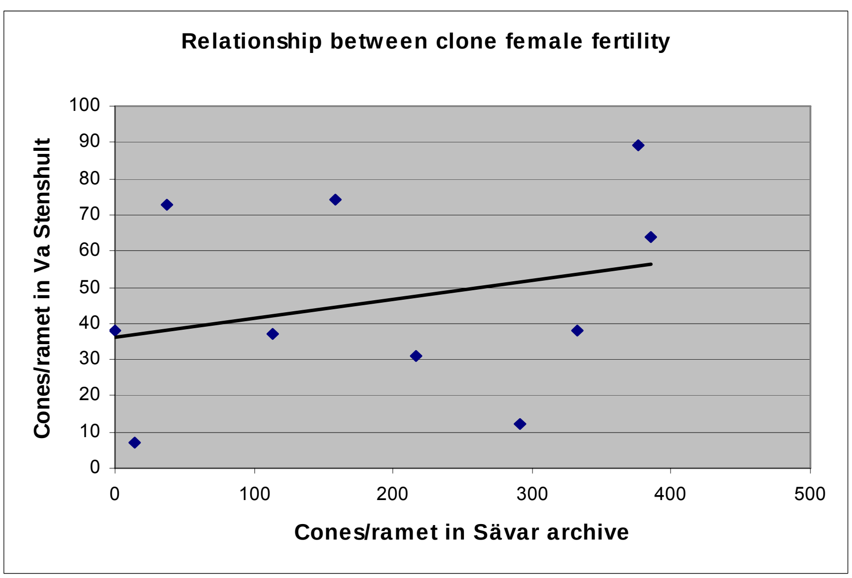
Figure B7. Regression function expressing relationship between the number of cones per ramet in Robertsfors seed orchard and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,216

Slope = 0,428

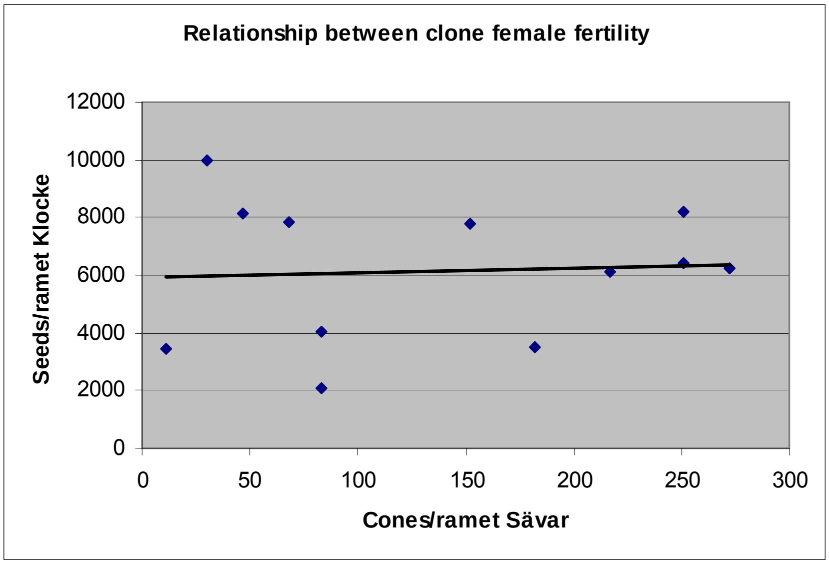
Figure B8. Regression function expressing relationship between the number of cones per ramet in Va Stenshult clone experiment and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,287

Slope = 0,053

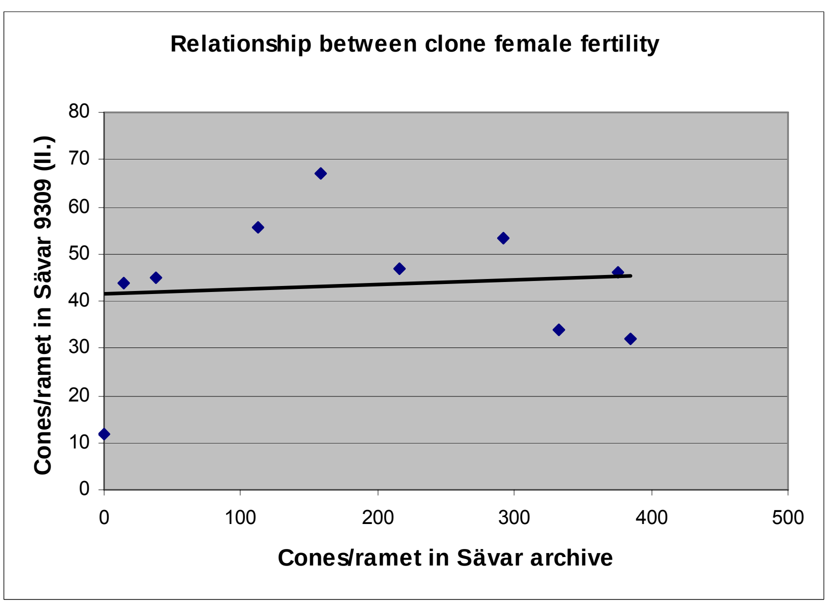
Figure B9. Regression function expressing the relationship between the relative number of seeds per ramet in Klocke seed orchard and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,059

Slope = 1,649

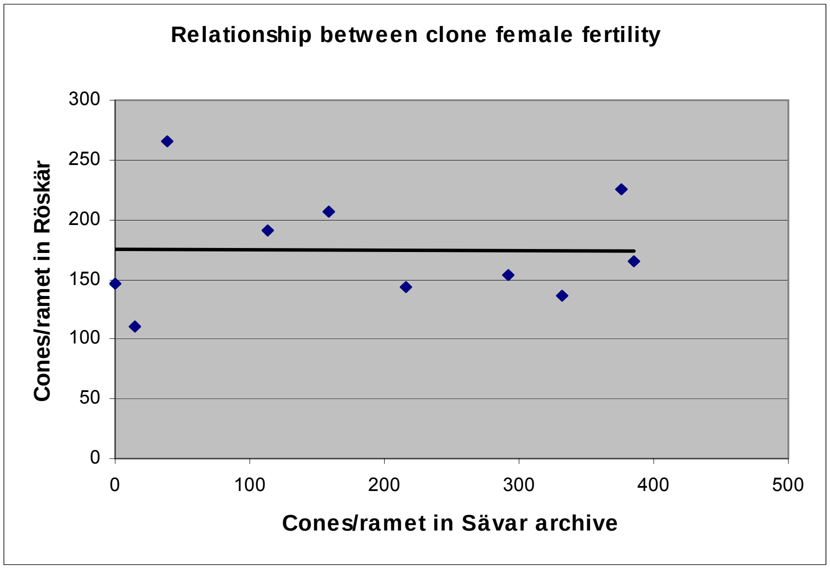
Figure B10. Regression function expressing the relationship between the number of cones per ramet in Sävar 9309 II clone experiment and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,097

Slope = 0,009

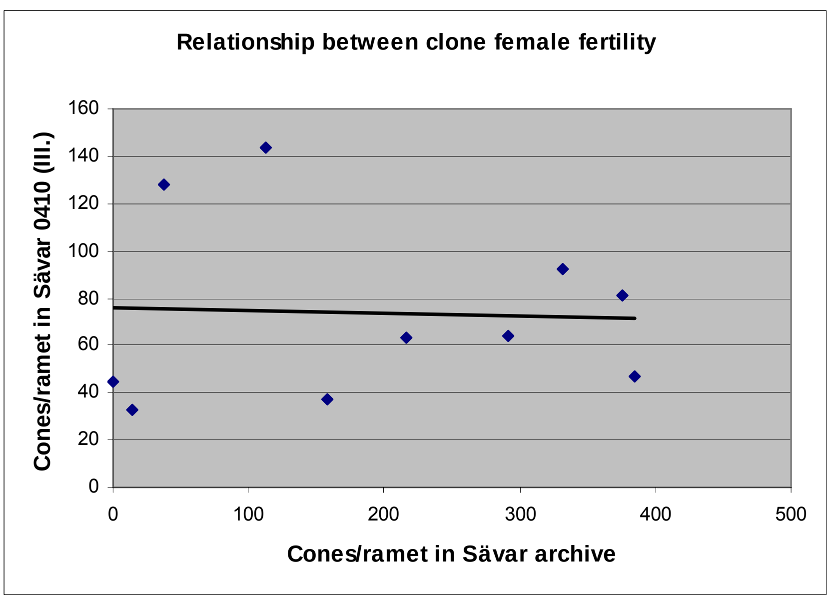
Figure B11. Regression function expressing relationship between the number of cones per ramet in Röskär clone experiment and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,004

Slope = -0,001

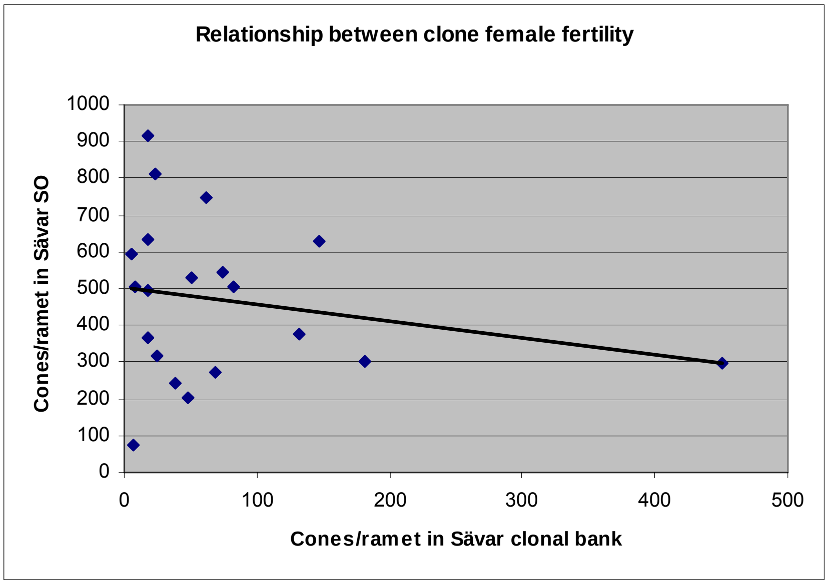
Figure B12. Regression function expressing the relationship between the number of cones per ramet in Sävar 0410 III clone experiment and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,047

Slope = -0,012

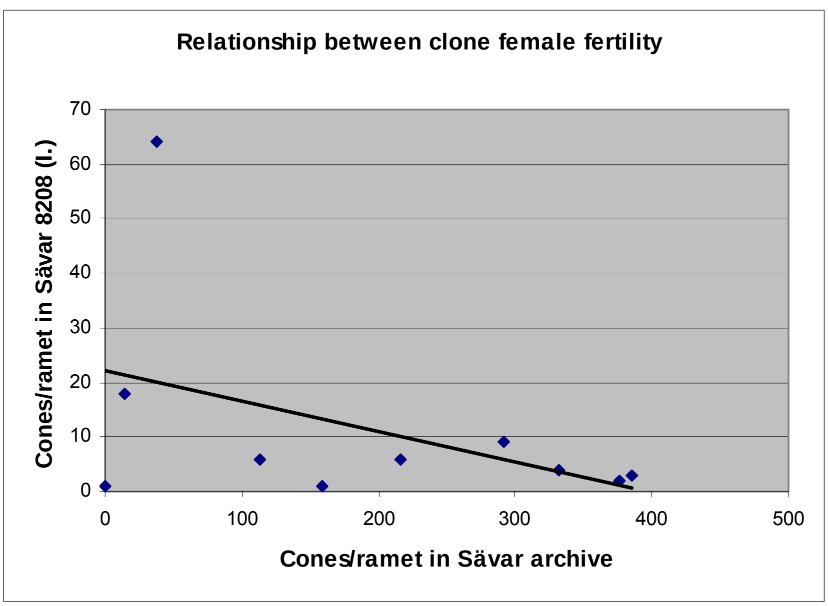
Figure B13. Regression function expressing the relationship between the number of cones per ramet in Sävar "SO" seed orchard and the number of cones per ramet on same genotype in Sävar clonal bank.



Correlation = 0,213

Slope = -0,454

Figure B14. Regression function expressing the relationship between the number of cones per ramet in Sävar 8208 I clone experiment and the number of cones per ramet on same genotype in Sävar clonal bank.

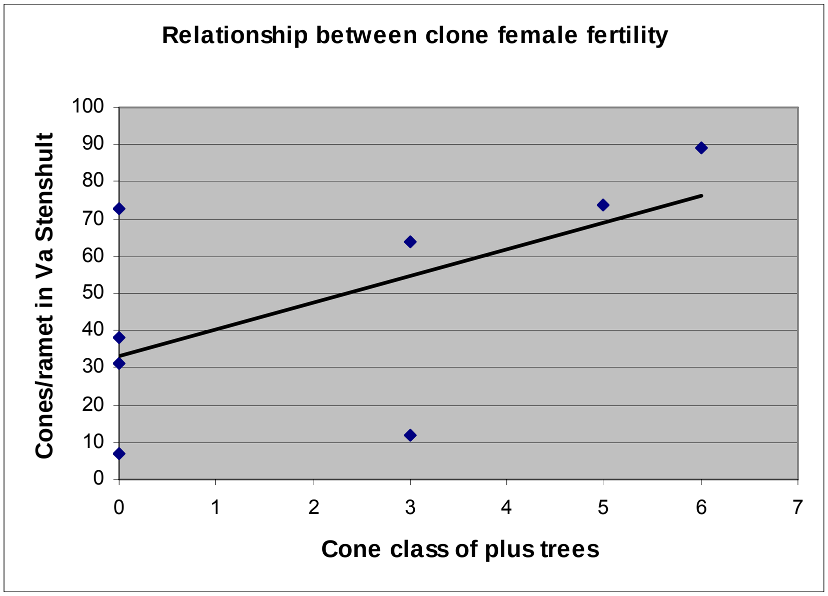


Correlation = 0,437

Slope = -0,056

The plus trees as base for comparison

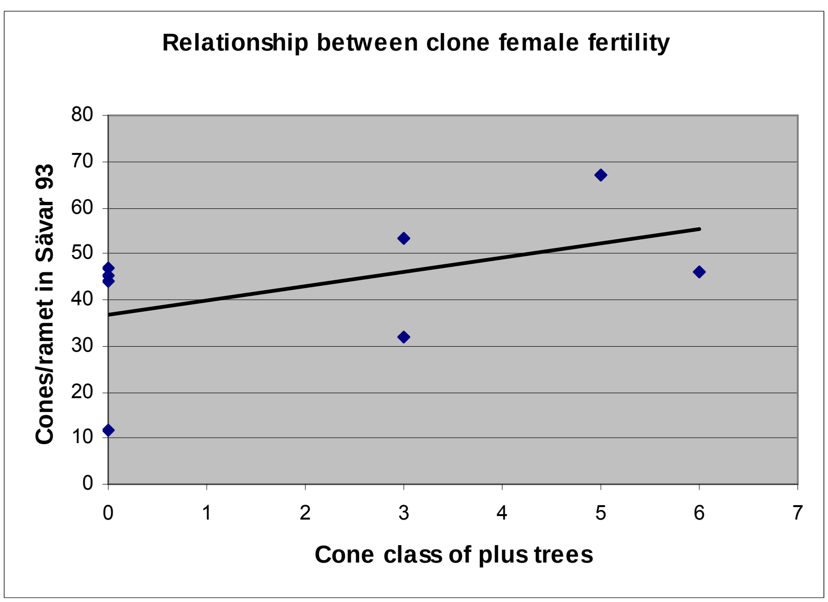
Figure B15. Regression function expressing the relationship between the number of cones per ramet in Va Stenshult clone experiment and the cone class of the plus trees from which these clones originate.



Correlation = 0,578

Slope = 7,172

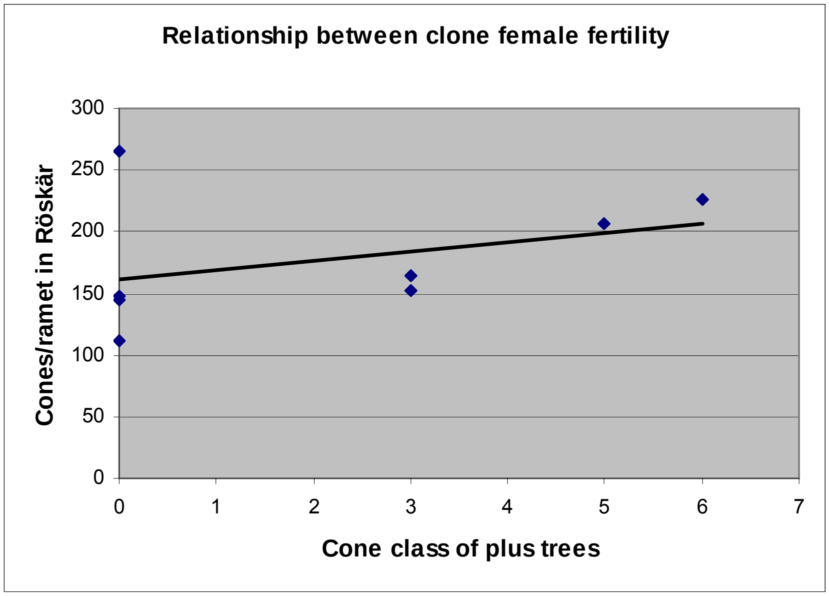
Figure B16. Regression function expressing the relationship between the number of cones per ramet in Sävar 93 clone experiment and the cone class of the plus trees from which these clones originate.



Correlation = 0,476

Slope = 3,082

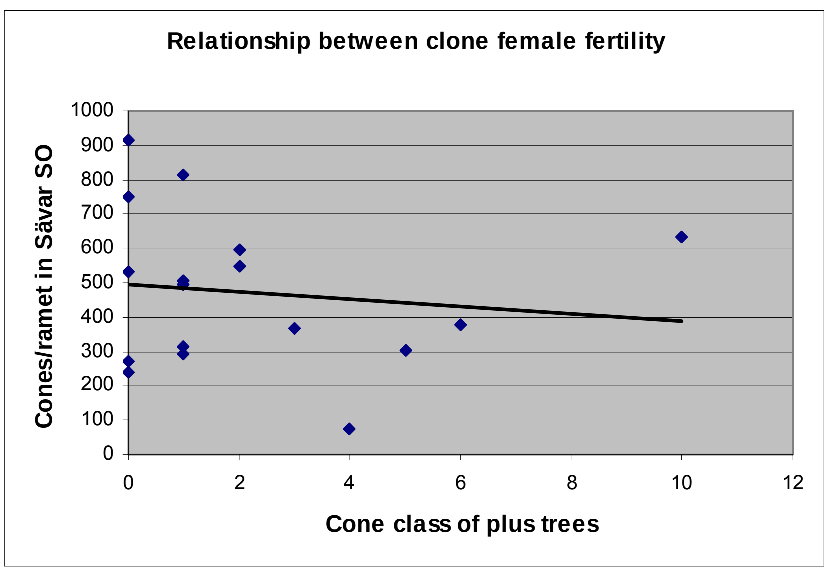
Figure B17. Regression function expressing the relationship between the number of cones per ramet in Röskär clone experiment and the cone class of the plus trees from which these clones originate.



Correlation = 0,376

Slope = 7,738

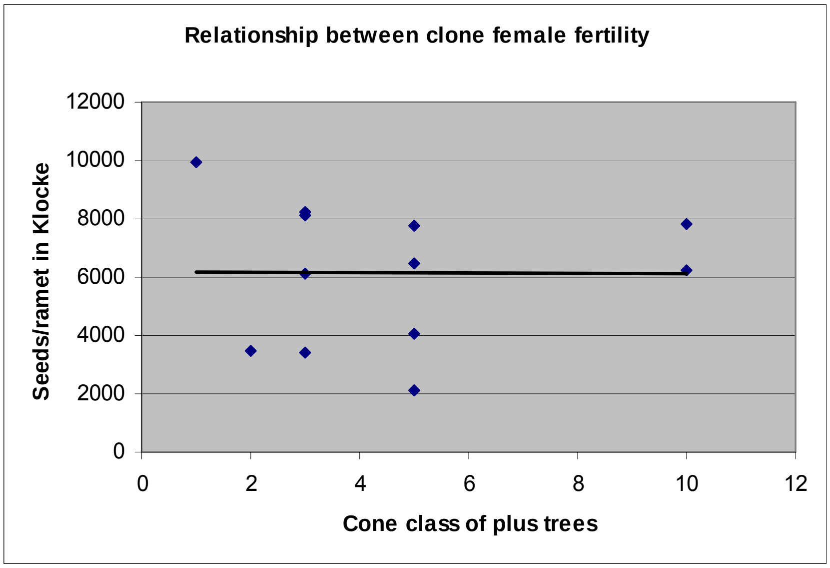
Figure B18. Regression function expressing the relationship between the number of cones per ramet in Sävar seed orchard and the cone class of the plus trees from which these clones originate.



Correlation = 0,135

Slope = -11,068

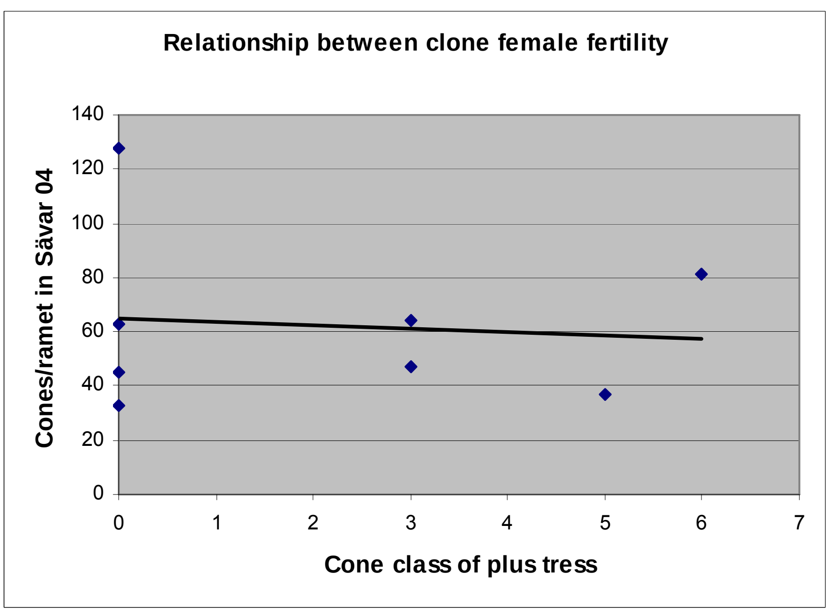
Figure B19. Regression function expressing the relationship between the relative number of filled seeds per ramet in Klocke seed orchard and the cone class of the plus trees from which these clones originate.



Correlation = 0,011

Slope = -9,036

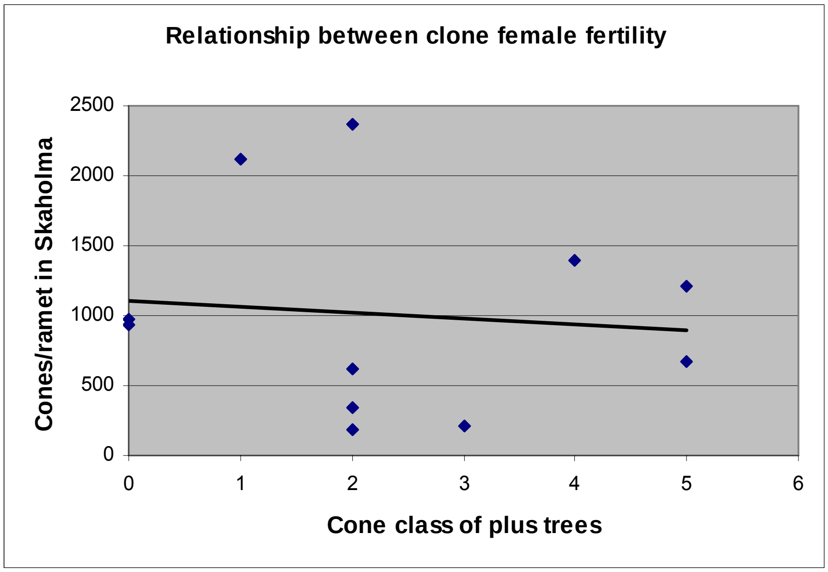
Figure B20. Regression function expressing the relationship between the number of cones per ramet in Sävar 04 clone experiment and the cone class of the plus trees from which these clones originate.



Correlation = 0,101

Slope = -1,265

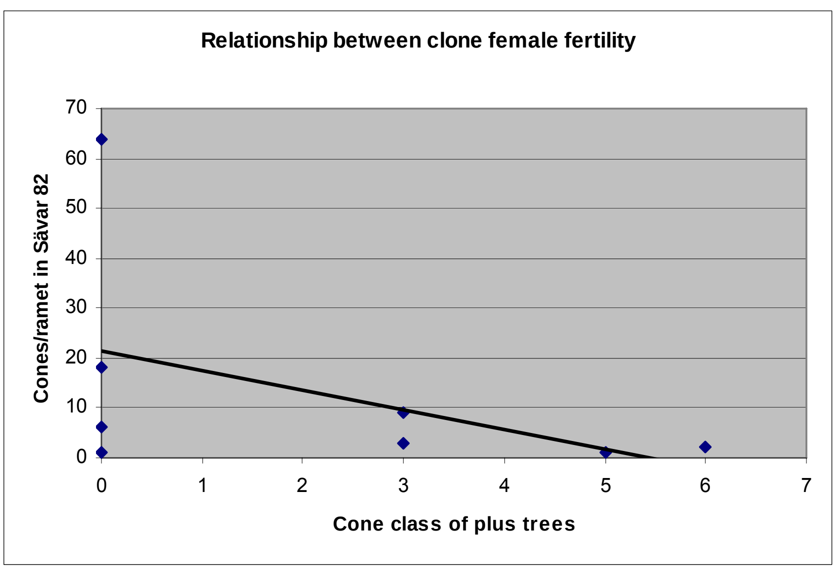
Figure B21. Regression function expressing the relationship between the number of cones per ramet in Skaholma seed orchard and the cone class of the plus trees from which these clones originate.



Correlation = 0,104

Slope = -43,146

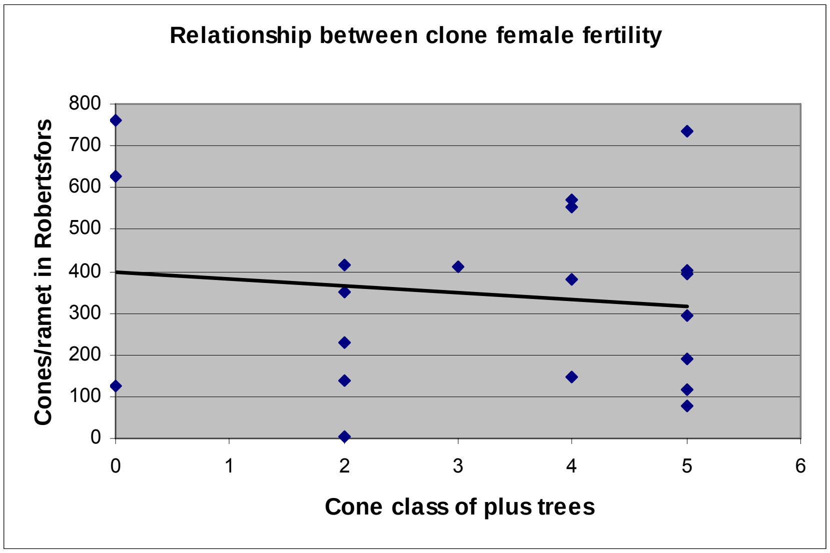
Figure B22. Regression function expressing the relationship between the number of cones per ramet in Sävar 82 clone experiment and the cone class of the plus trees from which these clones originate.



Correlation = 0,454

Slope = -3,918

Figure B23. Regression function expressing the relationship between the number of cones per ramet in Robertsfors seed orchard and the cone class of the plus trees from which these clones originate.



Correlation = 0,129

Slope = -15,635

Table B4. Relationship between clone female fertility in all localities outspread to Sävar CA.

Locality (There are 3 different measurements at Sävar marked with I, II, III)	Number of compared clones	Correlation coefficient $r_{.95}$ and resulting **r**	Fraction of response[1] of cone set on the locality compared to the clone archive at Sävar	Assumed age of grafts. Establishing year and measurement year within hyphens	Weights*	Adapted fraction of response
Skaholma	13	0,476>0,344	0,3584	43 (1960-2003)	2	0,7168
Robertsfors	30	0,306>0,215	0,1621	42 (1962-1982)	3	0,4863
Va Stenshult	10	0,549>0,287	0,2193	22 (1960-1982)	1	0,2193
Klocke	12	0,497>0,059	0,0323	21 (1969-1990)	2	0,0645
Sävar 9309 II.	10	0,549>0,098	0,0433	23 (1970-1993)	1	0,0433
Röskär	10	0,549>-0,004	-0,0015	21 (1961-1982)	1	-0,0015
Sävar 0410 III.	10	0,549>-0,047	-0,0314	34 (1970-2004)	1	-0,0314
Sävar SO 99	20	0,378>-0,213	-0,0716	30 (1969-1999)	3	-0,2148
Sävar 8208 I.	10	0,549>-0,437	-0,9466	12 (1970-1982)	0.5	-0,4733
	average	**0,081**	**-0,0262**			**0,0899**

**The weights are a subjective way to get what we believe is a fairer average. The seed orchard Skaholma, Robertsfors, Klocke and Sävar SO are rather mature with different clones where cones and seeds were evaluated, those where more clones were evaluated get a higher weight. Röskär, Va Stenshult and Sävar experiment use the same 10 clones and are thus less informative "per site". Sävar 82 was only 12 year old clones which had not reached full age for flowering yet, few and unequally distributed flowers and thus get a lower weight.*

1) Fraction of response was created with the idea to show what is the improving in the cone set in percentages created by the same genotype, if we look at the best clones in the clone bank (Equation 1).

Table B5. Relationship between clone female fertility in all localities outspread to the date from plus trees.

Locality (There are 3 different measurements at Sävar marked with I, II, III)	Number of compared clones	Correlation coefficient $\mathbf{r}_{.95}$ and calculated **r**	Fraction of response of cone set on the locality compared to the clone archive at Sävar	Assumed age of grafts. Establishing year and measurement year within hyphens	Weights*	Adapted fraction of response
Va Stenshult	8	0,621>0,578	0,3142	22 (1960-1982)	1	0,3142
Sävar 9309 II.	8	0,621>0,476	0,1514	23 (1970-1993)	1	0,1514
Röskär	8	0,621>0,376	0,0928	21 (1961-1982)	1	0,0928
Sävar SO 99	18	0,400>-0,135	-0,0004	30 (1969-1999)	3	-0,0012
Klocke	12	0,497>-0,011	-0,0067	21 (1969-1990)	2	-0,0134
Sävar 0410 III.	8	0,621>-0,101	-0,0432	34 (1970-2004)	1	-0,0432
Skaholma	11	0,521>-0,104	-0,1017	43 (1960-2003)	2	-0,2034
Sävar 8208 I.	8	0,621>-0,454	-0,6405	12 (1970-1982)	0.5	-0,3203
Robertsfors	20	0,378>-0,129	-0,1447	42 (1962-1982)	3	-0,4341
	average	**0,055**	**-0,0421**			**-0,0508**

**see table B4*

In no case of 18 relationships analyzed significant relationship between female fertility observed at Sävar clone bank or in plus trees and female fertility observed in the different seed orchards or trials studied. It is unlikely to be a strong relationship. The estimated correlation was 0.08 for the clone bank and 0.06 for the plus trees; this indicates that the relationship is very weak if it exists at all. Estimates about the potential percentage gain in cone production appeared negative or very low in a new seed orchard if using observed fertility as a selection instrument.

Discussion

Different clone behavior due to clone origin

For commercial plantations of Scots pine on harsh sites in northern Sweden it is common to use provenances origination north of the plantation site (e.g., Eiche 1966) to obtain an acceptable survival. Northern provenances (including grafts) grow slower compared to local, but that may be more than compensated by their better survival. Seed orchards of northern trees are usually localized at warm spots at low elevation and south compared to the origin. The main reason is to get better seed set and maturation, but easier access to staff and workers also matters.

An increase in the frequency of flowering has sometimes been observed when trees were moved to the south (Johnsson et al. 1950). Of course there are some limitations about the transfer distance which is associated with positive effects, - 1° or 2° latitudes seems often good, while longer transfers may cause so slow growth so the trees are more exposed to injuries and has more difficult to recover from injuries.

In the current experiment a tendency that trees with more southern origin have a larger diameter was observed, the BD trees seem to have the smallest average diameter and the slowest growth, reference to charts B3. Figure B4 shows the contribution of the clones by their diameters, instead of average value, which result is restricted to only one number. This figure illustrates that the best growing ability have the clones originate from Z County, contrariwise the growth of BD clones is slowed down. Z clones attain to the largest average diameter also. The clones from Y County look to grow slower than the Z ones, even if Y County is situated in warmer temperature zone. The clones originate from the same County where the clone bank is – AC, could be regarded as average growing.

The main reason for the distinct growing ability is probably that in more southern environments the vegetation period is longer, so southern trees are adapted to remain vegetative active longer in the autumn. Anyway the Y clones are not the best growing ones, that is perhaps sort of too southern origin and the trees may not be able to assimilate with the colder climate and soon or late they could be damaged by the lower temperatures.

The most damaged clones however were those from Z County and the highest survival was detected for the AC clones (clones originate from the same county, where the clone bank is). Survival of the grafted clones is indeed influenced not only by their origin, but there is always certain unacceptability between the rootstock and the cutting, which doesn't allow regular growth and development of the tree. Beyond the supply of rood stock most probably had varied during the years of establishing the clone bank and survival of the grafts may be influenced due to the different supplies. These facts can be hardly predicted or affected and its impact is randomly spread between all clones.

The difference is most evident among the Z and AC clones, which are near to each other on the "south-north scale". Z County indeed belongs especially to the mountain region, while AC subsumes all land types from the sea coast up to the mountains and in total the clime is lighter. The survival may be influenced by the climate in the clone bank, which is close to the sea where the springs are colder but this seems to be unlikely for the low survival of the clones come from Z County.

For the other counties it doesn't seem to be an evident difference in diameter, in the average amount of cones and the percentage of dead (bad) trees. Confidence interval was calculated for the average diameters of the grafts and found that BD average diameter can fluctuate by 32% in both directions (Figure B3) and discussed results can not hold out statistically.

Percentage of dead trees increases slightly with more southern origin, comparing Y, Z with AC, BD similar as we would expect in a normal plantation - higher mortality of southern provenances (e.g. Eiche 1966) (Figure B2). The reason for most of the mortality is unlikely to be climate, the climate at this low coastal area is expected to be more favorable than on typical forest land, and the management with removing competing vegetation and protecting the grafts from damage in different ways (e.g. fencing individual grafts to protect against rodents) is much more intensive than on forest land.

Size of the tree of course must be correlated with the number of cones i.e. larger trees with bigger diameter have more branches and more space to produce cones. Figure B5 shows that the clones from BD with the same diameter as the clones from the other three localities produce fewer cones per ramet. On the next three charts 5Ba, 5Bb and 5Bc the same comparison is made separately for each one of the counties. The trend lines foreshadow much higher cone production for clones originated from AC and Y Counties comparing with BD clones. However only a small number of BD clones have got larger stem than 10 cm and that's the place on the chart, where the difference between cone sets starts to be striking. Of course lower fruitfulness of BD clones can be present because of the trees shape - northern trees are often characterized by narrow crowns.

These results seem to indicate a possible disadvantage to place northern clones more than two degrees south of their origin with cone production point of view. They develop slowly vegetative which delays the time to cone production. However, the northern clones may be able to reach the same level of cone set when they get older. And we can not be certain that it is a response on the transfer, it could as well be that the BD clones set less cones independent on the transfer, this clone bank was not set up to find the reply to such questions.

Relationships between female fertility

Regression between the clones growing at Sävar clone bank and the same clones growing at the other localities is shown on charts B6-B14 and written down in table B4.

The same comparison was done with the plus tree cone sets and all other plantations i.e. table B5 and charts B15-B23. The relationship is expressed by the slope of the regression line and the statistical significance by calculated correlation coefficient r and r_{95}. There wasn't found statistically significant relationship at the P=0.05 level between the female fertility. Taken into consideration that only a small number of trees were compared and mainly no replications were done, kind of varying results could be intelligible. Anyway final results seem to be outcome of randomly collected data regarding high negative and positive correlations between the clone cone sets. It seems that cone production in one or only few years gives little information about the cone production capacity of the clone.

With regard to method used in practice – established clonal seed orchards use clones chosen according cone production ability of their ortets – results in this study are unexpected.

A high variance as well among as within clones was detected in Sävar clone bank, which foreshadows also big variation among the years. For only one location data about the cone set for more than one year were used it the study. Not even there a strong relationship was found and that can make the reliability of the results uncertain! The only possible way to have dependable information about the cone producing capacity of the clones is to collect data from more years. Anyway very weak relations found in this work can mean that such effort is not justified.

Some more comparisons of the female fertility

Comparison was done not only outspread to Sävar archive, but also between the other locations in order to see if there is any better relationship between their female fertility. In two of the cases there is a hint at relationship, but not statistically big enough. It is between cone fertility at Röskär and Va Stenshult and between first measurement in Sävar (1982) and Röskar (Dag Lindgren 1985). Even between the same clones growing in Sävar clone experiment no relationship was found in the cone production from three measurements (table B3). Only small amount of data (10 clone cone sets) were available to make comparisons. Indeed better relationship was found in female fertility in clones at Sävar archive and Robertsfors firstly and Sävar archive and Skaholma secondly, but again not statistically significant. There is likewise one case not outspread to Sävar archive but indicated strong relationship found in Röskär and Va Stenshult. Even if not significant, there could be a relation. There is a positive slope of the regression lines found in some observations.

Uncertainties

There are factors, which can contribute to uncertainty of the possibility to generalize the results. The results of this study were unexpected in some point of view, and it may be discussed if they are repeatable. There was only one site for the clonal archive and registrations were done only one year. The number of registered trees per clone was not very high. The grafts in the clone bank were not mature when registered.

Sävar clone bank

As it is a clone bank where the clones appear in rows without replications, probably planted in order as they were delivered, much of what here is interpreted as clonal variation may be “error” caused e.g. by that the grafting was done at different time, planting was done at different time, there are differences in the experimental field, operators and rootstocks may change during the grafting operation etc.

Counting of cones is generally unreliable. The most reliable is to collect all cones. For a big tree this may be time-consuming and cumbersome but visual estimates can be made.

Certain error can be made because we don’t have exact number of cones per graft but we have 8 classes and estimated cones were ranked to one of the classes.

Data about only a single cone set were collected and the trees were “only” 14 years old what could be regarded as to young age to be representative. Indeed a two ramets had 1100 cones and even if the average number of cones per ramet is smaller than in mature plantations the decision to collect all this data was made, its reliability must have been considered carefully.

The registrations were done specifically to aid to select seed orchard clones based on seed production, the uncertainties were known at the time of registration and the data were regarded as useful for clone selection. Uncertainties are expected to reduce the correlation but not make it close to zero as was found here. Neither a correlation was found for the mature plus trees in the forest, which is a quite different environment than the clone bank; this makes the results more certain.

Conclusions

It's hard to make prediction of fertility between clones. The main reason is the large variation occurring among the years, which is higher in younger plantations and in years with bad flowering.
It seems that the cone set data from one or a very few years give little information about the cone production capacity of the clone.

It doesn't seem meaningful to predict clones cone-set in a seed orchard by information on cone set of the same clones growing else and if clones had been selected based on cone-set, the gain in seed production would have been absent or negligible low.

It seems that clones with more northern origin grow slower and produce fewer cones than clones from more southern counties.
The survival ability of the clones is not influenced by their origin.

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