Blühen nur eine rein physiologische Eigenschaft, wie NITSCH und NITSCH (1967a und b) und NITSCH (1972) bei Plumbaco in in vitro-Kulturen beschrieben haben. Obgleich viele Einzelheiten des frühen Blühens bei den aus Kalluskulturen herangezogenen Birken noch offen bleiben, ist sicherlich anzunehmen, daß die beiden erwähnten Methoden, Selektion und vegetative Vermehrung der selektierten Mutterpflanzen, neue Möglichkeiten für die Blühstimulierung von Forstpflanzen bieten können. Ob die vegetative Vermehrung durch Stecklinge bei der Birke zu dem gleichen Erfolg führt, haben wir bisher nicht untersucht.

Anmerkung

Die Arbeiten wurden durch eine Beihilfe der Deutschen Forschungsgemeinschaft gefördert.

Zusammenfassung

Frühblühende Birken (Betula pendula ROTH.) wurden durch Kalluskulturen vegetativ auf dem modifizierten Murashige-Skoog-Medium vermehrt. Aus dem Kallus herangezogene Pflänzchen behielten das Merkmal des frühen Blühens bei und bildeten bereits nach fünf Monaten ihre ersten männlichen Blüten.

Schlagworte: Blühstimulierung, Gewebekultur, Differenzierung, Vegetative Vermehrung.

Summary

The early flowering birches (Betula pendula ROTH.) have been propagated vegetatively through tissue cultures on the modified Murashige-Skoog-Medium. The characteristic of the early flowering stayed in the plants grown up from the callus. These plantlets have the ability to form their first male flowers after five months.

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Some results from second generation crossings involving inbreeding in Norway spruce (Picea abies)

By ENAR ANDERSSON, RUTH JANSSON and DAG LINDGREN

Department of Forest Genetics, Royal College of Forestry, S 104 05 Stockholm

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Introduction

Studies concerning the effect of inbreeding in forest trees are of interest from several points of view. A major question is the evaluation of the hazards involved in selecting related individuals for use in advanced generation seed orchards. It is also important to discuss the significance of selfing within a clone. Another aspect is the possibility of using inbreeding as a tool in plant breeding, which has been extremely successful in e. g. maize.

Probably the oldest existing experiment with selfed progeny of Norway spruce (Picea abies) was planted 1916 by SYLVÉN. The experiment and results are described by SYLVÉN (1910), LANGLET (1940), ERIKSSON et al. (1973) among others. The crosses were performed in 1909. Five trees at Hassle, Västergötland were self-pollinated. Open-pollinated seeds were also collected. The seeds were sown 1910 and the plants obtained were planted in the field in 1916, near Akersberga (20 km NE of Stockholm). The spacing was 3 X 3 m. No thinning has yet been carried out. During the past few years flowering has been rather good, and a series of crosses has been performed. The first results were presented by ANDERSSON (1965). This material has been used for short studies in nurseries. Results have been presented by ERIKSSON (1972) and ANDERSSON and LINDGREN (1973). The latter paper includes a study of the genetic variance between the trees at Akersberga and a more detailed study of the variance within sibships. Further results, including data on cone weight, seeds per cone, germination of filled seeds, growth rhythm in the nursery and general combining ability, will be presented in a later paper.

The material is of particular importance to the understanding of the pattern of inheritance in forest trees, since it comprises various offspring from parents related in different ways. The aim of this paper is to present some early results of these experiments and discuss their implications for forest genetics. However, it must be pointed out that one of the main aims of this study was the establishment of long-term field trials, which cannot be evaluated for many years.

Material and Methods

Designations. Offsprings from four of SYLÉN's initial trees (F_0) are included in the Åkersberga trial. The F_0 trees will be designated A, B, C and D (SYLVÉN used 1, 3, 4, 5 for the corresponding trees). Unfortunately, the initial trees have not been preserved.

The two filial generations are called F_1 and F_2. Following one or two generations of selfing they are designated S_1 and S_2 respectively. (Many authors prefer I_1 and I_2. However, it appears that S_1 and S_2 have been used most frequently in forest-genetical literature.) The progeny following open pollination is designated OP_1. The following types of crossing designations will be used: $[S_{1p} \times S_{1p}]$ (C_{12} combination according to *Fig. 1)* denotes cross between full sibs in a selfed progeny and $[S_1 \times S_1]$ (C_8) denotes cross between unrelated individuals originating from selfing. The trees growing at Åkersberga belong to F_1. They are given the following designations (cf. LANGLET 1940) *(Table 1).*

Table 1. — Designation and origin of trees in the Åkersberga trial

F_0	selfing (S_1)	F_1	open pollination (OP_1)
A	Å 1—16		Å 45— 80
B	Å 17—33		Å 81—101
C	Å 34—42		Å 102—139
D	Å 43—44		Å 140—151

The Å is excluded when no misunderstandings can occur. The different mating patterns possible between the F_1 trees at Åkersberga are illustrated in *Fig. 1.* The mating patterns are designated C_1—C_{14}. F stands for coefficient of inbreeding. The F_2 materials are usually referred to the year of crossing.

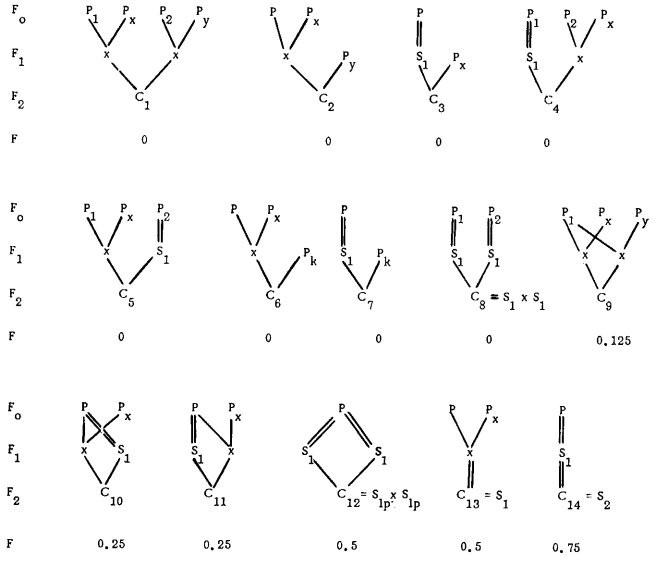

Figure 1. — All different types of crossings at Åkersberga. — Explanations: Mother to the left. P, P_1 and P_2 stand for SYLVEN's F_0 trees A, B, C or D. P_x and P_y stand for unknown father (wind pollination). P_k stands for a known father which is not related to SYLVEN's. F_0 trees. S_1 is an individual originating from selfing. Selfing is indicated by double lines. x is another individual in the F_1 generation. The possible F_2 generation combinations are designed by C_1—C_{14}.

The indications of significance levels are those commonly used: ns ($p > 0.05$, not significant) * ($0.05 > p > 0.01$) ** ($0.01 > p > 0.001$) and *** ($0.001 > p$).

Experimental technique

The F_1 trees at Åkersberga were isolated and artificially pollinated by climbing the trees according to ANDERSSON (1965). There were many flowers on S_1 trees. The seeds were extracted and counted. The number of filled seeds was determined by X-ray radiography. Only seeds classified by radiography as "filled" were sown. The number of germinating seeds and the number of chlorophyll mutants were determined two weeks after sowing.

Available materials

Early crossings

Some results of crossings carried out in 1954 were presented by ANDERSSON (1965). Seeds from crossings in 1952 were sown in 1953 and planted in 1961 at Röskär. It is possible that S_3 plants may be generated in the near future.

Crossings performed in the years 1966—1970 were treated in the following way:

1966

Propagated in phytotron at 16 hours day 20° and 8 hours night 15° C. In some cases there were no replications of individual materials. Sown at the end of December 1967. Height was measured on 30. 5. 1968.

1967

Sown in plastic greenhouse on 17. 6. — 19. 6. 1968. Some material from 1966 was also included. Height was measured on 20. 9. — 22. 9. 1969. Transplanted to plastic greenhouse in eight replications.

1968—1969

Sown in glass greenhouse in the middle of April 1970. Transplanted to plastic greenhouse in the middle of June 1970. Measured at August 1971.

1970

Sown in glass greenhouse on 8. 4. — 16. 4. 1971. Transplanted to plastic greenhouse at the beginning of June 1971. Measured on 13. 9. — 15. 9. 1972. The material was divided into three main blocks, containing materials with the same maternal grandmother. Within each main block there were six replications.

Results and Discussion

The effect of inbreeding on height growth

The inbreeding coefficient of different kinds of matings performed is shown in *Fig. 1.* The inbreeding coefficient varies between 0 and 0.75. The comparison of the values of different crossings might be discussed. The crossings may differ as regards general and specific combining ability, and the inbreeding depression may depend on the inbred genome. Here the degree of inbreeding has simply been estimated as the performance of inbred progeny versus non-inbred, and no effort has been made to adjust for e. g. general combining ability and seed weight.

For the material from the years 1968—69, several different categories of cross are available, covering the whole range of inbreeding coefficients. Materials including crosses with inbreeding of genome A are shown in *Fig. 2* (a

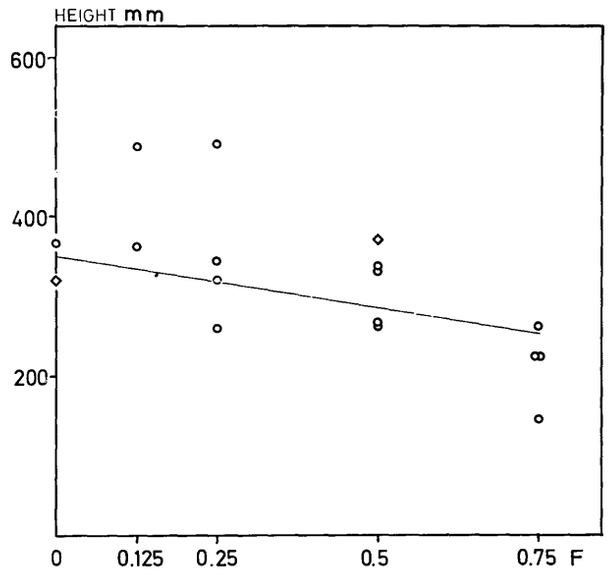

Figure 2. — Height as a function of the coefficient of inbreeding. The points represent different combinations including initial tree A. The diamonds are more closely related to A than the circles are. At F = 0 only mean values are given.

preliminary version of the figure was presented by ERIKSSON 1972). For both A and B there is a significant decrease of height growth for an increasing coefficient of inbreeding (For A ** and B * significance). As regards trees C and D, and for the other years, information is far more limited.

The calculated regression coefficients of height on the inbreeding coefficient are given in *Table 2.*

The regression coefficients (b) are based on a scale in which the height at F = 0 is taken as 100 (per cent). The expected inbreeding depression following selfing (in per cent) is given by ½ b. The crosses are regarded as experimental units. The number of crosses with F > 0 gives a rough indication of the reliability of b.

There is a strong indication that inbred offspring from the initial tree C did not suffer so much from inbreeding in 1968—69. This is especially remarkable, since in the S_1 generation there is a marked inbreeding depression, and an inbreeding depression was also found in the limited material of 1967. A possible explanation might be that the inbreeding depression of tree C is regulated by rather few genes. It appears that different genomes may respond in different ways following inbreeding.

Table 2. — Inbreeding depression in height

Year of cross	(F_0 tree)	b	number of crosses with F > 0	
1966		—11.7	4	
1967		—66.3	13	
1968—69	A	—37.6	16	
	B	—36.3	17	
	C	+21.4	4	
	D	—51.5	3	
1970		—29.0	6	
		F_1 at Åkersberga (cf ERIKSSON *et al.* 1973)		
	early age	—110.0	4	* (8, 11, 16 and 27 years, mean value)
	late age	—55.0	4	

There seem to be differences between the several ways of propagating the material. The material from 1966, which was sown in the phytotron, has the lowest inbreeding depression. The materials from 1968—69 and 1970, which were sown in a glass greenhouse and transplanted to jiffy pots in a plastic greenhouse, are intermediate and the material from 1967, which was sown direct in a plastic greenhouse, has the highest inbreeding depression among the progeny trials. A still higher inbreeding depression was obtained in the field trial at Åkersberga. (The inbreeding depression of height at an advanced age is smaller than at an early age, since the outbred trees have already reached a height at which height growth begins to decline.) However, the S_1 and OP_1 seedlings forming the basis of the Åkersberga trial had almost the same appearance after the first year, when they were cultivated in pots (SYLVÉN 1910). These observations indicate that it is not possible to select against inbred plants in nurseries if the plants grow under favourable conditions. They might subsequently show poor growth when planted out. The data from this investigation suggest that the present trends of nursery techniques, using plastic greenhouses, optimal temperature, humidity and nutrition, might be questionable from a genetic point of view. Investigations aiming to elucidate the genetic consequences of our present methods of growing seedlings on a large scale must in any event be assigned hight priority. ERIKSSON and RUDIN have recently started such an investigation, making use of the isozyme technique.

Variation within sibships

Theoretically, it is expected that the genetic variance within sibships will be lower in crosses between inbred unrelated parents than in crosses between non-inbred parents (cf. ANDERSSON and LINGGREN 1973).

In the experiments the following variations within sibships (within plots) were obtained *(Table 3).* Only crosses between unrelated individuals are included. The standard deviation is expressed in relation to the mean plant height (coefficient of variation).

Table 3. — Variation within sibship

Years of cross	Mating type	σ (per cent of mean)	significance for difference
1966	$S_1 \times S_1$ (C_8)	17.0	
	$S_1 \times OP_1$ (C_3, C_4, C_5, C_7)	18.2	n.s.
1967	$S_1 \times S_1$	26.5	
	$S_1 \times OP_1$	25.5	n.s.
1968—69	$S_1 \times S_1$	19.7	***
	$S_1 \times OP_1$	20.1	***
	$OP_1 \times OP_1$ (C_1)	23.2	
1970	$S_1 \times S_1$	13.4	***
	$S_1 \times OP_1$	15.5	

It may be stated that the variance within sibships with one or two inbred parents is smaller than that found with non-inbred parents. This confirms theoretical expectations.

The percentage of filled seeds

The percentage of filled seeds has been calculated on the basis of radiography of the seeds of each cross. The results are summarised in *Table 4.* For comparison the results of ANDERSSON (1965) and SYLVÉN (cf. LANGLET 1940) are also

included. The data from ANDERSSON are based on table XLB embryo type 0—1 and the data from SYLVÉN on germinating seeds. The corresponding percentage of filled seeds might be somewhat larger. The crosses are grouped according to the mother and the mating type *(Fig. 1)*.

The general impression of the proportion of filled seeds is that there are great fluctuations of random nature from cross to cross. These large random variations make detailed analysis difficult. The mean values given in the table are often much influenced by a single extreme value.

Table 4

Mean values of different types of crosses (number of crosses in the different categories within brackets)

Year of cross: 1909 (SYLVÉN 1916)

Germinating seeds (%)

mother	S_1	OP_1
A	4.4 (1)	9.8 (1)
B	4.5 (1)	3.8 (1)
C	3.5 (1)	11.3 (1)
D	1.5 (1)	5.0 (1)

Year of cross: 1954 (ANDERSSON 1965)

Seeds with good embryos (%)

mother	$S_2(C_{14})$	S_1 (C_{13})	OP (C_3)	OP (C_2)
S_{1A}	0.7 (2)		39.1 (2)	
S_{1B}	0.2 (2)		29.9 (3)	
OP_{1A}		1.3 (1)		61.6 (4)
OP_{1B}		2.9 (1)		55.9 (5)

Filled seeds (%)

Year of cross: 1966

$S_2(C_{14})$	S_1 x unrelated OP(C_3)	
0.08 (4)	8.39 (12)	14.2 (2)
0.00 (1)	0.00 (1)	
0.00 (1)	F=0.25(C_{10},C_{11}) other (C_1,C_5,C_6)	OP(C_2)
	2.2(1) 1.44 (4)	32.6(1)
	0.1 (1) 10.2 (3)	
	0.6 (1) 1.71 (9)	62.8 (1)

Year of cross: 1967

mother	$S_2(C_{14})$	$S_{1p}xS_{1p}(C_{12})$	S_1 x unrelated (C_3,C_4,C_7,C_8)	OP (C_3)
S_{1A}	0.28 (5)	1.48 (3)	7.30 (19)	21.7 (4)
S_{1B}	0.55 (4)	2.55 (3)	7.65 (21)	
S_{1C}	4.13 (3)	1.72 (2)	9.66 (10)	10.0 (2)

Year of cross: 1968

	$S_2(C_{14})$	$S_{1p}xS_{1p}(C_{12})$	S_1 x unrelated (C_3,C_4,C_7,C_8)
S_{1A}	0.77 (5)	2.54 (4)	8.26 (11)
S_{1B}	0.50 (4)	0.76 (2)	9.54 (6)
S_{1C}	0.00 (1)		8.60 (2)

	$S_1(C_{13})$	F = 0.25* (C_{10},C_{11})	F=0.125 (C_9)	other (C_1,C_5,C_6)	OP (C_2)
OP_{1A}	7.95 (2)	18.3 (2)	28.5 (1)	21.0 (7)	
OP_{1B}	2.99 (2)	6.8 (1)	11.8 (2)	14.8 (8)	
OP_{1C}	4.15 (2)	27.2 (1)	2.66 (2)	13.6 (9)	0 (1)
OP_{1D}	1.67 (1)			15.2 (4)	

* including crosses of type C_{11} which have an S_1 mother

Year of cross: 1969

mother	S_2	S_{1p} x S_{1p}	S_1 x unrelated	OP (C_3)
S_{1A}	0.18 (3)	2.41 (3)	33.3 (6)	56.8 (3)
S_{1B}	2.24 (2)	10.3 (3)	13.9 (6)	51.9 (1)
S_{1C}	-	0.0 (1)	3.48 (2)	

	S_1	F=0.25*	F=0.125	other	OP (C_2)
OP_{1A}	1.61 (1)	20.9 (3)	23.4 (1)	12.2 (3)	
OP_{1B}	0.07 (1)	8.00 (4)	14.3 (1)	10.6 (6)	49.7 (1)
OP_{1C}	0.00 (1)	-	53.6 (1)	35.0 (4)	53.7 (1)
OP_{1D}	0.20 (1)		49.9 (1)	8.39 (5)	55.8 (1)

Year of cross: 1970

mother	S_2	S_{1p} x S_{1p}	S_1 x unrelated
S_{1A}	0.25 (3)	3.24 (6)	18.1 (21)
S_{1B}	0.27 (2)	-	-
S_{1C}	0.26 (1)	-	-

	other (C_3)	OP (C_2)
OP_{1A}	26.4 (24)	40.8 (1)
OP_{1B}	21.8 (24)	34.5 (1)
OP_{1C}	19.1 (19)	20.5 (3)
OP_{1D}		36.9 (1)

To evaluate the effect of inbreeding, it is desirable to compare with the results from "normal" crosses. However, in this context one must consider what constitutes a "normal" cross. First, open-pollination must be excluded as a suitable reference:

1. The pollen cloud might be physiologically different compared to the pollen used for artificial pollination.
2. Artificial pollination is done once. At a certain moment only a limited part of the ovules within a spruce strobilus are receptive. Therefore a lower seed yield is usually expected following artificial pollination compared with wind pollination.
3. Open pollination may include a substantial proportion of selfing or crossing with relatives. In Åkersberga the related trees (S_1 sibs, resp. half sibs) are growing side by side and this implies a high probability of inbreeding.
4. The general pollen cloud may not contain enough pollen for sufficient fertilization at the proper moment.

Results from controlled crossings between unrelated individuals may be more reliable. However, even this reference might have some drawbacks. This is especially so if there are marked differences between mothers and fathers as regards the ability to produce filled and vital seeds.

There seems to be no really important differences between the individual fathers and mothers. However, there is a possibility that the S_1 lines have a somewhat reduced percentage of filled seeds. Since the differences appear not to be systematic, it was considered best to calculate a mean value for all artificial crosses without inbreeding coefficient (C_1, C_4, C_5, C_6, C_7, C_8) for each year. The following results were obtained *(Table 5)*.

Table 5. — Filled seeds in reference crosses

	%	number of crosses
1966	5.11	(30)
1967	7.92	(50)
1968	13.06	(47)
1969	17.89	(32)
1970	21.59	(88)
1909	9	
1954	18	

To facilitate a comparison with the material of 1909, when poor germination was found following open pollination, and 1954, with good germination following open pollination, values were included in *Table 5* based on a reasonable guess.

Most of the filled seeds germinated, thus 88 per cent of the filled seeds in the material from 1966 germinated. On the basis of the values given in *Table 5*, the relationship between germination of inbred seeds versus non-inbred may be calculated. The mean values of the different crossing combination possibilities for all years pooled are found in *Table 6*.

Table 6. — The effect of inbreeding coefficient on amount of filled seeds (all materials pooled)

crossing	combination cf *Fig. 1*	F	mean (% of F = 0)	number of crosses
S_2	C_{14}	0.75	6.93	43
$S_{1p} \times S_{1p}$	C_{12}	0.5	26.4	27
S_1	C_{13} +1909	0.5	25.4	17
	C_{10}, C_{11}	0.25	80.5	14
	C_9	0.125	136.6	9
		0	100	

It must again be emphasised that the accuracy of the percentage values is affected by a few crosses with high percentages of filled seeds.

The comparatively good seed production of a cross between so closely related individuals as sibs originating from selfing emphasises the danger that may be involved in including close relatives in a second-generation seed orchard. Trees as closely related as ordinary full sibs *(Table 6)* give an almost normal yield of filled seeds. There appears to be no strong mechanism for reducing seed production following mating of relatives.

The very large variation in filled seeds between different crosses, different years and open pollination versus artificial pollination emphasises the uncertainties involved in the relationship between the production of filled seeds and inbreeding. Most investigators base their conclusion on a comparison with open pollination or a few crosses. In Norway spruce the random variation between crosses is evidenty so large that calculations and conclusions concerning the percentage of filled seeds must be based on an extremely large material to be meaningful. Those difficulties are well illustrated by the present investigation. In spite of a large number of crosses a considerable uncertainty still exists if the right reference values have been chosen.

The expected decrease of yield of filled seeds caused by embryonic lethals following inbreeding

The decreased yield of filled seeds following inbreeding is usually interpreted as an effect of recessive lethals. The consequences of this assumption will be discussed.

A hypothesis is assumed (cf. Koski 1971): A tree has a certain number of recessive factors. They behave independently of one another. Homozygosity of any factor means embryonic death. There exists a certain number of genetically identical haploid ova. A pollen grain fertilizes only one ovum. Thus several zygotes (embryos) may occur within one seed, but only one remains in the fully developed seed. If at least one zygote is not homozygous for any recessive factor a good seed will be produced.

If recessive lethals cause empty seeds this ought to be reflected in the relationship between different kinds of matings (S_1, S_2, $S_{1p} \times S_{1p}$ etc). The relationship between those different matings ought to give information about the number of embryonic recessive lethals (= n) and the number of zygotes per ovule (= k). The normal allele is denoted A and the recessive lethal a.

The calculation difficulties originating from polyembryony in spruce are considerable. No complete calculations will be carried out. Since the most probable number of k = 1.7 (Koski 1971) only the cases with k = 1 and k = 2 will be regarded. It must be pointed out that k covers a range of values within a seed lot, and that differences in k may occur between materials.

If there is only one embryo, selfing of an Aa individual will segregate 1 AA : 2 Aa : 1 aa. The last one dies and the segregation of survivors will be ⅓ AA : ⅔ Aa. If two embryos are involved the situation is more complicated: The following 8 types of ovules may arise (with the same probability) *(Table 7)*.

Table 7. — The different ovules possible following selfing of an Aa individ producing two embryos

case	embryo 1	embryo 2	Probability of survival of at least one embryo n = 1	n = 10
1	AA	AA		
2	AA	Aa		
3	Aa	AA	1	0.1366
4	Aa	Aa		
5	aA	aA		
6	aA	aa	1	0.0751
7	aa	aA		
8	aa	aa	0	0

The probability of elimination (i. e. empty seed) was calculated by aid of the formula given by Koski (1971) and Bramlett and Popham (1971)

$$P_n(k) = \frac{1}{2^n} \sum_{i=1}^{n} \binom{n}{i} (1 - 2^{-i})^k$$

There: n = number of embryonic recessive lethals,
k = number of embryos per ovule,
$P_n(k)$ = probability of elimination of the ovule,
i refers to the possibility that exactly i lethals are inherited on the female side.

As regards case 1—5 the ovule can be eliminated only by the other 9 lethals, thus $P_9(2) = 0.8644$.

As regards case 6—7 one embryo is eliminated. The probability that the other embryo is eliminated by any of the other 9 lethals is $P_9(1) = 0.9249$ ($= 1 - 0.75^9$).

The segregation ratio of the survivors may differ considerably from the "Mendelian" *(Table 8)*. If n = 1, k = 2 the proportion of AA will be (cf *Table 7*) $5/7 \times 4/10 = 0.286$ (cf *Table 8*). The general formula of the proportion of AA (k = 2) will be:

$$\frac{1}{2.5 + \dfrac{[1 - P_{n-1}(1)]}{[1 - P_{n-1}(2)]}}$$

S_1 is expected to act as a sieve against recessive lethals. The expected proportion of "surviving" lethals following selfing under different assumptions is the same as the segregation proportion of Aa.

Table 8. — Segregation ratio following selfing

		AA:	Aa
k = 1		0.333	0.667
k = 2	n = 1	0.286	0.714
	n = 5	0.317	0.683
	n = 10	0.328	0.622
	n = 15	0.331	0.669
	n = 20	0.332	0.668
	$n \to \infty$	0.333	0.667

The following number of survivors (filled seeds) following different mating procedures is expected with different assumptions concerning k and the number of lethals in the initial F_0 generation (n_0) *(Table 9).*

Table 9. — Theoretically expected proportion of filled seeds

		S_1	mean number of surviving lethals	S_2	sibs $S_{1p} \times S_{1p}$
k = 1	n_0 = 1	0.750	0.667	0.833	0.888
	n_0 = 5	0.237	3.333	0.387	0.532
	n_0 = 10	0.056	6.667	0.148	0.282
	n_0 = 15	0.013	10.00	0.046	0.149
k = 2	n_0 = 1	0.875	0.714	0.902	0.923
	n_0 = 5	0.379	3.92	0.490	0.593
	n_0 = 10	0.104	6.22	0.282	0.496
	n_0 = 15	0.026	10.03	0.103	0.249
	n_0 = 20	0.0063	13.4	0.041	0.139
Experimental pattern (cf *Table 6)*		0.254		0.069	0.264

The mean number of survivors in S_2 and $S_{1p} \times S_{1p}$ (C_{12} in *Fig. 1)* crosses is estimated from the mean number of lethals in S_1. E. g. S_2 ($n_0 = 10$, k = 2) is calculated as

$$1 - P_{6.22}(2) = 1 - 0.78\, P_6(2) - 0.22\, P_7(2) = 0.282.$$

(The values of P_n (k) for decimal n is calculated by interpolation as indicated in the example).

It would be more accurate, but less convenient, to base the calculation on a summation of different possibilities of the actual numbers of transmitted (S_2) or common ($S_{1p} \times S_{1p}$) lethals.

Concerning $n_0 = 1$ such a summation of the two different possibilities is easy (1 or no lethal transmitted or common).

In calculating the results of $S_{1p} \times S_{1p}$, the expected number of genes which will be mated Aa × Aa was first calculated, and then this number was introduced into the P_n (k) expression.

E. g. $n_0 = 10$, k = 2. The probability that a certain one of the 10 initial genes will occur in the configuration Aa × Aa in the $S_{1p} \times S_{1p}$ cross is $0.622 \times 0.622 = 0.387$. The expected number of common genes will then be $10 \times 0.387 = 3.87$, and the probability of survival $1 - P_{3.87}(2) = 0.496$.

It is evident from *Table 9* that the agreement between theory and experimental results is extremely poor. In theory, survival in S_2 would be considerably in excess of survival in S_1, but the reverse is found experimentally. If the S_2 survival of 7 per cent was accepted, a S_1 survival of the order of 1—2 per cent would have been expected, but 25 per cent is found. On the other hand, an S_1 survival of 25 per cent would indicate an S_2 survival in order of 40 per cent instead of 7 per cent. Furthermore, it would be expected that $S_{1p} \times S_{1p}$ was considerably in excess of S_1, but only a small difference is found. It might be objected that OP_1 trees need not reflect the number of lethals in the F_0 trees. However, SYLVÉN's data on selfing of the actual F_0 trees in 1909 would rather indicate a lower number of lethals in F_0 trees compared to OP_1 trees (cf *Table 5).*

It seems that the suggested hypothesis involving independent recessive lethals has to be discarded, at least in this particular case. An acceptable hypothesis must explain why S_2 trees are less self-fertile than S_1 trees. The explanation may involve epistasis, over-dominance or some presyngamic action. Probably clues to the solution are to be found in the extremely strong dependence of the coefficient of inbreeding and the independence of the way used to reach the coefficient of inbreeding.

Chlorophyll mutations

Chlorophyll mutations have been described in many forest species, i. e. by LANGNER (1953), EICHE (1955), KOSKI (1973). A literature review has been presented by FRANKLIN (1970). Chlorophyll mutations are detected as white, yellow or light green seedlings. The mutants mostly die soon, since they are unable to carry out photosynthesis. The mutant gene is expected to be inherited as a simple recessive character.

In the present material both albina (pure white) and chlorina (light green) seedlings were found. The frequencies of chlorina were never high and no regular pattern was found. We are not convinced that the chlorina phenotypes have a true genetic background. Concerning albina the situation seems to be clear. The percentage of albina mutant seedlings of all seedlings is shown in *Table 10.* For the material obtained from the crosses of 1967, there are no data available. The data from the other years are pooled in *Table 10* (except for wind pollination).

From the F_2-segregation ratios the F_1 genotypes may be determined *(Table 10).* A F_1 tree is classified as heterozygous (Aa)

1. If selfing produced 25 per cent albina seedlings
2. If crossing to another Aa tree produced 25 per cent albina seedlings
3. If wind pollination produces some albina seedlings

To be classified as AA the prerequisite is not to produce any mutants in a crossing with a known Aa tree.

The crossings were divided into the following categories: Aa × Aa (or Aa selfed), Aa × AA or AA × Aa and AA × AA (or AA selfed).

AA × AA. 64 crossing combinations, no single albina was found. Indicates that mixing of seeds between lots is not common.

Aa × AA. Some albina seedlings were found also in these combinations, although the percentage was low, as indicated in *Table 11.*

The following cases were detected *(Table 11):*

Table 11. — Crossing combination of type Aa × AA segregating chlorophyll mutants

cross	total	albina
2 × 37	10	1
9 × 29	143	1
49 × 18	249	1
49 × 29	255	1
49 × 134	104	1

There are further two probable cases (52 × 40 and 73 × 37). However, since the genotype of the mother trees

Table 10 Albina segregation (per cent)

F_0	F_1 ♀	geno type ♂	A						B									C							D	A			B			C			D					Open pollination				Other prov			
			2	4	7	9	15	16	18	21	22	26	27	28	29	31	33	34	37	38	39	40	41	42	43	49	64	77	81	96	101	117	134	135	140	144	146	149	151	66	67	68-69	70	O 2011 -	O 2012 -	P 2001 AA	P 2002 AA
	2	Aa	50										0		0			0	10																											
	4	Aa		0		31						0	0		0			0	0					0		28					0			0					2.1		1.0			0		
	7	Aa																																												
A	9	Aa			11	23	26	0	0			0	0		0.2		0	0	0	0		0	0	0		29		19		0				0			0		4.2		3.5				0	
Aa	15	Aa	46		33	11	0	0	0	0		0			0				0			0	0	0					0				0			0		6.6		1.1					
	16	AA					0	0						0				0	0																	0			0							
	18	AA						0	0								0	0		0								0													0				0		
	21	AA																																												
	22	-																																												
	23	AA				0							0																																	
	26	AA																																												
B	27	AA					0						0		0				0	0									0			0											0			
AA	28	-											0																																	
	29	AA										0			0														0														0			
	31	-																																												
	33	AA															0																													
	34	AA																	0																							0						
	37	AA																																													
	38	AA																																												
C	39	-																																												
AA ?	40	AA											0																													0						
	41	AA																																													
	42	AA																																												
D																																														
AA	43	-																																												
	49	Aa	33			14			0.4				0		0.4			0				0		0		9.3	19	28	0		0		1.0			0	0			2.4			9.3			0	
	51	-							0			0						0	0																											
	52	-										0			0				0			0.4																									
A	57	-							0						0			0				0																									
	64	-							0			0						0	0																											
	69	-																	1,5																											
	73	-										0			0				0			0																									
	77	Aa																												0			25			0										
	80	Aa				21		0										0				0																									
	81	AA						0									0									0			0		0		0			0	0				0			0	0	
	83	AA	0			0	0								0																															
B	91	AA				0	0											0	0																											
	95	AA					0	0														0																									
	96	AA				0		0										0				0																									
	98	AA				0	0											0	0																											
	101	AA					0	0				0							0			0					0			0		0			0											
C	104	Aa																																						11							
	108	-																																												
	117	Aa	22										0		0			0																							3.3			10				
	118	Aa					28	0				0			0																												5.9			
	119	AA				0		0	0						0																															
	134	AA				0	0					0																		0			0			0										
	135	Aa	23				39	0				0			0				0							23			0	0		0	23		0	0						0.8	3.8				0	
	140	AA																																												
	142	AA	0			0	0	0																																						
D	144	AA																	0																											
	146	AA																									0						0			0									
	149	AA																																												
	151	AA																		0							0			0			0			0		0				0	0			

is uncertain, they are not included. Probably 52 and 73 are of genotype Aa.

There are two possible explanations for the occurrence of mutants in these crosses. Either there is a contamination of the pollen or new mutations take place.

It is noted that all five cases found (and both suspected) occur in crosses Aa × AA and not in AA × Aa. This would have been expected if pollen contamination was responsible for the segregation. If mutations are responsible, the mutation frequency must be lower on the female side.

The frequency of pollen grains changing genotype is calculated in the following way: In all 11 269 seedlings originating from Aa × AA crosses have been analysed (1966: 890; 1968 + 69: 4355; 1970: 6024). Half of them have the a-gene from the mother, and thus 11 269/2 = 5634.5 pollen grains are analysed for transformation A → a.

Among these five were transformed, hence the ratio of transformed pollen grains is : $5/5634.5 = 8.87 \times 10^{-4}$.

If mutations are responsible, the given figure may be interpreted as a mutation ratio. It is a comparatively high mutation ratio to be in a single gene. For instance, it might be compared with 0.02×10^{-4} waxy resembling pollen grains found in *Larix leptolepis* (Eriksson *et al.* 1966).

The other alternative is that the true father is not the intended. Such a pollen contamination may arise in many ways. The male flowers are collected on the trees, and there may be pollen of other origin in the air. The flowers are forced in different rooms, but pollen may float or be transported by tools or persons between the rooms. There may be pollen in the air when the pollinations are performed, which contaminates during the artificial pollination.

It may be stated that the risk of contamination is low. For a quantitative estimate, it is necessary to guess the probability that contaminating pollen will carry the a-allele. Assume that the a-allele is as common in the pollen cloud as in the genotype-determined trees (cf. *Table 10).* Then the probability of contamination with an a-carrying pollen grains is 12/82 = 0.146. Then the contamination rate would be $\frac{8.87}{0.146} \times 10^{-4} = 6.1 \times 10^{-3}$.

A contamination rate below 1 per cent must be regarded as quite satisfactory with regard to the experimental difficulties involved. A possible exception is selfing of highly self-sterile individuals.

Aa × Aa

All crosses of this type are listed in *Table 12.*

As seen from *Table 12,* only two of 27 segregation ratios deviated significantly from the Mendelian 3 : 1 expectation, and the significances were not higher than could be explained by chance alone. The overall segregation ratios is in excellent agreement with expectation.

Thus the behaviour in F_2 of this albina gene seems to be an unusually good example of simple recessive inheritance in forest trees.

Table 12. — Segregating crosses of type Aa × Aa

Cross	F_2 seedlings normal	albina	Significance
2 × 2	1	1	ns
4 × 4	2	0	ns
4 × 9	62	28	ns
4 × 49	194	76	ns
9 × 7	8	1	ns
9 × 9	20	6	1 ns
9 × 15	61	21	ns
9 × 49	72	29	ns
9 × 77	13	3	ns
15 × 2	7	6	ns
15 × 7	8	1	ns
15 × 15	6	0	1 ns
49 × 2	2	2	ns
49 × 9	6	1	ns
49 × 49	39	4	*
49 × 64	196	45	*
49 × 77	161	64	ns
77 × 135	165	54	ns
80 × 9	173	53	ns
117 × 2	18	5	ns
117 × 9	173	54	1 ns
117 × 15	14	6	ns
118 × 15	13	5	ns
135 × 4	181	55	ns
135 × 15	11	7	ns
135 × 49	95	29	ns
135 × 135	41	12	ns
Sum	1 742	568	ns

total segregation = 24.6 per cent
Remarks 1 Pooled of several years
ns, *, **, *** Statistical significance for deviation from 3 normal: 1 albina (if total below 30 binomial, otherwise χ^2)

The genotypes of F_1 and F_0

It is expected that a heterozygote will segregate 1 AA : 2 Aa among the survivors following selfing and 1 AA : 1 Aa following open pollination, if the pollen cloud does not contain the a-allele. (The aa genotypes are eliminated.)

The genotype of C is almost certainly AA, since all six classified S_1 trees are AA. The probability that such a segregation would occur if C is Aa is $(1/3)^6 = 1/729$. It is therefore extremely surprising to find 2 AA : 4 Aa in the wind pollinated offspring from C. The explanation must be that the unknown fathers contribute the a-allele. The tree C was growing close to other spruce trees (Sylvén 1910). It might therefore be possible that the OP_1 offspring are more or less full sibs, and therefore it is not necessary that the a-allele is extremely common in the population.

That offspring from tree A and C both have the same allele indicates a common origin. The chlorophyll mutations are semilethal and will probably not stay in a population for any length of time. If several trees have the same chlorophyll mutant allele, it is an indication of a common ancestor a limited number of generations ago.

The heterozygotes seem to be over-represented in F_1. The S_1 offspring of A is expected to segregate 1 AA : 2 Aa, but segregates 1 AA : 5 Aa; the OP_1 offspring of A is expected to segregate 1 AA : 1 Aa but segregates 0 AA : 3 Aa; and finally, the OP_1 offspring of C cannot segregate more Aa than 1 AA : 1 Aa and ought to give fewer Aa, but the actual segregation is 2 AA : 4 Aa. This might be connected with heterosis in some way.

Wind pollinated offspring

Mutant seedlings were found in the OP offspring of all heterozygous F_1 trees. The occurrence of mutants in wind-pollinated offspring seems to be evidence enough to classify a tree as a heterozygote. The mutants occurring may be the result of selfing or of pollination from other heterozygotes. The frequency of the a-allele in the investigated trees was 12/82 = 0.146. About $0.5 \times 0.146 \approx 7$ per cent mutants would therefore be expected. Furthermore, related trees are planted together in the Åkersberga trial. If selfing was

the background, the less self-fertile S_1 trees would yield fewer mutants, but they do not. It might be concluded that there is no reason to believe that spontaneous selfing is responsible for the main part of the mutants found in wind-pollinated heterozygotes.

Two-clone seed orchard with S_1 trees

In this connection the possibility of establishing two-clone seed orchards with S_1 trees might be discussed. (This will be denoted $S_1 \times S_1$ orchard below.) There may be some advantage in following a tree-breeding program involving selfing of plus-trees, selection of the best individual from the best inbred progenies and including two clones selected in this way in a two-clone seed orchard. For larch this has been discussed by KEIDING (1968). The follwoing points may be discussed:

1. Outcrossing of inbred lines has been very successful in breeding agricultural crops, especially maize (cf. e. g. MÜNTZING 1965).

2. In forestry it is very time-consuming to carry out inbreeding through several generations. The main advantage with outcrossing of inbred lines is that a larger variation between the matings is obtained and thus a lower number of possible matings may be considered to obtain a given genetic gain.

The first generation of inbreeding may represent a stage around half-way to complete homozygosity in this respect (ANDERSSON and LINDGREN 1973). For forest trees it may well be that only the first generation of inbreeding pays.

3. The variation in the genetic quality of the seeds from an $S_1 \times S_1$ orchard would be smaller than that from an ordinary two-clone seed orchard. For several purposes this might be considered an advantage. If not, it is simple to achieve variation by mixing seeds of different origin. The low variation also means that more reliable estimates of the genetic quality may be obtained. Furthermore, the quality will not change from year to year.

4. The decreased variance within a sibship means that fewer individuals per cross give the same precision in a progeny test of S_1 clones.

5. Evidently S_1 trees can produce satisfactory amounts of male and female flowers, and are able to produce almost as good seeds as non-inbred trees.

6. An $S_1 \times S_1$ orchard would take considerably longer time to establish than a two F_0 clone seed orchard based on progeny tests. However, on the basis of experience from a small trial sown in 1953, including S_2 plants from Åkersberga, the inbred plant does not begin flowering later than normal. They grow more slowly. This might partly be regarded as an advantage (if slow growth does not reduce seed production), since it is more easy to harvest the cones from short trees.

7. A selection of the best individuals within a selfed sibship will increase the general combining ability compared to the F_0 parent.

8. The advantage of an $S_1 \times S_1$ orchard is more evident the higher is the specific combining ability, if each considered cross is included in a progeny test of the S_1 trees. However, even in the situation with only a progeny test of the F_0 and only general combining ability, an $S_1 \times S_1$ orchard may be the best choice, since it makes the best possible use of the general combining ability, and since the gain in selecting the best individuals adds to the gain obtained by choosing the best F_0 clones.

9. Probably the advantage of an $S_1 \times S_1$ orchard is most pronounced in provenance-cross of species-cross seed orchards. Their use has therefore been suggested by KEIDING (1968) for crossing Japanese and European larch.

10. The possibility of selfing must be carefully considered in planning a two-clones seed orchard. If S_1 trees are less self-fertile than F_0 trees, as is indicated in this investigation, it is of advantage to use S_1-trees in advanced seed orchard generations.

11. To make selfings is often a part of a progeny test program (to evaluate the self-fertility of the clone). The S_1 plants will give information about the general combining ability of the parent and thus be of value even if there is no intention of using them in a seed orchard. Therefore, the initial costs for keeping this option open are low.

12. S_1 can probably not be conveniently obtained from all F_0 plus trees. Therefore the method cannot be generally used.

13. Decreased vitality of homozygotes and the presence of recessive lethals may decrease the degree of homozygosity obtained following selfing.

14. If pollen contamination occur, the best individuals of an S_1-progeny may be just those originating from the contamination.

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Summary

Crossings have been performed in different ways between individuals obtained from selfing and open pollination of a number of parent trees.

The inbreeding depression on height growth in the nursery was dependent on growth conditions, the depression being more pronounced in bad conditions than in good. The variation within sibship was smaller in families with inbred parents compared with families with outbred parents.

The percentage of filled seeds was lower after two generations of inbreeding than after one. Mating of full and half sibs produced almost normal amounts of filled seeds.

The segregation of an albina mutation was studied in 27 different crosses between heterozygotes. A good fit to 3 : 1 segregation was found.

Key words: Chlorophyll mutations, selfing, nursery test, filled seeds, inbreeding depression, variation within sibship, two clone orchard, biclonal orchard.

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Choosing Mating Designs to Efficiently Estimate Genetic Variance Components for Trees

I. Sampling Errors of Standard Analysis of Variance Estimators[1])

By G. Namkoong and J. H. Roberds[2])

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The choice of a mating design for estimating genetic variances in forestry should be dictated by the likelihood of achieving the estimation objectives within the usual restrictions of time, space, cost, and biological limitations. Poor designs can cause poor estimates or failure to detect even very high genetic variances. Since experiments with trees are relatively expensive and time consuming, foresters try to estimate several parameters in the same experiment. This paper compares the relative efficiencies of standard, balanced, mating designs for estimating additive and dominance genetic variances. The main variables considered are mating design, total number of crosses, total number of parents, number of half-sib versus full-sib families, level of the genetic variances, and the use of sub-blocking. These are special cases of the general problem of estimating variance components (for a good review see Searle, 1971) expressed in a particular useful way for plant breeders.

The need for precision is often dependent on the relative size of the parameter which is being estimated. When the genetic variance is twice the nongenetic variance, a precise estimate of the genetic variance may not be necessary. When genetic variance is low for a trait however, interest may lie mainly in other traits with high heritabilities. In that case, estimation errors should be evaluated over all levels of heritability. Clearly, many other value functions can be used to evaluate designs in terms of the heritability. Since errors of estimation are also dependent on heritability, we examine efficiency over a continuous range of heritabilities.

Mating Designs

Consideration is restricted to designs using only one common generation of parental materials originating as a random sample from a single random mating population. The designs are the nested (A/B), factorial (AB), and diallel (AA) as designated by Cockerham (1963). All these designs are well known, and are described elsewhere (see Cockerham, 1963; Gardner, 1963). All permit estimates of full-sib, half-sib, and error variance components which can be interpreted in terms of genetic variances of the sampled population. Two useful variants of the diallel design are investigated here; the partial diallels of Hinkelmann and Stern (1960), and Kempthorne and Curnow (1961), and the disconnected diallels of Braaten (1965). For a given number of crosses, the partial diallels are always more efficient than the nested design (A/B) for both additive and dominance genetic variance components (Kempthorne and Curnow, 1961). Similarly, the disconnected diallels are more efficient than the factorial (AB) for estimating the additive genetic variance component at some heritabilities for the same number of crosses. These mating designs are schematically drawn in *Figure 1*. Their analyses of variance are listed in *Table 1* with their derived estimates of the additive genetic variance, σ_A^2, and the dominance genetic variance, σ_D^2, under the simplifying assumptions of no epistasis, no genotype by environment interaction, regular diploid inheritance, and no disequilibria of any kind.

We shall compare ratios of the sampling variances of the estimates of the additive genetic variance, $V(\hat{\sigma}_A^2)$ to the σ_A^2 itself. Similar ratios for the dominance genetic variance are $V(\hat{\sigma}_D^2)/\sigma_D^2$. Designs and various allocations of males and females are compared first for an equivalent number of crosses, then for an equivalent number of parents. If the work of making the crosses or conducting the field experiment is the limiting factor, then comparison should be based on equivalent numbers of crosses. If a set of parents is previously established, then the designs should be compared with equivalent numbers of parents. In such cases, there is some increased cost with increased numbers of crosses, but the additional costs are likely to be a non-linear function of the numbers. We do not investigate these costs but merely indicate that cost analyses should be applied to our functions.

Randomized complete blocks within gross environmental classes are assumed in this discussion and as many different environments as possible will be sampled. Hence,

[1]) This paper was inspired by the work of Dr. Klaus Stern, and is dedicated to him and to advancing the fruits of his labors. — Joint contribution of the Department of Genetics, North Carolina Agricultural Experiment Station, Raleigh, and the Southeastern Forest Experiment Station. Paper No. 4266 of the Journal Series.

[2]) Research Geneticist and Geneticist, respectively, Forest Service, U.S.D.A., and North Carolina State University, Raleigh.