

Nr 126 · 1975

The relationship between  
self-fertilization, empty seeds and  
seeds originating from selfing as  
a consequence of polyembryony

*Sambandet mellan självbefruktning, tomfrö och  
självpollineringsfrö som en följd av polyembryoni*

DAG LINDGREN

Department of Forest Genetics,  
Royal College of Forestry, Stockholm

## Abstract

*ODC 165.3 ÷ 164.8*

*The question of how to evaluate the relationship between self-fertilization, empty seeds and selfed seeds is discussed. Formulas are worked out for different situations. The method of making calculations in the various cases is illustrated by examples. The following situations are considered:*

*There is one embryo per ovule.*

*There are two polyzygotic embryos per ovule*

- genetic death is caused by homozygosity of recessive embryonic lethals*
- the probability of genetic death is not correlated between embryos of the same ovule.*

*The presence of polyembryony is of considerable importance for the relationship between the factors mentioned.*

*Firstly the situation where all outbred embryos are viable is considered and later the model is extended to the situation where outbred embryos are allowed to die.*

Ms. received 1975-01-30

LiberFörlag/Allmänna Förlaget

ISBN 91-38-02288-5

Berlingska Boktryckeriet, Lund 1975

# Contents

<b>Abstract</b> . . . . .	2	<b>6 Discussion</b> . . . . .	18
<b>1 Introduction</b> . . . . .	5	6.1 Outbred embryos may compete more successfully than selfed . . . . .	18
<b>2 Terminology</b> . . . . .	6	6.2 What is the function of polyembryony from an evolutionary point of view? . . . . .	19
<b>3 Symbols</b> . . . . .	7	6.3 Risk of contamination in a selfed progeny . . . . .	19
<b>4 Complete survival following outcrossing</b> . . . . .	8	6.4 The presence of recessive embryonic lethals . . . . .	20
4.1 Model assumptions . . . . .	8	6.5 Further remarks . . . . .	20
4.2 Possible cases . . . . .	8	<b>7 Concluding remarks</b> . . . . .	21
4.3 Evaluation of the consequences of the models . . . . .	9	<b>Acknowledgements</b> . . . . .	22
4.4 Values of $P_n(1)$ and $P_n(2)$ . . . . .	11	<b>Sammanfattning</b> . . . . .	23
4.5 Examples 1—3 . . . . .	12	<b>Literature cited</b> . . . . .	24
<b>5 Outbred embryos may die</b> . . . . .	13		
5.1 Model assumptions . . . . .	13		
5.2 Evaluation of the consequences of the models . . . . .	13		
5.3 Examples 4—5 . . . . .	15		

# 1 Introduction

Multiple archegonia, each containing a mature egg cell, are frequently formed in ovules of many conifers. The egg nuclei in the ovule are genetically identical. The egg cells in the same ovule may be fertilized by different pollen grains. Several genetically different zygotes may be formed and develop into embryos. During the development, the number is normally reduced to a single embryo at the time of cone maturity. Some embryos probably abort due to genetic reasons. This may be specially important following self-fertilization by the action of recessive, lethal or sublethal genes.

Abortion of all embryos leads to gametophytic tissue deterioration. The seed coat, however, may develop normally as it consists of diploid maternal tissue. The result is an unsound (empty, hollow) seed.

In pine, the formation of a seed coat is triggered by pollination (although the presence of a seed coat does not prove that fertilization has taken place).

Empty seeds are developed in spruce without pollination.

Cytological studies have shown that neither pollen germination, growth of the pollen tube, nor fertilization capability were reduced as a result of self-pollination. It is generally agreed that embryo abortion between fertilization and germination does account for reduced yields of filled seeds after selfing (cf references reviewed by Franklin 1970).

Calculations relating to the proportion of selfed seedlings, the amount of self-fertilization in open pollination, and the number of filled seeds following selfing and open pollination, are important in forest genetics. Polyembryony may interfere in such calculations. Sarvas (1962) pointed out these effects. The present paper will discuss how calculations can be performed if there are two embryos per ovule instead of one.

In order to have a clearer understanding, the situation where all outbred embryos survive, is studied first. Then the more complicated situation allowing for death of outbred embryos is studied.

## 2 Terminology

Some of the terminology used in this paper deviates from the conventional, and some is not in common use.

Embryo	Refers in this paper to all stages from the formation of the zygote to the mature embryo.		
Polyembryony	Exclusively polyzygotic polyembryony comprising zygotes potentially able to develop into a seedling. Cleavage polyembryony is not regarded. A seed with one mature embryo may well be polyembryo-		nic in the meaning of this paper. If the death of one particular embryo leads to an empty seed by collapse of all material within a seed coat, this seed is to be regarded as monoembryonic. The terms monoembryony and biembryony may be used.
		Selfed embryo	Embryo originating from selfing.
		Selfed seeds	Seeds with the single remaining mature embryo originating from selfing.

### 3 Symbols

S	= Self-fertilized		
O	= Outcrossed		
+	= Dies (empty seed)		
*	= Survives (filled seed)		
s	= Probability of self-fertilization		
k	= Number of embryos per ovule		
P	= Probability of death of an embryo following self-fertilization	i	= (Model 6) number of recessive lethals transmitted to the maternal genome.
$P_n(k)$	= (Model 3, 6) probability of death of all k embryos following self-fertilization	n	= (Model 3, 6) number of recessive embryonic lethals (embryonic lethal equivalents)
$P^1$	= (Model 4, 5) probability of death of an embryo following outcrossing	$Q_1$	= Proportion of empty seeds
m	= (Model 6) "matches", the mean number of lethals common to a certain individual and the pollen contributing population. In a controlled cross with known mother and father, m corresponds to the number of recessive embryonic lethals common to the father and the mother.	$Q_2$	= Proportion of selfed filled seeds compared to all filled seeds
		$Q_{1i}, Q_{2i}$	i indicates the model used (i = 1—7)

## 4 Complete survival following outcrossing

### 4.1 Model assumptions

In order to carry out calculations, suitable mathematical models to describe the reality, must be evaluated. To do this an array of assumptions have to be made:

- There may be one or two genetically different embryos in the same ovule.
- If, and only if, all embryos die of genetic causes, will an empty seed occur. — Will be discussed in more detail in connection with “Outbred embryos may die”.
- Following outcrossing, all embryos are viable. If this requirement is not fulfilled the consequences will be studied in the section “Outbred embryos may die”.
- If both embryos are viable, the one which will give rise to a single mature embryo which is able to give rise to a seedling is selected by chance only. Thus, outbred embryos are not favoured by competition. (Will be discussed in the section “Outbred embryos may compete more successfully than selfed”.)

Three different models will be considered in this section:

Model 1. No polyembryony. (Monoembryony). There is only one embryo per ovule.

Model 2. There are two embryos per ovule. (Biembryony). The probability of genetic death of one embryo is inde-

pendent of the probability of death of the other embryo in the same ovule.

Model 3. The probability of genetic death of two embryos belonging to the same ovule is correlated. The correlation is caused by the following mechanism: The maternal part of the genome is identical to all embryos in the same ovule. An embryo dies if, and only if, it is homozygous to any of a number of recessive lethals. Thus embryonic lethality is caused by the action of independent Mendelian lethal genes (cf Koski 1971, Bramlett and Popham 1971).

### 4.2 Possible cases

First the simple model 1, disregarding polyembryony, is studied. An embryo may be selfed and die (S+), selfed and survive (S\*) or outcrossed and survive (O\*). The different cases which may occur are listed in Table 1. For each case the probability that a selfed seedling will arise is also listed, as well as the probability that the certain case will occur.

If there are two embryos per ovule, the situation will become more complicated.

If both embryos are selfed and die (S+) an empty seed will be the result. If both are selfed and at least one survives a selfed seedling will

Table 1. Fertilization and survival of ovules with one embryo.

Case	Embryo type	Survival of ovule	Probability of selfed seed	Probability of case
1	S+	+	—	sP
2	S*	*	1	s(1 - P)
3	O*	*	0	$\frac{1-s}{1}$

Table 2. Fertilization and survival of ovules with two embryos.

Case	Embryo		Survival of ovule	Probability of selfed seed	Probability of the studied cases	
	1	2			Model 2	Model 3
1	S+	S+	+	-	$s^2P^2$	$s^2P_n(2)$
2	S+	S*	*	1	$s^2(1 - P^2)$	$s^2(1 - P_n(2))$
3	S*	S+	*	1		
4	S*	S*	*	1		
5	S*	O*	*	1/2		
6	O*	S*	*	1/2	$2s(1 - s)(1 - P)$	$2s(1 - s)(1 - P_n(1))$
7	S+	O*	*	0	$2s(1 - s)P + (1 - s)^2$	$2s(1 - s)P_n(1) + (1 - s)^2$
8	O*	S+	*	0		
9	O*	O*	*	0		
					1	1

Table 3. Formulas of interest for different models.

	Model 1 (mono-embryony)	Model 2 (independence)	Model 3 (dependence)
$Q_1$	$sP$	$s^2P^2$	$s^2P_n(2)$
$Q_2$	$\frac{s(1 - P)}{1 - sP}$	$\frac{s(1 - P)}{1 - sP}$	$\frac{s(1 - s)(1 - P_n(1)) + s^2(1 - P_n(2))}{1 - s^2P_n(2)}$

arise. If one of the embryos is selfed and survives and the other is outcrossed there is one chance in two that a selfed seedling will arise (if there is no competition against selfed embryos). If all surviving embryos are outcrossed the seedling will originate from outcrossing. Those different cases are regarded separately and the probabilities of the different constellations are listed in Table 2. The probabilities for similar cases are summed up.

#### 4.3 Evaluation of the consequences of the models

Of interest to calculate are:

$Q_1$  = Proportion of empty seeds

$Q_2$  = Proportion of selfed seeds compared to all filled seeds.

Using the data of Table 1 and 2, values for  $Q_1$  and  $Q_2$  were determined according to Table 3.

$Q_{22}$  is obtained in the following way:

Probability of selfed seeds (Table 2, model 2) (case 2 + 3 + 4) + 1/2 (case 5 + 6) =

$$s^2(1 - P^2) + s(1 - s)(1 - P) = s(1 - P)(sP + 1).$$

Probability of filled seed = 1 - case 1 =

$$1 - s^2P^2 = (1 - sP)(1 + sP).$$

$$Q_{22} = \frac{s(1 - P)(sP + 1)}{(1 - sP)(1 + sP)} = \frac{s(1 - P)}{1 - sP}$$

It also seems logical that the proportion of selfed seeds will be the same, independent of polyembryony, when the probability of embryonic death is independent of the probability of death in the other embryos within the same ovule.

Model 2 and 3 correspond to one another. The formulas of model 3 may be changed into those of model 2 by replacing

$$P_n(1) \rightarrow P \text{ and } P_n(2) \rightarrow P^2$$

$Q_{23}$  approaches  $Q_{22}$  for small  $s$ .  $P_n(2)$  occurs only in connection with  $s^2$ , and will be small of the second order.

Figure 1 was drawn to illustrate the relationship between the proportion of empty seeds following selfing ( $Q_1(s=1)$ ); the probability of embryonic death ( $P, P_n(1), n$ ); and the proportion of selfed filled seeds ( $Q_2$ ) obtained after a reasonable amount of self-fertilizations (follow-



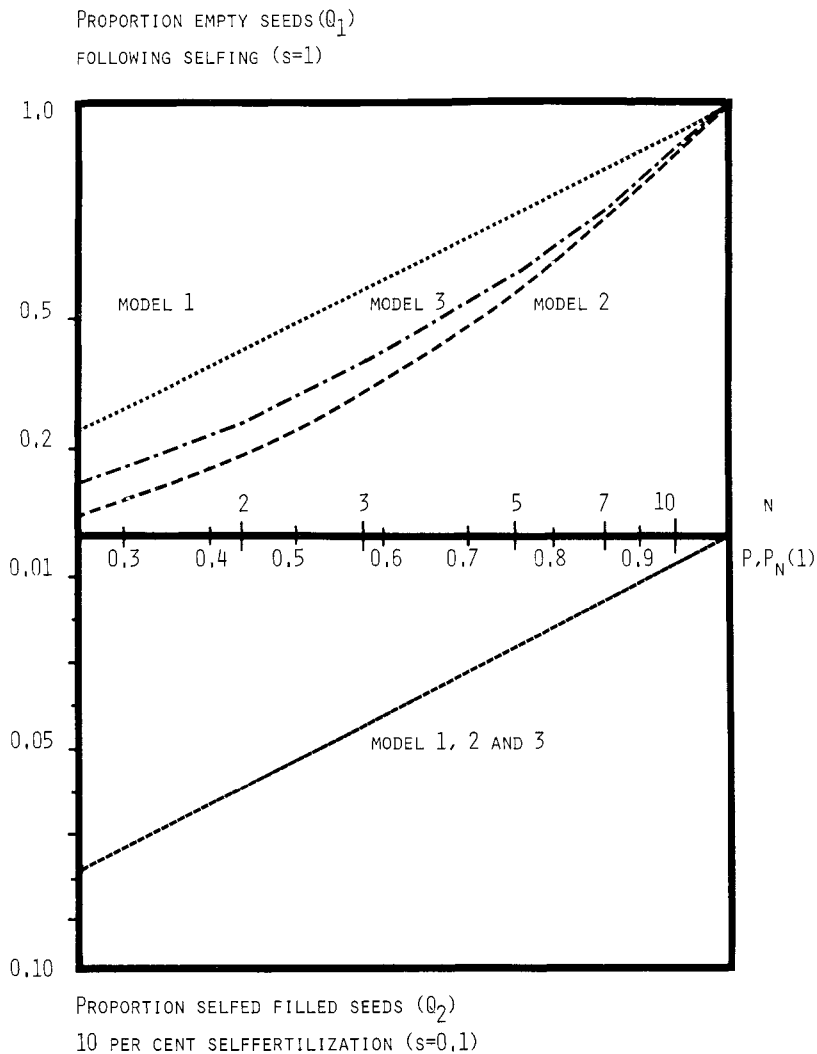


Figure 1. The proportion of empty seeds following selfing and the proportion of selfed seeds at 10 per cent self-fertilization as a function of the proportion of genetically dead embryos or number of recessive lethals (assuming no empty seeds in the outcrossed material).

ing open pollination,  $s=0.1$ , 10 per cent). All three models gave an almost identical relationship between  $P$  and  $Q_2$ . Moreover, this relationship was very close to linear ( $s$  is small,  $P^2_n(2)$  is preceded by  $s^2$ ). Almost identical and linear relationships are expected for all smaller  $s$  ( $s < 0.1$ ).

Concerning the possibilities of calculating  $P(n, P_n(1))$ , these are dependent on the model. The figure is drawn as a nomogram to facilitate

a comparison between the models. The experimentally determined amount of empty seeds following selfing may be read on the upward Y-axis. The corresponding  $P(P_n)$  may be the expected proportion of selfed filled seeds (seedlings) that corresponds to a certain  $P$  (embryo mortality) on the downward Y-axis. In the figure the curve for 10 per cent self-fertilization is drawn. The different models are almost identical for low  $s$ -values.

Table 4. Interpolation Table for calculating  $n$ ,  $P_n(1)$  or  $P_n(2)$ , when one of them is known.

$n$	$P_n(1)$	$P_n(2)$	$P_n(1)$	$n$	$P_n(2)$	$P_n(2)$	$n$	$P_n(1)$
0	.0000	.0000	.0000	0.000	.0000	.0000	0.000	.0000
1	.2500	.1250	.1000	0.400	.0500	.1000	0.800	.2000
2	.4375	.2656				.1250	1.000	.2500
3	.5781	.4004	.2000	0.800	.1000	.2000	1.533	.3500
4	.6836	.5198	.2500	1.000	.1250			
5	.7627	.6208	.3000	1.267	.1625	.3000	2.255	.4734
6	.8220	.7036	.4000	1.800	.2374	.4000	2.997	.5777
7	.8665	.7703	.5000	2.445	.3255	.5000	3.83	.6661
8	.8999	.8231	.5500	2.800	.3735	.5500	4.30	.7073
9	.9249	.8644	.6000	3.208	.4252	.6000	4.79	.7464
10	.9437	.8965	.6500	3.682	.4818	.6500	5.35	.7836
11	.9578	.9212	.7000	4.207	.5407	.7000	5.96	.8194
12	.9683	.9402	.7500	4.84	.6046	.7500	6.70	.8530
13	.9762	.9547	.8000	5.63	.6729	.8000	7.56	.8853
14	.9822	.9658	.8200	5.97	.7008	.8200	7.94	.8979
15	.9866	.9741	.8400	6.40	.7306	.8400	8.41	.9101
16	.9900	.9805	.8600	6.85	.7606	.8600	8.89	.9222
17	.9925	.9853	.8800	7.40	.7916	.8800	9.49	.9340
18	.9944	.9889	.9000	8.00	.8233	.9000	10.14	.9457
19	.9958	.9917	.9200	8.80	.8563	.9200	10.95	.9571
20	.9968	.9937	.9400	9.60	.8902	.9400	12.0	.9682
			.9500	10.45	.9075	.9500	12.7	.9736
			.9600	11.2	.9252	.9600	13.5	.9791
			.9700	12.2	.9433	.9700	14.5	.9844
			.9800	13.6	.9617	.9800	15.9	.9897
			.9900	16.0	.9805	.9900	18.4	.9950
			1.0000	$\rightarrow \infty$	1.000	1.0000	$\rightarrow \infty$	1.0000

#### 4.4 Values of $P_n(1)$ and $P_n(2)$

Values of  $P_n(1)$  and  $P_n(2)$  have been calculated by Koski (1971) and Bramlett and Popham (1971) independently. However, a higher accuracy than presented in the cited papers is necessary to carry out calculations. Such values will be found in Table 4. The values were obtained from Dr. Veikko Koski and have been checked by the author.

One of the main difficulties in handling the models described is that  $P_n(1)$  and  $P_n(2)$  are discontinuous variables. In this connection they may be defined as continuous variables. Noninteger  $n$  are regarded as a sum of the closest integer  $n$ .

Thus  $n=1.7$  gives the following values:

$$P_{1.7}(1) = 0.3 P_1(1) + 0.7 P_2(1) = 0.3812$$

$$P_{1.7}(2) = 0.3 P_1(2) + 0.7 P_2(2) = 0.2234$$

In a practical situation either  $P_n(1)$  or  $P_n(2)$  may be experimentally determinable, but the

other of the pair is required. Table 4 is constructed to facilitate such calculations.

The formulas of  $P_n(1)$  and  $P_n(2)$  are arrived at in the following way:

Assume exactly  $i$  of the  $n$  recessive lethals carried by an individual are transferred to the maternal genome. The probability of this is:  $\frac{1}{2^n} \binom{n}{i}$ . If this happens the probability that a selfed embryo will be homozygous for any of the  $i$  lethals is:

$$\left(1 - \frac{1}{2^i}\right). \text{ Thus } P_n(1) = \frac{1}{2^n} \sum_1^n \binom{n}{i} \left(1 - \frac{1}{2^i}\right).$$

The probability that two embryos of a biembryonic ovule will both be homozygous is:

$$\left(1 - \frac{1}{2^i}\right)^2. \text{ Thus } P_n(2) = \frac{1}{2^n} \sum_1^n \binom{n}{i} \left(1 - \frac{1}{2^i}\right)^2.$$

#### 4.5 Examples

*Example 1:* Following selfing of a clone, 50 per cent empty seeds were obtained. The level of self-fertilizations in that clone in a seed orchard has been estimated to be 10 per cent. What proportion of the filled seeds may be expected to originate from selfing after open pollination?

*Solution:* Principally, the problem might be solved with the aid of figure 1. Start at  $Q_1=0.5$ , go to the curve for resp. model, go down and read  $Q_2$ . (However, more reliable results may be obtained by using the corresponding formulas of  $Q_1$  and  $Q_2$ ) [ $Q_1(s=1)=0.5$ ; search  $Q_2(s=0.1)$  in analogy with Example 2]

	P	$Q_2$
Model 1	0.50	0.053
Model 2	0.71	0.031
Model 3	0.67	0.035

*Example 2:* Following selfing of a clone 77 per cent empty seeds were obtained and following open pollination 5 per cent. All empty seeds are caused by selfing. What is the expected level of self-fertilization and how many of the filled seeds will originate from selfing?

*Solution:* Using the formulas of Table 3, model 1.

$$Q_{11}(s=1)=P=0.77$$

$$Q_{11}(s)=0.77s=0.05 \Rightarrow s=0.0649$$

$$Q_{21}=0.0649(1-0.77)/(1-0.0649 \times 0.77)=0.0157$$

model 2

$$Q_{12}(s=1)=P^2=0.77 \Rightarrow P=0.8775$$

$$Q_{12}(s)=s^2P^2=0.77s^2=0.05$$

$$s^2=0.0649$$

$$s=0.2548$$

$$Q_{22}=\frac{0.2548(1-0.8775)}{1-0.2548 \times 0.8775}=0.04020$$

For model 3  $P_n(1)$  is calculated by aid of Table 4 ( $P_n(2)=0.77 \Rightarrow P_n(1)=0.8659$  by interpolation).

	s	$Q_2$
Model 1	0.0649	0.0157
Model 2	0.255	0.0402
Model 3	0.255	0.0425

*Example 3:* All ovules have two embryos ( $k=2$ ). Empty seeds are caused by recessive lethals (model 3). Each individual is a carrier of at least 5 lethals ( $n \geq 5$ ). What limits may be set for the correction factor of polyembryony (cf Lindgren 1974) when calculations of the proportion of selfed seeds in open pollinated progeny is based on knowledge of the self-fertilization level?

*Solution:* By using  $Q_{11}(s=1)$ ,  $P_n(2)$  is obtained instead of P. Then P is replaced by  $P_n(2)$  in formula  $Q_{21}$ . In an open pollinated progeny s is small.

For small s

$$Q_3=\frac{Q_{23}}{Q_{21}}=\frac{1-P_n(1)}{1-P_n(2)}$$

$$n=5 \text{ gives } Q_3=0.626$$

$$n \rightarrow \infty \text{ gives } Q_3 \rightarrow 0.500$$

Thus the sought factor is in the range 0.500—0.626.

# 5 Outbred embryos may die

## 5.1 Model assumptions

In the preceding section it was assumed that all outbred embryos would survive. This must not necessarily be the case. In this section the situation where outbred embryos could die will be considered. Three models will be studied which correspond to model 1, 2 and 3 with the exception that it is assumed that some outbred embryos might die.

Model 4. No polyembryony.

Model 5. There are two embryos per ovule. The probability of their genetic death is not correlated.

Model 6. There are two embryos per ovule. Their probability of genetic death is correlated in a way corresponding to the following mechanism. Selfed or outbred embryos die if, and only if, they are homozygous for any of a number of recessive lethal factors.

In addition an extension of model 1, 2 and 3 will be studied.

Model 7. Some seeds are empty for reasons not directly connected with the polyembryony. Additional empty seeds occur only according to model 1—3.

## 5.2 Evaluation of the consequences of the models

If there is only one embryo per ovule the embryo types described in Table 5 may arise (model 4).

If there are two embryos there are 16 possible combinations in analogy with Table 2. The resulting probabilities are presented in Table 6 (model 5).

The formulas for proportion empty seeds ( $Q_1$ ) and proportion filled seeds ( $Q_2$ ) obtained for model 4 and 5 with the aid of Table 5 and 6 have been compiled in Table 7.

The mother tree may have recessive lethals in common with the pollen contributing population. Outbred embryos may be homozygous for such lethals (cf Koski 1971). In a controlled cross, the number of lethals in common ("matches") may be determined from the percentage of empty seeds in the same way as the number of recessive lethals in an individual may be determined in a self. The situation following pollination with a mixture may be more difficult to handle, as the different fathers may constitute a different number of lethals in common.

The situation arising in a biembryonic ovule when one embryo is selfed and one is outcrossed is complicated. A complete analysis may be extended and difficult. The problem will be

Table 5. Fertilization and survival of ovules with one embryo (model 4).

Case	Embryo type	Survival of ovule	Probability of selfed seed	Probability
1	S+	+	—	sP
2	O+	+	—	(1-s)P <sup>1</sup>
3	S*	*	1	s(1-P)
4	O*	*	0	(1-s)(1-P <sup>1</sup> )

P = probability of death of selfed embryo

P<sup>1</sup> = probability of death of outbred embryo

Table 6. Possible constellations in ovules with two embryos.

Event	Probability of selfed seed	Corresponding probability model 5
Both embryos die, empty seed	—	$[P^1 + s(P - P^1)]^2$
One or two selfed embryos survive, if there is an outbred embryo it dies	1	$s(1 - P)[s(1 + P) + 2(1 - s)P^1]$
One selfed and one outbred embryo survive	1/2	$2s(1 - s)(1 - P)(1 - P^1)$
Only outbred embryos survive	0	$\frac{(1 - s)(1 - P^1)[2sP + (1 - s)(1 + P^1)]}{1}$

Table 7. Empty seeds and selfed filled seeds.

	Model 4 No polyembryony	Model 5 Independent embryos
Q <sub>1</sub> proportion empty seeds	$P^1 + s(P - P^1)$	$[P^1 + s(P - P^1)]^2$
Q <sub>2</sub> proportion filled selfed seeds	$\frac{s(1 - P)}{1 - P^1 - s(P - P^1)}$	$\frac{s(1 - P)}{1 - P^1 - s(P - P^1)}$

attacked in example 4 D, although no general formulas are given in this connection.

The situation allowing the death of outbred embryos in polyembryonic ovules branches into a manifold of subalternatives. The following alternatives will be treated in an example:

- A. A certain proportion of all seeds are empty for reasons not directly connected with the independent death of embryos. Examples of such mechanisms may be inefficient pollination (in eg. *Picea*, *Pseudotsuga* and *Larix* but probably not in *Pinus*), the action of seed bugs or cold damage (Sarvas 1962 p 123), stimulation of the integument tissue to develop a seed coat by non-viable pollen (Plym Forshell 1974) or a position of the seed in the cone preventing it from getting

enough nourishment (Plym Forshell 1974). (A corresponds to model 7).

- Alternative A may be divided into three subalternatives depending on whether there is only one embryo/ovule (A1), whether there are two independent embryos (A2) or two embryos which might be killed by the same recessive gene (A3).
- B. A certain proportion of all embryos, outcrossed and selfed, die from "other causes". There may be one (B1) or two (B2) independent embryos per ovule.
- C. Outbred embryos die due to interaction between the father and mother. There may be one or two independent embryos per ovule.

D. Outbred and selfed embryos die due to the action of recessive lethals, (cf Koski 1971, Bramlett and Pepper 1974). Thus the probabilities of death in embryos in the same ovule are correlated.

The main alternatives may be split partly into subalternatives, as demonstrated in the example.

All sorts of causes may act at the same time.

The method of calculation will be illustrated by an example, which will be treated in many different ways according to the assumptions.

### 5.3 Examples

*Example 4:* Following controlled pollination, a clone gives 10 per cent empty seeds and following selfing 80 per cent empty seeds. There may be one or two fertilized embryos per ovule. How does the proportion of empty seeds and filled selfed seeds depend on the proportion of self-fertilization?

With the mathematical designations:

$$Q_1(s=0)=0.10; Q_1(s=1)=0.80;$$

search  $Q_1(s)$  and  $Q_2(s)$

The results under different assumptions are illustrated in figure 2.

Alternative A. The proportion  $P_1$  of all seeds becomes empty for "other causes". The proportion of empty seeds  $Q_{17}=P_1+(1-P_1)Q_{11}$ , where  $Q_{11}$  refers to the model valid for the  $(1-P_1)$  seeds which do not become empty for other causes ( $i=1,2,3$ ).

$$Q_{1i}=\frac{Q_{17}-P_1}{1-P_1}$$

Concerning the proportion of selfed seeds ( $Q_{27}$ ) this will be the same as if there were no empty seeds for "other causes".

$$P_1=0.10 \text{ gives } Q_{11}=\frac{Q_{17}-0.10}{0.90}$$

$$s=1 \quad Q_{17}=0.80; \quad Q_{1i}(s=1)=0.777 \dots$$

Alternatively, calculations may be carried out based on the proportion of filled seeds compared to that following outcrossing. This is possible as:

$$1-Q_{1i}=\frac{1-Q_{17}}{1-P_1}$$

A1 One embryo per ovule

$$Q_{11}(s=1)=P=0.777 \dots$$

$$Q_{17}(s)=0.10+0.7s$$

$$Q_{27}(s)=Q_{21}=\frac{0.222s}{1-0.777s}$$

A2 Two independent embryos per ovule

$$Q_{12}(s=1)=P^2=0.777 \dots \Rightarrow P=0.883$$

$$Q_{17}(s)=0.10+0.7s^2$$

$$Q_{27}(s)=Q_{22}=\frac{0.117s}{1-0.883s}$$

A3 Two embryos per ovule, recessive lethals

$$Q_{12}(s=1)=P_n(2)=0.777 \Rightarrow P_n(1)=0.866$$

$$Q_{17}(s)=0.10+0.7s^2$$

$$Q_{27}(s)=Q_{23}=\frac{0.134s+0.088s^2}{1-0.777s^2}$$

Alternatives B and C are solved in the same way.

Probability of death of selfed embryo =  $P$

Probability of death of outbred embryo =  $P^1$

B1 One embryo per ovule

$$Q_{14}(s=0)=P^1=0.10; \quad Q_{14}(s=1)=P=0.80$$

$$Q_{14}(s)=0.10+0.70s$$

$$Q_{24}(s)=\frac{0.20s}{0.90-0.70s}=\frac{0.222s}{1-0.777s}$$

B2 Two independent embryos per ovule

$$Q_{15}(s=0)=P^{12}=0.10 \Rightarrow P^1=0.316$$

$$Q_{15}(s=1)=P^2=0.80 \Rightarrow P=0.894$$

$$Q_{15}(s)=[0.316+0.578s]^2$$

$$Q_{25}(s)=\frac{0.106s}{0.684-0.578s}=\frac{0.155s}{1-0.845s}$$

Alternative B may alternatively be dealt with as a reduction of the number of embryos per ovule.

Alternative D. Selfed as well as outbred embryos are killed by the action of recessive lethals. All ovules contain two embryos. Only empty seeds will be discussed in order to limit the discussion.

$$P_n(2)=0.80 \Rightarrow n=7.56 \text{ (Table 4)}$$

( $n$  has to be an integer. Thus  $n=7.56$  is interpreted that it is 56 per cent probability that  $n=8$  and 44 that  $n=7$ .)

The mean number of lethals common in foreign pollen contributors and the actual clon ( $m$ ) is determined analogous.

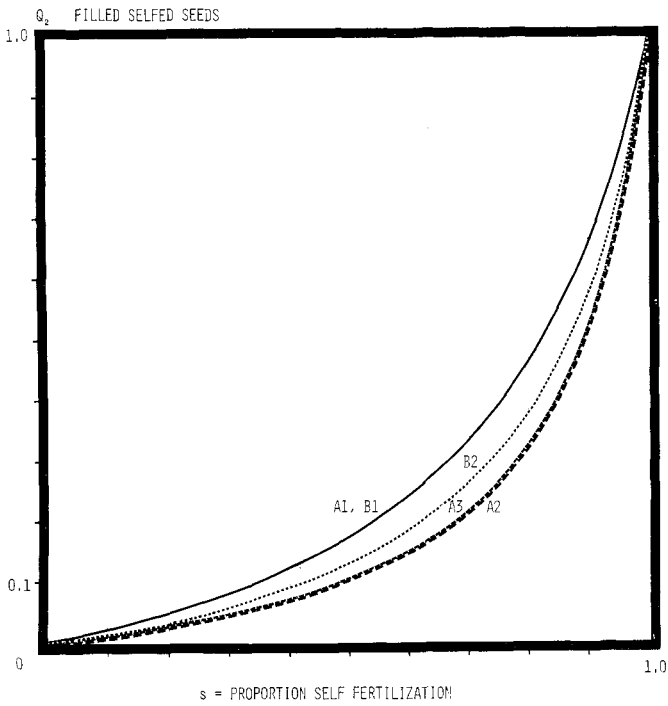
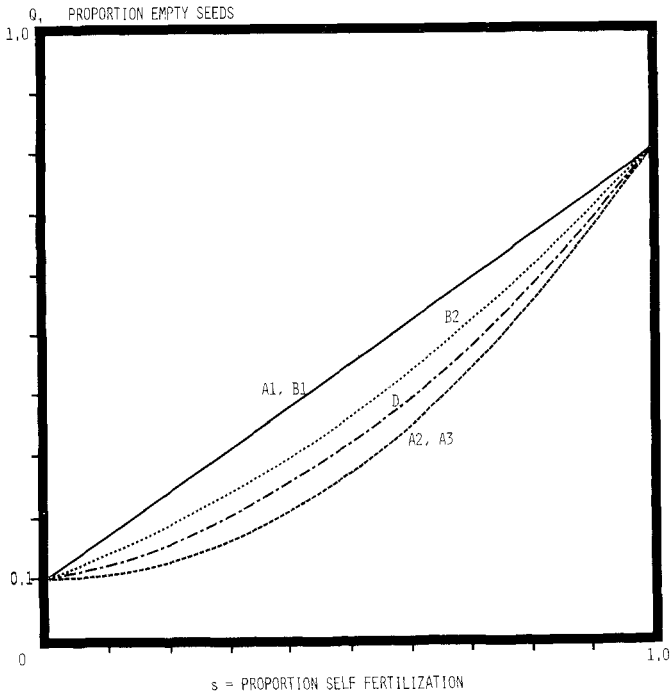


Figure 2. The proportion of empty seeds and the proportion of filled selfed seeds as a function of the proportion of self-fertilization. The different curves correspond to different assumptions concerning polyembryony (see text). The curves are drawn in the particular case (Example 4) that controlled outcross gives an empty seed yield of 10 per cent and controlled selfing an empty seed yield of 80 per cent.

$$P_m(2) = 0.10 \Rightarrow m = 0.800 \text{ (Table 4)}$$

In principle  $m$  has also to be an integer, but the pollen contributing population may be composed of parents which differ concerning  $m$ , and thus  $m$  may be regarded as an average value. A population composed of 80 per cent pollen from trees with  $m=1$  and 20 per cent pollen from trees with  $m=0$  would yield 10 per cent empty seeds following outcrossing, and is thus in agreement with experimental data.

The most interesting case is one outbred and one selfed embryo in the same ovule. If  $m=0$  no empty seeds will arise. If  $m=1$  the probability that the outcrossed embryo is homozygous is  $1/4$ , as the common lethal must be included in both joining gametes. The probability that the selfed embryo is also homozygous in the common lethal is  $1/2$ . The probability that it is homozygous for any of the other  $n-1$  lethals is  $P_{n-1}(1)$ . Thus the probability of getting both the outbred and the selfed embryo homozygous is:

$$1/4[1/2 + 1/2 P_{n-1}(1)]$$

The calculation may be divided into the following steps:

Case	n	Probability	Proportion empty seeds
0	7	0.088	0
0	8	0.112	0
1	7	0.352	0.2278
1	8	0.448	0.2333

The resulting probability of obtaining an empty seed is 0.185.

Open pollination may be approximated by a population in genetic equilibrium with equal gene frequency. In that case a Binomial or Poisson distribution may constitute a better approximation of the  $m$ -distribution than the distribution suggested. A calculation assuming Poisson-distribution gave the probability of empty seed 0.165. The true solution is probably within the interval 0.165—0.185. This is remark-

ably below the corresponding value of alternative B2:

$$0.316 \times 0.894 = 0.283.$$

To evaluate the probability of an empty seed as a function of  $s$  the following Table is constructed:

Case	Probability of case	Probability of empty seed in the particular case
both embryos selfed	$s^2$	0.800
one selfed, one outbred	$2s(1-s)$	0.185
both outbred	$(1-s)^2$	0.100

Probability of empty seed as a function of  $s$ :

$$0.800s^2 + 0.185 \times 2s(1-s) + 0.100(1-s)^2 = 0.100 + 0.169s + 0.531s^2$$

*Example 5:* Following selfing, a clone produces 80 per cent empty seeds; following outcrossing 10 per cent and following open pollination 20 per cent. What is the expected percentage of selfed seeds?

*Solution:* The problem might be graphically solved by the aid of figure 2. Start on 20 per cent empty seeds, choose the desired model and read the level of self-fertilization. Then read the proportion of filled selfed seeds for that level of self-fertilization. A calculation will be made for model 5, independent embryos:

$$Q_{1s}(s=0) = P^{12} = 0.10 \quad P^1 = 0.316$$

$$Q_{1s}(s=1) = P^2 = 0.80 \quad P = 0.894$$

$$Q_{1s}(s) = [0.316 + 0.578s]^2 = 0.20 \Rightarrow s = 0.227$$

$$Q_{2s}(s=0.227) = 0.0436$$

*Answer:* Around 4 per cent.



## 6 Discussion

### 6.1 Outbred embryos may compete more successfully than selfed

Several investigators (eg Ehrenberg *et al* 1955 and Plym Forshell 1974) have noted that embryos from selfed seeds often look worse than embryos from outbred seeds. The interpretation has been put forward that such observations indicate that the selfed embryos may generally have a lower competing capacity than those of outbred embryos.

The probability of obtaining a selfed seed if there are two genetically surviving embryos competing in one ovule; one selfed and one outbred, has been assumed to be 1/2 in the derivation of the formulas for the proportion of selfed seeds ( $Q_2$ ). It is easy to replace 1/2 by another experimentally determined factor, and derive formulas valid for other competing conditions.

However, when extending experimental results to conclusions concerning the competitive ability of selfed embryos, the polyembryonic nature of the ovules has to be considered. Following selfing most embryos die; most of the embryos giving rise to filled seeds will contain only one candidate for the mature embryo, and thus, even bad quality embryos may develop and give rise to seedlings. In the outbred ovules there may be several genetically surviving embryos which are able to develop into a mature embryo. In this competition a bad quality embryo has little chance of developing.

The reasoning will be exemplified by figures originating from Ehrenberg *et al* (1955, Table 1). On an average 92.8 per cent of the outcrossed seeds contained embryos belonging to the best embryo class (IV) and 7.2 per cent belonging to lower embryo classes (=weak embryos). Following selfing 83.8 per cent belonged to the best class and 16.2 per cent to the lower embryo classes.

It is assumed that if two embryos are com-

peting, an embryo belonging to the best embryo class always win over an embryo from a lower class. Otherwise the embryos have the same chance of winning.

The proportion of weak embryos= $X$ . In each outbred ovule there are two genetically surviving embryos. To obtain a weak embryo both initial embryos have to be weak. Thus:

$$X^2 = 0.072 \Rightarrow X = 0.268$$

Thus the probability of a good embryo following outcrossing is 73.2 per cent under these assumptions.

More embryos die following selfing. It is possible to find conditions giving 16.2 per cent weak embryos without assuming another value of proportion of weak embryos than that of outcrossing.

However, to be able to carry out calculations it might be assumed that the proportion between good and weak embryos obtained really are reflecting the true proportions. Then it is easy to calculate the probabilities of occurrence of different combinations of two embryos, one selfed and one outbred, based on data given by Ehrenberg *et al* (1955). Those calculations are carried out in the way demonstrated in Table 8.

The overall probability of a selfed seed is reduced to 0.455 instead of 0.5000, because of the assumed difference concerning the proportion of weak and good embryos. This might be regarded as an upper limit of the influence of the difference in embryo quality between selfed and outbred embryos.

There are several investigations dealing with mixtures of selfing and outcrossing pollen (Barnes *et al* 1962, King *et al* 1970 and others). The yield of selfed seeds is low compared to what was expected. This is usually interpreted as a reduced competing capacity in selfed embryos. However, such results may as well often be interpreted as an effect of polyem-

Table 8. Basic values for calculation of the influence of different embryo qualities in a biembryonic ovule.

Event	Probability of event	Probability of selfed seed
Both embryos belong to the best embryo class	0.778 (0.928 × 0.838)	0.5
The selfed belongs to the best, the outbred is weak	0.060 (0.838 × 0.072)	1
The outcrossed belongs to the best, the selfed is weak	0.150 (0.928 × 0.162)	0
Both are weak	0.012 (0.072 × 0.162)	0.5

bryony (*cf* figure 2). The following experiments are suggested for evaluating the competitive capacity of selfing pollen in pollen mixtures:

1. Use a completely self-fertile genotype as mother.
2. Use different pollination levels, either by applying different amounts of pollen or by correlating the obtained results with the amount of aborted ovules in the fertile part of the cones (might be done in *eg. Pinus sylvestris*, Sarvas 1962 p 106—119).

### 6.2 What is the function of polyembryony from an evolutionary point of view?

As the system of polyembryony has developed it is expected to have an evolutionary advantage. It does not directly decrease the amount of selfed offspring. For a constant mortality of selfed embryos ( $P$ ) and proportion of selfing pollen ( $s$ ), the proportion of selfed seeds ( $Q_2$ ) does not depend on the number of embryos per ovule ( $k$ ), at least not to any considerable extent. Thus  $Q_{21} = Q_{22}$ , and the curves for model 1, 2 and 3 in the lower part of figure 1 are identical. However, polyembryony may decrease the amount of selfed seedlings in a more indirect way as it will allow a population to limit the amount of selfing by recessive lethals without a corresponding loss of potential seed production.

Regard the situation with 30 per cent self-fertilization. With no recessive lethals 30 per

cent of the seedlings would die or develop into poor growing trees. If the species had been protected against self-fertilization by an infinite number of recessive lethals, 30 per cent of the seeds would become empty (all selfed embryos would die) if there was one embryo per ovule. But if there were two embryos per ovule only 9 per cent of the seeds would become empty. Thus a biembryonic tree would have a selective advantage against a monoembryonic, it would spread 30 per cent more filled outbred seeds (91 per cent against 70).

If the population has to stand a period of inbreeding (*eg.* spreading into a new area) the polyembryony in combination with recessive lethals improves the possibility to pass this period with a limited reduction of the heterozygosity.

If inbreeding occurred, the presence of recessive lethals may generally increase the level of heterozygotes compared to the situation without lethals.

Situations may occur (*eg.* isolated trees) when the own pollen is the only available. In such situations polyembryony improves the possibility to get any seeds at all.

### 6.3 Risk of contamination in a selfed progeny

In controlled selfings there is always a risk that some pollen contamination will occur. Andersson *et al* (1974) estimated the percentage

of contaminating pollen in controlled crosses to 0.6 per cent. However, it is notable that in the example presented in figure 2, one per cent contaminating pollen in a controlled selfing means that 4.4 per cent of the seedlings would originate from outcrossing if there is no polyembryony, and between 6.1 and 7.9 per cent if there are two embryos per ovule. Thus, there is a considerable risk that in selfed progenies there will be individuals originating from outcrossing with foreign pollen.

#### 6.4 The presence of recessive embryonic lethals

In model 3 and 6 it has been assumed that there is a correlation between embryos within the same ovule caused by the action of individually acting recessive genes. This mechanism of gene action may be doubted. Andersson *et al* (1974) have carried out second generation matings in Norway spruce. They found more empty seeds in  $S_2$  than in  $S_1$ , and the same amount of empty seeds in crosses between full sibs originating from selfing as in  $S_1$ . Those results do not agree with the hypothesis that empty seeds are caused by the action of individually acting recessive embryonic lethals. The number of embryonic lethals should be lower in the  $S_1$  generation than in  $S_0$ , if the obtained percentage of empty seeds are interpreted as caused by independent recessive embryonic lethals. It seems more reasonable that embryo lethality is caused by the combined action of many recessive sublethal genes, which could not separately kill an embryo (detrimental genes). However, even in this case a "genetic correlation" between embryos within the same ovule may arise. Correlation between embryos may probably arise because of environmental reasons, as well.

Although models have been developed assuming a mechanism including "number of recessive embryonic lethals" ( $=n$ ), it might be better to interpret " $n$ " as "embryonic lethal equivalents". The later term is more general, and might to a certain degree include the possibility of several interacting detrimental genes. It also considers the possibility that some of the embryonic lethals may be closely linked with others, and thus hidden.

#### 6.5 Further remarks

A general source of experimental uncertainty is that the polyembryonic conditions may not be identical within a material. Eg. open pollinated material may receive more or less pollen than control pollinated material which could lead to a lower or higher number of embryos per ovule. In a cold year in a hard climate the maturation of the seeds may be limited, and fewer embryos will be able to germinate under such conditions.

Complicating factors may be the possibility of embryonic death in different stages. Embryonic death at an early stage may allow another embryo to take over, but collapse of the terminal embryo at a later stage may lead to complete collapse of all material within the seed coat. There is also a chance that some genetic constitutions lead to incompatibility between embryo and endosperm thus causing endosperm death. Thus, a seed might be biembryonic for some embryonic lethals and monoembryonic for others. Another complication is that some eggcells may have a lower probability than others to develop into mature embryos, eg because of later fertilization.

Certainly the proportion of "selfed seedlings" may be reduced compared with "selfed seeds". The problem may be mainly one of definition. A viable embryo may be defined as "an embryo able to give rise to a seedling". Alternatively some measurable constants may be added expressing the probability that a filled seed will give rise to a seedling following selfing and following outcrossing. To avoid those difficulties it is preferable to limit discussion to the proportion of "selfed filled seeds". It is understood that these seeds contain a single embryo which is able to develop, and this embryo originates from selfing. The problem might be rather important. In the literature review by Franklin (1970) indications of low germination of filled selfed seeds compared with outcrossed are common.

The finding of example 3 could probably be extended to a general rule: the overestimation of the proportion of selfed seeds at a low rate of self-fertilization and a low percentage of filled seeds following selfing when assuming monoembryony equals  $k$  ( $=$  number of embryos per ovule).

## 7 Concluding remarks

Polyembryony has an important effect on the relationship between the levels of self-fertilization, production of selfed seeds and seedlings, and the production of empty seeds.

If the amount of empty seeds following selfing and the level of self-fertilization are known, then the amount of selfed seeds may be over-estimated, if no attention is paid to the polyembryony. If the amount of empty seeds following selfing and open pollination are known, the level of self-fertilization and the amount of selfed seeds may be underestimated.

Calculations have been carried out for one and two embryos per ovule. The situation with a mixture of different numbers of embryos in different ovules may be easily handled by simple addition.

Two embryos per ovule might be the most reasonable integer number from *Picea abies* and *Pinus sylvestris* (cf Sarvas 1962 and Koski 1971). Thus, without further knowledge of the polyembryony in the actual material, more accurate results may be obtained if the formulas assuming two embryos per ovule are used instead of those assuming one embryo per ovule.

If there are more than two embryos per ovule, further calculations must be carried out. If there are three possibilities for each embryo (S+, S\* and O\*), the number of possible cases is  $3^k$ . If four embryo types are regarded the corresponding number of cases is  $4^k$ . However, as for  $k=2$ , similar cases may be pooled.

## **Acknowledgements**

I am deeply indebted to Tony Squillace, Carlyle Franklin and Gösta Eriksson for reviewing the manuscript and giving valuable suggestions for amendments. I am grateful to Helge Johnsson and Marianne Rasmuson, who have contributed with valuable comments. I would also like to thank Veikko Koski for some of the figures presented in Table 4.

# Sammanfattning

## Sambandet mellan självbefruktning, tomfrö och självpollineringsfrö som en följd av polyembryoni

Hos barrträden förekommer ofta en speciell typ av multipel befruktning. I varje fröämne bildas flera genetiskt identiska äggceller, som kan befruktas med olika pollenkorn. Endast en av de på så sätt bildade zygoterna ger upphov till en mogen planta. Närvaron av flera genetiskt olika zygoter benämnes i denna uppsats polyembryoni. Polyembryonin innebär att om ett embryo dör på ett tidigt stadium så kan ett annat ta över. Om alla embryoner dör utbildas ett tomt frö. Embryodödligheten är större efter självbefruktning än efter korsbefruktning. Ofta uppskattas självbefruktningsfrekvensen med ledning av tomfröförekomsten. Förekomsten av polyembryoni är av betydelse vid beräkningar som relaterar självbefruktningsfrekvens, tomfröhalt och frekvensen plantor som härrör från självbefruktning. Dylika beräkningar är praktiskt betydelsefulla. Föreliggande arbete redogör för hur dylika beräkningar kan utföras i olika situationer och under olika antaganden. Metodiken illustreras med beräkningar och figurer.

I Figur 1 demonstreras hur tomfröfrekvensen ( $Q_1$ ) beror av den genetiskt betingade dödligheten av embryoner efter kontrollerad självbefruktning (P). De tre kurvorna svarar mot följande antaganden:

Modell 1. Ett embryo per fröämne.

Modell 2. Två embryoner per fröämne, båda med dödligheten P.

Modell 3. Två embryoner per fröämne, ett embryo dör om det är homozygot för någon av n recessiva letalgener i föräldra-genotypen.

I figurens nedre del visas hur frekvensen frön från självpollinering ( $Q_2$ ) beror av P vid självbefruktningsfrekvensen (s) 10%. För en given tomfröfrekvens blir den beräknade embryoletaliteten i hög grad olika för ett antagande av ett embryo per fröämne jämfört med två. Detta i sin tur leder till avsevärda skillnader vid uppskattning av frekvensen frön som härrör från självpollinering.

I Figur 2 åskådliggöres hur tomfröfrekvens ( $Q_1$ ) och frekvensen frön som härrör från självpollinering ( $Q_2$ ) beror av självbefruktningsfrekvensen (s). Förutsättningen är att man experimentellt bestämt tomfröhalten efter kontrollerad självpollinering till 80% och efter kontrollerad korsning till 10%. De olika kurvorna svarar mot olika antaganden hur polyembryonin fungerar.

A1, A2 och A3 svarar mot modellerna 1, 2 och 3 under antagandet att en viss del av fröna blir tomma av skäl som inte har med embryoletaliteten att göra.

B1 (ett embryo per fröämne) och B2 (två embryoner per fröämne) betyder att en del av embryonerna dör av icke genetiska skäl.

D innebär att embryodödligheten orsakas av recessiva letalfaktorer som förekommer i både moderträdet och det utifrån kommande pollenet.

## Literature cited

1. **Andersson, E., Jansson, R. and Lindgren, D.** 1974. Some results from second generation crossings involving inbreeding in Norway spruce (*Picea abies*). — *Silvae Genetica* 23: 34—43.
2. **Barnes, B. V., Bingham, R. T. and Squillace, A. E.** 1962. Selective fertilization in *Pinus monticola* Dougl. — *Silvae Genetica* 11: 103—110.
3. **Bramlett, D. L. and Pepper, W. D.** 1974. Seed yield from a diallel cross in Virginia pine. — Proceedings of a Colloquium "Seed yield from southern pine seed orchards". Ed. Kraus, J.: 49—55.
4. **Bramlett, D. and Popham, T.** 1971. Model relating unsound seed and embryonic lethal alleles in self-pollinated pines. — *Silvae Genetica* 20: 192—193.
5. **Ehrenberg, C., Gustafsson, Å., Plym-Forsshell, C. and Simak, M.** 1955. Seed quality and the principles of forest genetics. — *Hereditas* 41: 291—366.
6. **Franklin, C.** 1970. Survey of mutant forms and inbreeding depression in species of the family *Pinacea*. — USDA Forest Service Research Paper SE-61.
7. **King, J. P., Jeffers, R. M. and Nienstaedt, H.** 1970. Effects of varying proportions of self-pollen on seed yield, seed quality and seedling development in *Picea glauca*. — In proc. of "Sexual reproduction of forest trees", Finland 28,5—5.6 1970.
8. **Koski, V.** 1971. Embryonic lethals of *Picea abies* and *Pinus silvestris*. — *Commun. Inst. For. Fenn.* 75.3: 1—30.
9. **Lindgren, D.** 1974. Aspects on suitable number of clones in a seed orchard. IUFRO Joint Meeting of Working Parties S 2.04. 1—3, 30.8—5.9.1974. Proc. published by the Dept. of Forest Genetics, S-10405 Stockholm, pp. 293—305.
10. **Plym Forsshell, C.** 1974. Seed development after self-pollination and cross-pollination of Scots pine, *Pinus sylvestris* L. — *Studia Forestalia Suecica* 118: 1—24.
11. **Sarvas, R.** 1962. Investigations on the flowering and seed crop of *Pinus silvestris*. — *Commun. Inst. For. Fenn.* 53.4: 1—198.