Zusammenfassung

Bei Gaswechseluntersuchungen an 7 Monate alten Sämlingen von *Pinus merkusii* aus 2 Vorkommen Thailands (etwa 18^0 und etwa 15^0 nördlicher Breite) und aus einem Vorkommen der Insel Java (etwa 7^0 südlicher Breite) reagierten die thailändischen Herkünfte anders als die Herkunft aus Java. Gleichzeitig ergaben sich Wechselbeziehungen zu dem bei dieser Baumart auftretenden sog. Gras-Stadium.

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Contributions to the genetics of empty grains in the seed of pine (Pinus silvestris)

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Introduction

Already when artificial extraction of seed from conifers was started the observations were made that a certain amount of empty grains was contained in the seed. Schotte (1905) speaks about dull seeds. Hagem (1917) stated that pine seed lots from the Vestland in Norway contained 21.4 ± 3.1% empty grains on an average with a variation from stand to stand between 7 and 33%. Heikinheimo (1937) found that the average percentage of empty grains in seed from Finnish stands was 18.2%. In the seeds from other Finnish stands Sarvas (1962) counted 13.7% empty grains with a stand to stand variation between 10 and 18%. Dengler (1932) observed that selfing was followed by a very noticeable increase in the frequency of empty grains. This experience has been confirmed later on by a number of authors, i. e. Plym-Forshell (1953) and Koski (1971).

The penomenon in question is also known in other gymnosperms. Thus Allen (1942) stated that selfing resulted in but few germinable seeds in *Pseudotsuga menziesii,* which was supported by Duffield (1950). Orr-Ewing (1957) examined the anatomical development of the ovules, resulting from selfing in the said species, and stated that the embryos collapsed at an earlier or later phase. He interpreted these events as a consequence of homozygotisation of recessive deleterious genes following self-fertilization. This theory has also been generally adopted for *Pinus silvestris* (Johnsson 1961, Sarvas 1962, Koski 1971) as for other *Pinus* species, i.e. *P. banksiana* (Fowler 1964), and can be postulated as being valid for the gymnosperms in general.

Koski (1971) with the frequencies of empty grains after selfing as basis, has carried out calculations of the number of recessive embryonic lethals. According to these calculations there is an individual range from 2—20 with 9.4 as an average in the investigated material and 70—80 in the populations. In *Pinus virginiana* Bramlett and Pepper (1974) estimated that 150—180 such genes would provide the most satisfactory explanation of the frequency of empty grains after individual crosses.

In this report, data, obtained after open pollination and individual crosses, are presented and treated from a quantitative genetic point of view. The grafted clones, on which the studies are based, are derived from plus trees, selected in natural stands in South Sweden between the latitudes 57^0 and 58^0. Only in some few cases has more than one tree been selected in the same stand.

Materials and Results

The frequencies of empty grains in 30 clones after open pollination in a seed orchard during five successive years.

In a seed orchard with 30 clones, the seed quality of the separate clones has been analyzed during five successive years, beginning in 1967. The orchard was established in 1954 with three-year old grafts. Thus, the earliest analyzed seed crop had originated from the flowering 14 years after the grafting and 11 years after the establishment of the orchard. At that time the clones had a fairly abundant male flowering though of varying intensity. For the analysis, 50 cones were used, gathered at random from 10 ramets of each clone. Thus, the percentages as shown in *Table 1* are based on around 1,000 seeds. A glance at the Table reveals immediately sharp differences between the clones. Clone F 1000 has given such a high value as 44.3%, but clone H 1001, not more than 7.9%. The grand total is 19.1%, which agrees very well with the frequencies of empty grains in natural stands.

Table 1. — Percentages of empty grains after open pollination in a seed orchard in five successive years.

Clone	Percentages of empty grains in the year					Average
	1967	1968	1969	1970	1971	
E 2001	6.6	13.9	12.7	10.9	8.3	11.3
E 2002	21.3	20.0	8.9	25.0	15.9	18.8
E 1000	8.1	26.7	17.8	10.8	8.8	15.4
F 2011	18.7	10.3	9.6	29.8	10.6	19.4
F 1000	43.2	37.7	32.0	47.9	53.5	44.3
F 2007	22.9	40.8	26.7	24.7	16.5	28.6
F 3001	25.7	27.3	2.3	19.6	13.8	21.3
G 2004	29.4	31.4	18.4	24.6	18.4	27.3
G 2001	20.8	13.5	20.6	16.2	7.7	20.0
G 1001	29.3	21.7	12.8	17.1	12.0	22.3
G 3001	11.0	5.4	8.2	9.9	6.7	10.4
G 2003	9.4	18.0	7.0	7.0	5.8	10.3
H 2001	29.7	16.0	10.0	8.0	10.0	20.1
H 2008	24.7	21.7	14.8	13.4	16.7	19.6
H 2006	21.7	14.7	14.2	17.5	14.4	17.9
H 1004	22.8	38.4	18.3	35.8	19.5	26.5
H 1019	19.0	15.7	13.4	18.1	9.0	15.6
H 1005	10.4	24.5	9.7	17.6	13.5	16.1
H 1006	38.2	22.6	21.5	37.2	20.0	32.7
H 2010	24.3	31.8	22.5	25.6	19.6	27.3
H 2007	27.1	40.2	28.2	31.4	29.8	36.2
H 2016	32.3	22.4	33.0	19.9	8.5	23.1
H 3001	24.2	33.7	21.2	17.5	16.1	24.3
H 3002	19.1	16.4	11.2	12.9	8.7	17.9
H 1001	12.6	6.0	5.5	8.9	3.4	7.9
H 1020	9.5	19.2	9.3	17.1	10.8	14.8
H 2004	20.1	29.3	13.8	14.9	10.3	20.1
H 2003	13.0	17.0	28.9	18.5	10.1	18.3
H 1002	22.0	14.1	14.9	16.8	5.6	18.3
H 1003	22.0	37.7	21.6	42.0	33.0	31.9
Average	21.3	22.9	16.3	20.6	14.6	19.1

With the theory that the empty grains are caused by recessive embryo-lethal genes as starting point, the differences between the clones can be interpreted in two ways: they might express either varying degrees of selfing — a clone with a very abundant male flowering will, of course, be self-pollinated to a greater extent than a clone with weak male flowering — or a varying load of lethal genes.

An analysis of variance of Table 1 falls out as below:

	DF	MS	Expectations
Years	4	375.05	$\sigma^2 + \sigma^2_{YC} + 30\,\sigma^2_Y$
Clones	29	303.98	$\sigma^2 + \sigma^2_{YC} + 5\,\sigma^2_C$
Years × clones	116	35.07	$\sigma^2 + \sigma^2_{YC}$

The F-quotient for clone differences is 8.668*** and the causal variances are: $\sigma^2_C = 53.8$, $\sigma^2_Y = 11.3$ and $\sigma^2_T = 100.2$. Thus, 53.7% of the clonal differences are caused by peculiarities, characteristic for the individual clone, namely a varying degree of selfing or a varying genetic load.

The breeding values for the parents can be calculated according to the formula $\bar{x}_i = \bar{x} + \hat{g}_i \cdot \bar{x}_i =$ clone mean, $\bar{x} =$ grand total, $\hat{g}_i =$ breeding value for clone i. The $\hat{g}_i$-values calculated form Table 1 are given in *Table 2* in the column, denoted "o.p."

Table 3. — Percentages of empty grains, obtained after crosses with 4 common testers.

♀ ♂	F 1000	H 2016	H 2004	G 3001	Average
E 2001	11.3	22.2	18.8	11.0	15.8
E 2002	12.5	10.5	9.9	24.4	14.3
E 1000	18.2	4.9	10.5	21.5	13.8
F 2011	20.5	10.3	9.6	24.5	16.2
F 1000	—	52.3	42.0	45.6	46.6
F 2007	9.7	13.3	33.9	12.4	17.3
F 3001	26.3	16.4	16.6	20.0	19.8
G 2004	24.7	16.4	22.0	20.0	20.7
G 2001	25.1	8.0	8.6	24.4	16.5
G 1001	23.6	22.8	20.0	19.3	21.4
G 3001	25.5	7.2	2.6	—	11.8
G 2003	20.0	3.4	8.5	10.0	10.5
H 2001	16.1	15.9	15.3	22.7	17.5
H 2008	25.5	13.2	9.6	17.1	16.3
H 2006	26.2	—	12.5	20.8	19.8
H 1004	32.4	18.3	36.2	16.8	25.9
H 1019	16.2	8.4	13.3	20.1	14.5
H 1005	18.3	7.0	15.4	12.1	13.2
H 1006	40.2	11.8	18.3	15.4	21.4
H 2010	26.5	11.3	10.3	18.6	16.7
H 2007	42.7	39.7	32.9	32.2	36.9
H 2016	19.8	—	19.0	35.4	24.7
H 3001	16.6	8.4	9.4	13.2	11.9
H 1001	6.1	6.3	6.4	9.3	7.0
H 1020	15.6	5.9	11.5	—	11.0
H 2004	13.6	12.5	—	—	13.1
H 2003	22.1	25.2	10.2	11.7	17.3
H 1002	18.0	11.0	13.2	20.2	15.6
H 1003	43.0	15.3	37.6	28.4	31.1
Average	22.5	13.9	16.8	18.5	18.6

Table 2. — Individual additive values ($\hat{g}$), obtained after open pollination in a seed orchard (σ.p.) and after crosses with 4 common testers (c.).

Clone	$\hat{g}$		Clone	$\hat{g}$		Clone	$\hat{g}$	
	σ.p.	c.		σ.p.	c.		σ.p.	c.
E 2001	— 8.7	— 2.5	G 3001	—10.9	—6.1	H 2007	+12.2	+18.6
E 2002	— 0.9	— 4.0	G 2003	— 9.7	—7.8	H 2016	+ 4.1	+ 5.3
E 1000	— 4.7	— 4.5	H 2001	— 4.5	—0.8	H 3001	+ 3.4	— 6.4
F 2011	— 3.3	— 2.1	H 2008	— 0.9	—2.0	H 3002	— 5.5	
F 1000	+23.7	+29.6	H 2006	— 2.6	+0.5	H 1001	—11.9	—11.3
F 2007	+ 7.2	— 1.0	H 1004	+ 7.8	+7.6	H 1020	— 6.0	— 7.3
F 3001	— 1.4	+ 1.5	H 1019	— 4.1	—3.8	H 2004	— 1.5	— 5.3
G 2004	+ 5.3	+ 2.5	H 1005	— 4.0	—5.1	H 2003	— 1.6	— 1.0
G 2001	— 3.4	— 1.8	H 1006	+ 8.8	+3.1	H 1002	— 4.5	— 2.7
G 1001	— 0.6	+ 3.1	H 2010	+ 5.6	—1.6	H 1003	+12.1	+12.8

r = + 0.87, t = 9.169***

The frequencies of empty grains after individual crosses wiht four common testers.

The investigation, reported above, has made it clear that significant differences in empty grain percentages occur between the clones after open pollination in the orchard. However, it has not been possible to conclude whether these differences depend on varying degrees of selfing or are caused by varying loads of recessive genes, lethal for the embryos. The clones in this orchard, except one, have been crossed as mothers with four clones as fathers. The empty grain percentages, emerging from these crosses are collected in *Table 3*. Selfing is, of course, eliminated when individual crosses are performed correctly.

In this case, the grand total is 18.6%, i.e. slightly lower than after open pollination (19.1%). Also here conspicuous clonal differences come to light, moving from 7.0% (clone H 1001) up to 36.9% (clone H 2007). In Table 2, column "c" the g_i-values, estimated from the individual crosses, are recorded. The agreement between these and the corresponding $\hat{g}_i$-values, derived from open pollination, are, one must confess, surprisingly good. The coefficient of correlation between the two groups of data is +0.87***. This must mean that the four common testers, chosen at random, have approximately the same genetic load as the population. Now, it is quite evident that the clonal differences are caused by nothing but varying loads of lethal genes.

The analysis of variance for these individual crosses gives:

	DF	MS	Expectations
Males	3	273.66	$\sigma^2 + \sigma^2_{\mathrm{MF}} + 29\,\sigma^2_{\mathrm{M}}$
Females	28	247.81	$\sigma^2 + \sigma^2_{\mathrm{MF}} + \;\;4\,\sigma^2_{\mathrm{F}}$
Males × females	78	43.22	$\sigma^2 + \sigma^2_{\mathrm{MF}}$

From this the F-quotients are calculated as $F_M = 5.734$*** and $F_F = 6.332$***. The causal variances amount to: $\sigma^2_M = 7.95$, $\sigma^2_F = 51.15$ and $\sigma^2_T = 102.32$. The variance $\sigma^2_F = 51.15$ is an estimate of $\frac{1}{4}\,\sigma^2_A$ (the additive variance) as is $\sigma^2_c = 53.8$ after open pollination. The total variance of the series of crosses is 102.32 to be compared with 100.20, obtained after open pollination.

A comparison between $\sigma^2_F = 51.15$ with $(\sigma^2 + \sigma^2_{FM}) = 43.22$ makes it clear that the interaction variance, σ^2_{FM}, must be small in relation to the additive variance, i.e. the general combining ability, GCA, must dominate over the specific combining ability, SCA. As no repeated observations are at hand, σ^2 and σ^2_{FM} cannot be calculated separately.

The frequencies of empty grains by selfing resp. cross-pollination.

For 21 of the 30 clones, treated above, the frequencies of empty grains after selfing have also been stated. The percentages lie between 58.0% and 99.2% with a mean value of 86.6% as compared with 18.6% for individual crosses. The correlation between the $\hat{g}_i$-values, estimated from the crosses, and the percentages of empty grains after selfing is +0.36 but non-significant. However, in a more extensive material, this coefficient would probably have shown greater significance.

The percentages of empty grains in individual crosses, according to a partial diallel Table.

In an other seed-orchard the 40 clones, constituting the orchard, have been crossed for progeny testing in a partial diallel Table according to Kempthorne and Curnow (1961) with s = 5 (every clone is one of the parents of 5 progeny families). Consequently, the number of progeny families is 100. The crosses have been repeated twice in two successive years. The percentages of empty grains are recorded in *Table 4*. Also in this material striking clonal differences come into view. Thus, clone H 1027 has given values between 53.9% and 93.3%, clone P 1014, on the other hand, only values between 5.6% and 15.3%. The grand total is 23.5%.

Analysis of variance:

	DF	SS	MS	Expectation
Years	1	33.62	33.62	—
Families	99	63,262.29	639.01	$\sigma^2 + 2\sigma^2_{\mathrm{YFa}} + 2\sigma^2_{\mathrm{Fa}}$
Years × families	99	4,388.53	44.33	$\sigma^2 + 2\sigma^2_{\mathrm{YFa}}$

This gives F = 14.415*** and $\sigma^2_{\mathrm{Fa}} = 297.34$.

In this case it is possible to split the SS for families in GCA and SCA. This operation has been performed according to Kempthorne and Curnow (1961).

The CA-analysis:

	DF	SS	MS	Expectation
GCA	39	2,766.56	46.11	$\sigma^2 + 2\sigma^2_s + 2 \cdot s(p-2)/(p-1)\sigma^2_g$
SCA	60	60,495.73	1,551.17	$\sigma^2 + 2\sigma^2_s$
Sume	99	63,262.29		

The superiority of GCA to SCA is very convincing. σ^2_g is 154.47 and even if σ^2 should be 0, σ^2_s should not exceed 23.06 and σ^2 is certainly not 0.

The $\hat{g}_i$-values have been estimated as:

Clone	$\hat{g}_i$	Clone	$\hat{g}_i$	Clone	$\hat{g}_i$	Clone	$\hat{g}_i$
G 2009	18.8	F 1006	—2.1	H 1026	— 5.5	G 1003	— 8.0
F 2024	—4.9	F 2028	—2.7	R 1005	29.5	F 2020	6.9
F 3001	1.2	F 2023	—1.3	G 1005	— 4.5	P 1014	—11.1
H 3008	—2.1	F 2030	—1.7	F 2021	— 6.6	R 2007	— 5.2
F 1003	—1.2	F 2016	—2.7	F 2015	— 4.0	F 2031	—11.1
F 1005	—3.5	F 2018	—2.0	F 2012	— 0.9	F 2033	— 5.4
N 2012	3.0	H 1004	—5.3	F 2025	7.1	F 1009	3.1
H 1027	61.3	H 1003	—4.4	G 3003	— 2.2	F 1007	16.1
F 2027	0.7	G 1004	—2.6	H 1022	—15.6	H 3007	— 6.9
G 2008	—1.4	G 3002	—4.3	H 1015	—11.8	H 1024	— 6.7

The variation between the clones in this set is greater than for orchard No. 49, treated above. Here the standard deviation for the $\hat{g}_i$-values is 12.87 as against 8.35 and 7.73 for open pollination and crosses in orchard No. 49.

The frequencies of empty grains in a complete 6 × 6 diallel.

In *Table 2* an indication is to be seen that reciprocal differences cannot be excluded. As female F 1000 has given $\hat{g} = 29.6$, as male only 3.7. In order to study this question

♂ \ ♀	G 1004	G 3002	H 1026	R 1005	G 1005	F 2021	F 2015	F 2012	F 2025	G 3003	H 1022	H 1015	G 1003	F 2020	P 1014	R 2007	F 2031	F 2033	F 1009	F 1007	H 3007	H 1024
G2009	39.9 35.9	45.7 34.5	38.6 34.6	67.6 71.1	40.0 40.2																	
F2024		11.3 7.1	11.0 9.6	62.5 39.9	13.7 14.6	22.0 11.7																
F3001			19.3 16.6	78.8 39.3	17.4 17.0	19.3 17.3	11.8 28.3															
H3008				51.3 57.8	13.1 19.3	11.7 14.5	21.2 14.7	13.7 23.5														
F1003					16.3 21.7	14.0 7.0	20.1 23.0	25.0 17.8	32.2 26.7													
F1005						18.0 12.6	9.2 24.6	16.5 21.9	26.3 25.7	11.7 20.2												
N2012							14.3 22.5	25.2 38.2	29.2 36.9	16.8 28.0	11.2 10.9											
H1027								79.8 78.3	89.6 93.3	83.0 85.2	72.6 53.9	80.7 85.0										
F2027									31.1 35.2	14.3 29.9	10.1 5.9	10.4 11.7	14.9 17.4									
G2008										14.6 29.3	8.2 11.1	5.7 4.3	16.5 9.8	23.1 36.5								
F1006											11.7 5.9	10.0 3.6	12.0 11.0	21.3 40.6	11.8 7.4							
F2028												6.9 10.7	22.5 5.2	15.0 29.5	7.6 10.5	17.9 23.8						
F2023													19.7 12.1	22.8 40.2	15.3 7.8	12.7 10.2	11.2 13.3					
F2030														20.3 36.7	14.6 12.6	16.3 13.9	9.8 10.3	15.2 16.7				
F2016															10.2 5.6	26.1 14.7	7.4 8.0	14.6 9.4	39.7 12.7			
F2018																13.1 13.7	14.3 4.8	17.3 5.5	27.3 15.8	48.6 49.7		
H1004																	12.6 5.9	25.1 18.2	25.7 21.9	15.8 18.5	16.7 12.4	
H1003																		17.7 8.6	20.7 21.5	34.5 39.5	15.6 9.9	12.6 10.7
G1004																			22.5 24.1	36.3 34.9	13.4 12.7	13.8 23.8
G3002																				42.1 39.5	11.0 7.2	11.6 14.9
H1026																					13.7 8.5	16.8 14.4
R1005																						47.2 27.0

Table 4. — Percentages of empty grains in the seeds from a partial diallel series of crosses. In the cells: above, values from 1973 — below, values from 1974.

more closely, a complete 6 × 6 diallel with selected clones from orchard No. 49 has been achieved. For this purpose, three clones with high $\hat{g}$-values were chosen, viz. H 2007 (18.6%), H 1003 (12.8%) and H 1004 (7.3%) together with three clones with such low values as: H 3001 (—6.4%), G 2003 (—7.8%) and H 1001 (—11.3%). The resulting frequencies of empty grains are summarzied in *Table 5.*

Table 5. — Percentages of empty grains in the seeds from a 6 × 6 complete diallel

♀ \ ♂	H 2007	H 1003	H 1004	H 3001	G 2003	H 1001	$\bar{x} - I_1$
H 2007	68.6	63.0	32.7	32.0	39.0	30.6	39.5
H 1003	26.0	93.8	27.5	19.6	25.0	24.4	24.5
H 1004	22.3	46.3	87.1	14.1	45.0	18.1	29.2
H 3001	13.6	43.8	12.3	84.9	9.6	7.2	17.3
G 2003	9.9	51.4	11.7	7.5	72.8	8.3	17.8
H 1001	5.8	33.9	7.8	13.8	16.9	78.1	15.6
$\bar{x} - I_1$	15.5	47.7	23.0	17.4	27.1	17.7	24.0

The analysis of variance with the I_1-combinations excluded, and with expectations according to "Model 1" (GRIFFING, 1956) as the parents are systematically chosen has given:

	DF	SS	MS	Expectation
GCA	5	3,233.26	646.65	$\sigma^2 + 2p/p\text{-}1)\, S\, g^2_i$
SCA	9	227.15	25.24	$\sigma^2 + 2(\frac{2}{p(p\text{-}3)})\, SS\, r_{ij}^2$
Rec.	15	3,172.01	211.47	$\sigma^2 + 2(\frac{2}{p(p\text{-}1)})\, SS\, s_{ij}^2$

Nor in this case can σ^2 be estimated, as repeated observations do not occur. However, the following F-quotients can be calculated:

$$F_{GCA/CA} = 25.620^{***}\ F_{GCA/Rec.} = 3.058^0 \text{ and } F_{Rec./SCA} = 8.378^{***}.$$

Also in this case it can be questioned whether any specific influences at all are present. The low MS for SCA, 25.24, may very well be wholly attributed to error. The sums of squares, contained in the expectations, can be written:

$$S\,\hat{g}_i^2 = 269.44 — 5/12\sigma^2,\ SS_{ij}2 = 113{,}58 — 9/\sigma^2 \text{ and}$$
$$SS\, r_{ij} = 1{,}586.03 — 15/2\sigma^2.$$

The $\hat{g}_i$ and $\hat{g}_j$ values (the individual as female resp. male) have been estimated thus:

Clone	Female	Male
H 2007	+13.9	— 5.9
H 1003	+ 6.8	+24.2
H 1004	+ 3.9	+ 4.8
H 3001	— 8.5	— 8.3
G 2003	— 5.9	+ 2.3
H 1001	—10.0	— 7.9

Particularly great differences are shown by H 2007 and H 1003. The former gives more empty grains as female, the latter as male. The coefficient of correlation between the female $\hat{g}_i$-values and the $\hat{g}_i$-values after open pollination *(Table 3)* is +0.82*.

After selfing, the following percentages of empty grains have been obtained: 68.6%, 93.8%, 87.1%, 84.9%, 72.8% and 78.1%. From $\hat{g}_i + \hat{g}_j$ the following percentages should have been obtained: 32.0%, 55.0%, 23.1%, 7.5%, 20.4% and 6.1%.

Discussion

The materials, treated in this report, which have been obtained after open pollination and individual crosses according to different schemes, common testers, partial diallel and complete diallel, incontestably show that GCA is decisive for the frequency of empty grains in the seed. Manifestations of SCA have hardly been possible to trace. If it is presumed that a considerable number of recessive embryo-lethal genes are present in the populations (Koski, 1971, Bramlet and Pepper, 1974), specific gene-combinations must also be rare.

Fowler (1964) has drawn attention to the fact that the occurrence of several archegonia in an ovulum changes the segregation ratios for embryonic lethals. The effects of this multiple fertilization on the frequencies of homozygous recessives have later on been calculated by Koski (1971) as well as by Bramlett and Popham (1971) (cf also Lindgren, 1975). According to Sarvas (1962) the frequencies of 1, 2, 3, 4 archegonia per ovulum are 18.7%, 70.7%, 10.1%, 0.5% with such a small individual variation that the individual averages do not vary more than from 1.7 to 2.4. This implies that in a cross between two trees, which have only one recessive lethal in common, the percentage of empty grains should amount to 14.1, which should be the lowest possible frequency in any individual cross.

However, considerable lower values are not uncommon *(Tables 3, 4 and 5)*. This fact could be explained by the assumption that trees exist, which have a greater number of archegonia than stated by Sarvas or — which seems to be more probable — that recessive genes with incomplete penetrance (semilethals) are also in operation. Lindgren (1975) has expressed the opinion that "embryo lethality is caused by the combined action of many recessive sublethal genes, which could not separately kill an embryo".

The clear indication of the occurrence of reciprocal differences, which has come forth in the complete diallel, merits serious attention. In a 3 × 5 diallel of *Pinus virginiana*, Bramlett and Pepper (1974) have also found one parent, which gave significantly (*) higher frequency of empty grains as female than as male. In their case not only SCA but als GCA were insignificant.

In the 6 × 6 diallel, treated here, there are not only individuals, which give greater amounts of empty grains as females than as males, but also individuals, giving more empty grains as males than as females. The latter case could be caused by different numbers of archegonia per ovulum. If the parent A has two archegonia and B only one, and two recessives are common, the cross A × B should give 27% empty grains and B × A 44%. In the case of the parent giving more empty grains as female, some maternal factor must be presumed. It is plausible to speculate about recessive genes with lethal effects on the haploid endosperm. Assume that A as well as B have two archegonia and two embryo-lethals in common but A has one endosperm lethal in addition and is heterozygous for this gene. The reciprocal crosses in such a case will give the following results.

A × B gives (27 + 73/2) = 63.5% empty grains
B × A gives 27.0% empty grains

More and more convincing facts have been collected showing that in *Pinus silvestris* — and probably in all gymnosperms — an inbreeding preventing mechanism has been developed, having as its main element a great number of recessive genes, which in homozygous conditions are lethal for the embryos. These genes are additive to their effect, specific effects being lacking or in any case rare. This mechanism is complicated by the occurrence of several archegonia per ovulum, all originating from the same macrospore. Further complications are: the penetrance of the recessive is probably not always complete (semilethals); the frequencies of the recessive alleles in the populations are probably lower than those of the dominant alleles; and maternal effects, possibly caused by endosperm-lethal recessives.

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Summary

The frequencies of empty grains in the seed of *Pinus silvestris* have been studied after open pollination as well as after individual crosses. The following series of crosses have been performed: 29 females with 4 males as common testers, 40 parents with 5 crosses per parent in a partial diallel Table, and 6 parents, crossed in a complete diallel Table.

Striking individual differences in the frequencies of empty grains are present.

It has been shown beyond doubt that the origin of empty grains is dependent on homozygotization of recessive genes, which are lethal to the embryos. The heredity is highly additive (high general combining ability) with great individual variation between the additive values.

No specific effects have come to light (low specific combining ability). This is considered to result from the great number of loci involved, making specific gene combinations rare.

Considerable differences in percentages of empty grains may occur between reciprocal crosses. That an individual gives a higher percentage of empty grains as female than as male can hypothetically be explained by the assumption that this individual carries not only embryo-lethal recessives but also endosperm lethal recessives. If, on the other hand, the percentages of empty grains prove to be higher, when the individual functions as male, a larger number of archegonia per ovulum can be the cause.

This mechanism of empty grains, which in its effect prevents inbreeding, and is thus a parallel to the s-allele mechanism in the angiosperms, undoubtedly consists of a great number of pairs of alleles. The mechanism is complicated not only by the occurrence of more than one archegonia per ovulum but probably also by the recessive alleles having lower frequencies in the populations than

the dominant ones. Furthermore, all recessive genes may not have complete penetrance (semi-lethals) and, what is more, endosperm lethal recessives may also be involved.

Key words: Empty grains, recessive embryolethal genes, recessive endospermlethal genes, general- and specific combining ability, individual breeding values, reciprocal differences.

Zusammenfassung

Die Frequenzen der Hohlkörner in Kiefernsamen *(Pinus silvestris)* sind nach freiem Abblühen und nach Individualkreuzungen untersucht worden. Die folgenden Kreuzungspläne wurden verwendet: 29 Mütter mit 4 durchgehenden Vätern, 40 Eltern mit Kreuzungen jeder Eltern in einem partiellen diallelen Plan und einem vollständigen Diallel mit 6 Eltern.

In den Prozentsätzen der Hohlkörner liegen große Individualunterschiede vor.

Es hat sich eindeutig gezeigt, daß die Entstehung der Hohlkörner im wesentlichen von der Homozygotisierung rezessiver Gene abhängig ist, die den Embryo zum Absterben bringt. Die Additivität ist hoch (hohe allgemeine Kombinationseignung) bei großer Streuung der additiven Werte.

Spezifische Kombinationseignung hat sich nicht gezeigt. Dies kann dadurch erklärt werden, daß eine große Anzahl loci gegeben ist, und deshalb spezifische Genkombinationen selten werden.

Es können erhebliche Verschiedenheiten im Hohlkornprozent reziproker Kreuzungen auftreten. Daß ein Individuum einen höheren Hohlkornanteil hervorruft, wenn es als Mutter verwendet wird, beruht möglicherweise darauf, daß ein solches Individuum nebst embryolethalen auch endospermlethale Gene besitzt. Ist dagegen der Hohlkornanteil höher, wenn das Individuum als Vater fungiert, kann dies andeuten, daß das Individuum über eine hohe Anzahl Archegonien pro Ovulum verfügt.

Dieser Lethalgenmechanismus, der in seiner Auswirkung Inzucht verhindert und folglich den s-Allel Systemen der Angiospermen entspricht, ist zweifellos von einer großen Anzahl Allelpaare aufgebaut.

Der Mechanismus ist nicht nur durch das Vorkommen von mehreren Archegonien pro Ovulum kompliziert, sondern auch dadurch, daß die rezessiven Allele wahrscheinlich eine niedrigere Frequenz in den Populationen haben als die dominanten Allele. Es ist auch anzunehmen, daß nicht alle rezessiven Gene eine vollständige Penetranz besitzen (Semilethale), und daß es nicht nur embryolethale, sondern auch endospermlethale rezessive Gene gibt.

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A simple method of estimating rates of self-fertilization by analysing isozymes in tree seeds

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If it is possible to identify an individual pollen of a certain tree by detecting its genes in the diploid embryo tissue of seeds, one should be able to estimate rates of self-fertilization, as well as fertilization probabilities in general. The knowledge of the parameters is the well-known basis for investigations concerning the mating system of trees, especially the estimation of coefficients of inbreeding and kinship. The applicability of isoenzyme techniques for identifying genes in needle and endosperm tissues of trees is proved by numerous investigations (review see Feret and Bergmann, 1976). In addition, experiments with the purpose of estimating rates of self-fertilization were performed by Rudin (1976) by means of needle analysis of *Pinus silvestris.*

Because self-fertilization affects the whole genome equally, it can be sufficiently demonstrated by investigating one polymorphic gene locus.

Several conditions required

Using diploid material for isozyme analysis, monomeric enzymes should be preferred, as they do not cause hybrid band-configurations in heterozygous individuals so that the genotype can be identified directly by the isozyme phenotypes in the zymogram. Therefore, our investigations began with the analysis of the monomeric system of leucine aminopeptidase (LAP) in Norway spruce *(Picea abies* (L.)

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