Model Relating Unsound Seed and Embryonic Lethal Alleles in Self-Pollinated Pines

By David L. Bramlett and Thomas W. Popham[1]

Polyembryo is an important aspect of oogenesis in ***Pinus*** (Ferguson 1904; Buchholz 1931; Chamberlain 1935). Multiple archegonia, each containing a mature egg cell, are frequently formed in an individual pine ovule (Stockwell 1939; Sarvas 1962). Unlike most plants, all the egg nuclei in the pine ovule are genetically identical as they have arisen from the division of a single haploid megaspore. With room for several pollen grains in the pollen chamber of each ovule, multiple fertilization of identical egg cells in a single ovule frequently occurs (Sarvas 1962).

Competition between the developing polyzygotic embryos normally reduces their number to a single embryo at the time of cone maturity (Buchholz 1931). Many embryos in the pine ovule abort before maturity for reasons other than polyembryonic competition. The primary cause of noncompetitive embryo abortion is thought to be the action of homozygous embryonic lethal genes (Sarvas 1962). If the ovule has two or more fertilizations, all but one of the polyzygotic embryos in an ovule could abort and the ovule still form a sound seed. If the ovule has only a single fertilization, embryo abortion leads to gametophytic tissue deterioration. The seed coat, however, may develop normally as it is the product of the diploid cells of the female parent. The result is an unsound (empty) seed. Excluding non-genetic factors an unsound seed from a polyzygotic ovule occurs only when all the zygotes contain recessive embryonic lethal genes. In pines, self-fertilization frequently reduces the yield of sound seed as compared to cross-fertilization (Squillace and Kraus 1962; Fowler 1965; Snyder and Squillace 1966; Snyder 1968; Franklin 1969). This result is attributed to the increased percentage of homozygotes after selfing producing a greater likelihood of homozygous recessive embryonic lethals (Sarvas 1962).

Table *1.* — Combinations of male and female gametes with two embryonic lethal alleles and two fertilizations per ovule.

Male Gametes		Female Gametes AB	AB	Ab	Ab	aB	aB	ab	ab
AB	AB	AABB	AABB	AABb	AABb	AaBB	AaBB	AaBb	AaBb
AB	Ab	AABB	AABb	AABb	AAbb[1]	AaBB	AaBb	AaBb	Aabb[1]
AB	aB	AABB	AaBB	AABb	AaBb	AaBB	aaBB[1]	AaBb	aaBb[1]
AB	ab	AABB	AaBb	AABb	Aabb[1]	AaBB	aaBb[1]	AaBb	aabb[1]
Ab	AB	AABb	AABB	AAbb[1]	AABb	AaBb	AaBB	Aabb[1]	AaBb
Ab	Ab	AABb	AABb	AAbb[1]	AAbb[1][2]	AaBb	AaBb	Aabb[1]	Aabb[1][2]
Ab	aB	AABb	AaBB	AAbb[1]	AaBb	AaBb	aaBB[1]	Aabb[1]	aaBb[1][2]
Ab	ab	AABb	AaBb	AAbb[1]	Aabb[1][2]	AaBb	aaBb[1]	Aabb[1]	aabb[1][2]
aB	AB	AaBB	AABB	AaBb	AABb	aaBB[1]	AaBB	aaBb[1]	AaBb
aB	Ab	AaBB	AABb	AaBb	AAbb[1]	aaBB[1]	AaBb	aaBb[1]	Aabb[1][2]
aB	aB	AaBB	AaBB	AaBb	AaBb	aaBB[1]	aaBB[1][2]	aaBb[1]	aaBb[1][2]
aB	ab	AaBB	AaBb	AaBb	Aabb[1]	aaBB[1]	aaBb[1][2]	aaBb[1]	aabb[1][2]
ab	AB	AaBb	AABB	Aabb[1]	AABb	aaBb[1]	AaBB	aabb[1]	AaBb
ab	Ab	AaBb	AABb	Aabb[1]	AAbb[1][2]	aaBb[1]	AaBb	aabbl)	Aabb[1][2]
ab	aB	AaBb	AaBB	Aabb[1]	AaBb	aaBb[1]	aaBB[1][2]	aabb[1]	aaBb[1][2]
ab	ab	AaBb	AaBb	Aabb[1]	Aabb[1][2]	aaBb[1]	aaBb[1][2]	aabb[1]	aabb[1][2]
Number of lethal alleles per female gamete, r		0		1		1		2	
Aborted ovules		0		4		4		9	
Number of ovules		16		16		16		16	

[1] embryo abortion: homozygotic combination of lethal alleles.

[2] unsound seed: all embryos aborting in a single ovule.

A model of the probability of unsound seed can be developed with the number of loci heterozygous for embryonic lethal recessive alleles in the selfed parent, *n;* the number of fertilizations which take place in each ovule, *f;* and the number of loci containing recessive embryonic lethal alleles in a particular female gamete, *r.*

For the development, it is assumed that when $n > 1$, independent segregation occurs. There are 2^n possible genetically different male gametes for each female gamete possible in an ovule; of these, $2^{(n-r)}$ would result in sound seed when the gametes unite. When multiple fertilization of identical female gametes occurs, as in pines, there are f sets of gamete combinations. Thus, in any ovule where multiple fertilization occurs, there are $(2^n - 2^{(n-r)})^f$ gamete combinations possible which result in unsound seed. There are $\binom{n}{r} = \frac{n!}{r!\,(n-r)!}$ ways of having ovules which have gametes with *r* loci with recessive embryonic lethal alleles. The product of the number of ovules with gametes which contain *r* recessive embryonic lethal alleles and the number of ways unsound seed could occur in such an ovule gives the number of ways an unsound seed could be produced in that type ovule. Summing over all values of *r*, $\sum_{r=0}^{n} \binom{n}{r} (2^n - 2^{(n-r)})^f$, gives the total number of ways unsound seed may occur.

When multiple fertilization occurs, there will be 2^{nf} sets of male gametes available for each fertilization and 2^n possible genetically differing female gametes. The number of genetically differing ovules possible is $2^{nf} \times 2^n$. The nominal number of ovules which would result in unsound seed divided by the number of ovules possible gives:

PROBABILITY OF UNSOUND SEED =

$$\frac{\sum_{r=0}^{n} \binom{n}{r} (2^n - 2^{(n-r)})^f}{2^{(nf+n)}}$$

An illustration of this is given in ***Table*** 1 for a parent with two loci containing recessive embryonic lethal alleles and with two fertilizations per ovule. In the example, there are 17 ways unsound seed can occur in the 64 possible ovules. The probability of unsound seed would then be .27. ***Table 2*** gives the probabilities of unsound seed for up to 20 recessive embryonic lethal genes and up to 5 fertilizations according to the above equation.

As the number of fertilizations per ovule increases, the probability of unsound seed decreases rapidly for light lethal gene loads. For higher lethal gene loads, the probability is decreased very little for each additional fertilization.

The number of lethal genes may be estimated with ***Table 2*** for any self-pollinated pine if the number of fertilizations per ovule and the percent unsound seed are known, and if independent segregation is assumed. This

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Table 2. — Probability of unsound seed after self-fertilization in pine as a result of the homozygosity of embryonic lethal alleles after multiple fertilizations per ovule.

Lethal Alleles	Number of Fertilizations				
	1	2	3	4	5
1	.25	.12	.06	.03	.02
2	.44	.27	.17	.11	.08
3	.58	.40	.29	.22	.16
4	.68	.52	.41	.33	.27
5	.76	.62	.52	.44	.38
6	.82	.70	.61	.54	.48
7	.87	.77	.69	.63	.58
8	.90	.82	.76	.71	.66
9	.92	.86	.81	.77	.73
10	.94	.90	.86	.82	.79
15	.99	.97	.96	.95	.94
20	1.00[1]	.99	.99	.99	.98

[1]) Rounded to 1.00; actual value = .9968.

table may be useful in evaluating potentially superior trees for seed orchards in terms of their embryonic lethal allelic load. This table gives a more conservative estimate of the number of lethal genes than has been previously reported. Fowler (1965) estimated that he had a minimum of 15 lethal genes in *Pinus banksiana* on the basis of 63 percent unsound seed after selfing, with the assumption of two fertilizations per ovule. *Table 2* shows that the estimated number should be five rather than 15.

Summary

Two major factors appear to affect the probability of unsound seed in self-pollinated pines: the number of fertilizations in each ovule and the number of mendelian lethal alleles carried by the selfed parent. A model is developed which expresses the probability of unsound seed as a function of these two factors.

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Estimating Frequency of Natural Selfing Based on Segregating Mutant Forms

By E. C. Franklin[1])

Segregating mutant forms have been used in several cases to estimate the frequency of natural self-fertilization based exclusively on frequency of mutant phenotypes (Squillace and Kraus, 1963; Fowler, 1965 a; Fowler, 1965 b; Rudolph, 1966). The point that these estimates do not necessarily reflect the total frequency of self-fertilization has received insufficient emphasis. To estimate total frequency of self-fertilization, adjustments must be made for embryonic mortality. Otherwise, discrepancies can be substantial, as illustrated by a case reported for *Pinus sylvestris* L. where only 7 percent viable self-fertilized seed were obtained following approximately 26 percent natural self-fertilization (Sarvas, 1962).

Methods

Wind-pollinated seed were collected in 1967 from 16 trees proven to be heterozygous for one or more mutant alleles on the basis of controlled self- and cross-pollinations made in 1965 (Franklin, 1969 a). Up to 1000 seed from each of these seed lots were sown in a greenhouse. Counts of abnormal phenotypes were made daily during germination and every 3 to 4 days for a month thereafter.

Percentage of natural self-fertilization (S) was estimated according to the following relationship:

$$S = \frac{\text{Number of self-fertilized ovules}}{\text{Number of cross plus number of self-fertilized ovules}} \times 100$$

Computations are illustrated in the following example. Controlled self-pollination of a certain tree resulted in 120 normal and 30 mutant seedlings from a total seed yield of 1000. Controlled crossing on the same tree resulted in 800 normal seedlings from a total seed yield of 1000. A wind-pollinated collection from the same tree yielded 994 normal and 6 mutant seedlings.

From the data on controlled self-pollination, it is estimated that each mutant seedling appearing in a wind-pollinated family represents $\frac{120 + 30}{30} = 5$ self-fertilized seedlings.

Also, each self-fertilized seedling in a wind-pollinated family represents $\frac{1000}{150} = 6.67$ self-fertilized ovules. Similarly, each cross-fertilized seedling in a wind-pollinated family represents $\frac{1000}{800} = 1.25$ cross-fertilized ovules.

Based on these predictions, the following estimates can be made for the wind-pollinated collection —

Number of self-fertilized seedlings: (6) (5) = 30
Number of self-fertilized ovules: (30) (6.67) = 200
Number of cross-fertilized seedlings: 1000 — 30 = 970
Number of cross-fertilized ovules: (970) (1.25) = 1212

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