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 uscd 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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Percent selfed seedlings: $\frac{30}{30 + 970}(100) = 3$

Percent self-fertilization: $\frac{200}{200 + 1212}(100) = 14$

Data from the 16 trees, which segregated for a total of 20 mutant forms[2]) were utilized for computations such as those illustrated.

Results and Discussion

Frequency of natural self-fertilization in upper crowns averaged 7 percent. Estimates from individual trees ranged from zero to 22 percent. An estimate of 7 percent natural self-fertilization was found for the same stand by another method based on yields of filled seed (FRANKLIN, 1971).

Two aspects of the computations deserve special emphasis. First, the observed ratio of mutant to normal forms is an unbiased estimate of the actual ratio for a particular family regardless of the amount of embryonic mortality due to environmental or genetic factors. If a particular segregation ratio is assumed, such as 3 normal to 1 mutant — the classical Mendelian ratio, the undetected presence of genetic linkage, for example, would result in biased estimates of the frequency of natural selfing (SORENSON, 1967). Second, the use of germination percentages from controlled pollinations allows the estimation of actual percentages of self-fertilization, in addition to percentages of seedlings resulting from self-fertilization. Both values are necessary for a more complete understanding of the genetic structure of populations.

[2]) For descriptions of mutant forms and their segregation ratios see FRANKLIN (1969 b).

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Estimates of Frequency of Natural Selfing and of Inbreeding Coefficients in Loblolly Pine

By E. C. FRANKLIN[1])

Knowledge of the genetic structure of loblolly pine (*Pinus taeda* L.) stands is needed as more sophisticated models and methods of selection, testing, and seed production are proposed for this species. Two important facets of genetic structure are the average frequency of self-fertilization and the average inbreeding coefficient of the sexually mature stand. The present experiments were conducted to estimate the frequency of natural self-fertilization in upper and lower crowns of loblolly pine trees in a natural old-field stand. Estimates of inbreeding coefficients of seed populations at the time of fertilization, and of the population of parental trees, were also sought.

Methods

Artificial self- and cross-pollinations[2]) were performed on 132 trees on an old-field site in the North Carolina Piedmont in spring 1965 (FRANKLIN, 1969). Control-pollinated cones were obtained from 118 of those trees. In addition, wind-pollinated cones were collected from the upper crowns of 114 trees included in the control-pollinated group and from the lower crowns of 23 open-grown, large-crowned trees included in the wind-pollinated group.

Percentage of filled seed was found to be the best metrical trait for estimating frequency of selfing (KATSUTA, 1964).

The following relationship was used:

$$\text{Percent natural selfing} = \frac{c - w}{c - s} \times 100$$

where c, w, and s are percentages of filled seed after controlled cross-, wind-, and controlled self-pollination.

Estimates of the inbreeding coefficients cannot always be simply estimated because they are functions of the amount of inbreeding in the current generation, plus the average inbreeding coefficients of the parent trees. However, in this experiment the inbreeding coefficient of the parent trees was found to be so low (0.0003) that it could be safely ignored.[3]) Therefore, the inbreeding coefficients for upper and lower crown seed lots at fertilization could be found by multiplying the percentage of self-fertilization by one-half, which is the inbreeding coefficient after one generation of selfing.

Results and Discussion

Frequency of natural self-fertilization in upper crowns was estimated to be 7 percent, and in lower crowns 34 percent. Estimated population inbreeding coefficients were as follows: upper crown at time of fertilization, 0.03; lower crown at time of fertilization, 0.17; and among parent trees in the natural stand, 0.0003.

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[2]) Cross pollen consisted of a five-tree mix from a stand 10 miles distant.

[3]) For estimation procedures see FRANKLIN, E. C.: Artificial self-pollination and natural inbreeding in *Pinus taeda* L. Ph. D. Diss. N. C. State Univ. at Raleigh, 127 pp. (1968). Available from University Microfilms, Ann Arbor, Michigan 48106. (Order No. 68-14, 653).