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## Unsound Seeds in Conifers: Estimation of Numbers of Lethal Alleles and of Magnitudes of Effects Associated with the Maternal Parent

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### Abstract

A model is developed for estimating the genetic load and the magnitude of maternal environmental effects in conifers from observed proportions of unsound seeds produced. Results indicate that maternal effects typically account for 5–30% of seed mortality. The average number of lethals carried is around 10, consistent with previous estimates. Observations relating to variances in proportions of unsound seeds lead to the conclusion that the average genetic load, and hence the mutation rate, probably varies over different forest locations. Implications for sampling methodology are presented.

*Key words:* unsound seed, lethal alleles, maternal effects, genetic load.

### Zusammenfassung

In der vorliegenden Arbeit wird ein Modell entwickelt, welches bei Koniferen sowohl zur Schätzung der genetischen Belastung als auch zur Schätzung des Ausmaßes mütterlicher Umwelteffekte dienen kann. Hierbei wird der beobachtete Anteil der produzierten nicht lebensfähigen Samen genutzt. Die Ergebnisse weisen darauf hin, daß 5–30% der Samen-Mortalität durch Effekte des Sameneltern verursacht werden. Die mittlere Anzahl von Letal-Allelen liegt bei 10, was früheren Untersuchungen nicht widerspricht. Beobachtungen hinsichtlich der Varianzen in den Anteilen nicht lebensfähiger Samen lassen den Schluß zu, daß die mittlere genetische Belastung, und daher die Mutationsrate, wahrscheinlich über die verschiedenen Standorte variiert. Folgerungen für die Stichprobennahme werden ebenfalls dargestellt.

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### Introduction

Observations of high proportions of unsound seeds produced by conifers, especially following self-pollination, have led to surprisingly high estimates of the average number of recessive embryonic lethal alleles carried by individual trees (see FRANKLIN, 1970, for review). However, effects that are external to the embryonic genotypes also determine seed viability. Such effects as the age and vigor of the maternal parent, the water and nutrient supply to the female reproductive organs, and the maternal parent's resistance to pests all influence seed development. Although some interaction between the maternal support apparatus and the embryonic developmental capacity may exist, we wish to consider only those effects on female fecundity that can be considered to be independent of the embryonic genotype. In this paper we propose a model that incorporates embryonic genetic effects and maternal effects. We present analyses of data on proportions of unsound seeds for *Pinus taeda* and *Pinus virginiana* observed by BRAMLETT (personal communication) and by BRAMLETT and PEPPER (1974). Our results indicate that maternal effects typically account for 5 to 30 percent of the mortality and that the number of embryonic lethals is therefore lower than previously estimated. However, the estimated reduction is slight, typically no more than 10% relative to estimates obtained from models that do not incorporate maternal effects.

Previous investigations have placed the average number of lethals per tree at about 10, though there seems to be considerable variation around this mean number. For instance, SORENSEN (1969) estimated numbers of lethals in 35 sample trees to range from a low of 3 to a high of 27, with a mean number of 11.2 and a variance of 32. We shall argue that variances which exceed the associated means suggest that the lethal load varies from one location to another in the forest. This variation in lethal load implies that the forest from which the samples were drawn has a hetero-

geneous structure, and that some parts contain a greater average number of lethal alleles than others.

### Background

BRAMLETT and POPHAM (1971) and KOSKI (1971) independently obtained a formula for the probability,  $p$ , that a particular seed, produced by self-fertilization from a tree carrying  $n$  lethal alleles, is unfilled due to homozygous lethal alleles at some locus. Assuming a constant number,  $f$ , of fertilizations per ovule, this formula

$$p = \sum_{r=0}^n \binom{n}{r} \frac{1}{2^n} (1-2^{-r})^f \quad (1)$$

is obtained by observing that the female component of the seed may contain any number of lethals from 0 to  $n$  inclusive, where the probability of exactly  $r$  lethals is  $\binom{n}{r} \frac{1}{2^n}$ .

With  $r$  female lethals, the seed is then unsound if in each fertilization the male component exhibits a lethal allele in at least one of these  $r$  loci. The complementary event, that in a particular male zygote no lethals are found in these  $r$  loci, has probability  $(1/2)^r = 2^{-r}$ , giving  $1 - 2^{-r}$  as the probability of an 'unsound fertilization'. Multiplying all relevant probabilities and summing over  $r$  yields the indicated form for  $p$ .

In addition to constant  $f$ , this derivation depends on assumptions of independent segregation, no 'new' mutations and no unsound seed due to causes other than lethal alleles. BISHIR and PEPPER (1977) circumvent the assumption of constant  $f$  by introducing a random number,  $F$ , of fertilizations. This leads to a more inclusive formula

$$q = \sum_{f=0}^a P(F=f) \sum_{r=0}^n \binom{n}{r} \frac{1}{2^n} (1-2^{-r})^f \quad (2)$$

for the probability a seed is unsound. Here  $a$  denotes the maximum possible number of fertilizations, typically 5 in conifers.

In this paper we require a slightly modified form of formula (2), in which  $P(F=f)$  is replaced by  $P(F=f | F > 0)$ , the conditional probability that  $F=f$ , given that  $F$  is positive. This formula

$$q = \sum_{f=1}^a P(F=f|F>0) \sum_{r=0}^n \binom{n}{r} \frac{1}{2^n} (1-2^{-r})^f \quad (3)$$

represents the probability that a selfed seed is unsound in a tree carrying  $n$  lethals, if that seed has in fact been fertilized.

### The Model Including Maternal Environmental Effects

Denote by  $b_i$  the probability that a particular seed from tree  $i$  is unsound because of maternal environmental effects, by which we mean those effects on seed viability that are stochastically independent of the numbers of lethals carried. Let  $Q_i$  be the probability that a selfed seed from tree  $i$  dies because of lethal homozygosity. A formula for  $Q_i$  is obtained from (3) by replacing  $n$  with  $n_i$ , the number of lethals carried by tree  $i$ . Then the probability that a selfed seed from tree  $i$  is unsound is

$$s_i = b_i + (1 - b_i)Q_i \quad (4)$$

That is,  $s_i$  is the probability of environmental 'failure' plus the probability the environment does not fail (environmental 'success' includes fertilization of at least one archegonium) but there is death due to homozygous lethal alleles. For seeds obtained by crossing female  $i$  with male  $j$ , the corresponding probability is

$$c_{ij} = b_i + (1 - b_i)Q_{ij} \quad (5)$$

where  $Q_{ij}$  is the probability of genetic death from this cross. The quantity  $Q_{ij}$  is obtained from (3) by replacing  $n$  with  $n_{ij}$ , the number of loci at which lethals appear in both tree  $i$  and tree  $j$ .

Meanings attached to the parameters  $b_i$ ,  $Q_i$  and  $Q_{ij}$  depend upon the particular experimental observations to which the model is applied. Differences in the beginning and end points of observable deaths influence the choices. In pine, for instance, unfertilized seeds shrivel and die and are typically not included in experimental counts. In this case,  $b_i$  is associated with effects occurring between fertilization and time of observation, which KOSKI (1971) takes before germination, although FRANKLIN (1972) shows that genetic deaths can continue to occur through early life. In spruce, on the other hand, unfertilized seeds are usually externally indistinguishable from fertilized, and are included in counts of unsound seeds. The model may be applied in any case, interpretations being assigned to the model parameters according to the situation.

### Parameter Estimation

For a sample of seeds from crosses among  $t$  trees, there are  $t$  of the  $b_i$ 's (one corresponding to each tree) and  $t$  of the  $n_i$ 's. Since  $n_{ij}$  always equals  $n_i$ , there are only

$$\binom{t}{2} = t(t-1)/2$$

of the  $n_{ij}$ 's, giving a total of

$$t + t + \binom{t}{2} = (3t + t^2)/2$$

parameters. These enter the model through the probabilities  $s_i$  and  $c_{ij}$  in formulas (4) and (5) above. Unfortunately, of the  $t^2$  probabilities in the model (all the  $s_i$  and  $c_{ij}$ ), only  $(3t + t^2)/2 - 1$  are mathematically independent, one fewer than the total number of parameters. Since experimental data typically consist only of observed proportions of unsound seeds obtained from selfing and crossing sample trees, i.e., of sample values of the probabilities  $s_i$  and  $c_{ij}$ , it is not possible to estimate all parameters from these data.

It is, however, possible to eliminate certain parameters in a way that has little effect on those remaining. NAMKOONG and BISHIR (1987) demonstrate: 1) that the total number,  $M$ , of loci at which lethals occur is at least  $10^4$ , and 2) that for two randomly chosen trees the average number of common lethal loci is about  $100/M$ , and thus less than 0.01. This means the probability of at least one such common lethal locus cannot exceed 0.01, and thus that  $Q_{ij}$  is 0.01 or less. We emphasize that these observations apply to trees randomly chosen from the forest, not to neighboring or otherwise closely related trees.

The  $Q_{ij}$  being so small, we arbitrarily set them to zero, thus eliminating all  $n_{ij}$  from the model. Then, using a least squares criterion, we choose as estimates those values of  $b_i$  and  $Q_i$  that minimize

$$S = \sum_{i=1}^t (u_{ii} - s_i)^2 + \sum_{i=1}^t \sum_{j \neq i}^t (u_{ij} - c_{ij})^2, \quad (6)$$

the sum of squared differences between observed proportions  $u_{ij}$  and  $u_{ij}$  and the model probabilities  $s_i$  and  $c_{ij}$ . Under the assumption that all  $Q_{ij}$  are zero, we have

$$S = \sum_{i=1}^t (u_{ii} - b_i - Q_i + b_i Q_i)^2 + \sum_{i=1}^t \sum_{j \neq i}^t (u_{ij} - b_i)^2 \quad (7)$$

The least squares estimates are now easily obtained:

$$\hat{b}_i = \frac{1}{t_i - 1} \sum_{j \neq i} u_{ij} \quad (6)$$

and

$$\hat{Q}_i = (u_{ii} - \hat{b}_i) / (1 - \hat{b}_i) \quad (7)$$

(If certain crosses are missing, the divisor  $t - 1$  in (6) is replaced by  $t_i - 1$ , where  $t_i$  is the number of other trees actually crossed with female tree  $i$ .)

If the assumptions upon which the model is based are true, namely that unsound seeds are due either to homozygous lethals or to maternal effects, these causes being stochastically independent, then the parameter estimates should be quite accurate. Assuming seeds are independently unsound, the observed proportion  $u_{ij}$ , for  $j \neq i$  is associated with a binomial distribution having parameters  $N_{ij}$  = number of seeds observed and  $c_{ij} = b_i$  = probability a particular seed is unsound. The variance of  $u_{ij}$  is thus  $b_i(1-b_i)/N_{ij}$ , for which the maximum possible value is  $N_{ij}/4$ , attained when  $b_i = 1/2$ . With typical sample sizes of 400 or more seeds per cross, the variance of  $u_{ij}$  is smaller than  $1/1600$ . Thus, the variance of  $\hat{b}_i$  is less than  $1/1600(t_i - 1)$ , or less than  $1/6400$  in a sample of 5 trees. In such a sample, the standard deviation of  $\hat{b}_i$  would be less than  $1/80$  — on the order of 0.01, giving probability about 0.95 that  $\hat{b}_i$  lies within  $\pm 0.02$  of  $b_i$ .

The variance of  $\hat{Q}_i$  is more difficult to obtain, due to the random quantity  $\hat{b}_i$  in the denominator. From equation (7),  $\hat{Q}_i$  can be interpreted as the observed proportion of unsound seeds among those that are not unsound due to maternal effects. The observed proportion  $u_{ij}$  is associated

Table 1. — Percent unsound seeds in a diallel cross from a natural stand of Virginia pine (Adapted from BRAMLETT and PEPPER, 1974).

Tree # (Female)	Tree # (Male)				
	1	2	3	4	5
1	93	25	21	20	23
2	8	99	3	7	9
3	7	21	93	7	7
4	6	6	4	66	2
5	7	3	7	4	67

with a binomial distribution having parameters  $N_i$  and  $s_i$ . Hence, if  $\hat{b}_i$  is close to  $b_i$ , the variance of  $\hat{Q}_i$  is approximately  $s_i(1-s_i)/N_i(1-b_i)^2$ . Using an argument similar to that in the preceding paragraph, with  $N_i = 400$  and  $b_i \leq 0.3$ , we conclude that the standard deviation of  $\hat{Q}_i$  would not exceed 0.03. Actually, it might be considerably smaller since  $s_i$  is more typically around 0.9 than near 0.5. Thus, a 95% confidence interval for  $Q_i$  is  $\hat{Q}_i \pm 0.06$ , or narrower.

Once  $\hat{Q}_i$  is known we use a graph such as Figure 1 to obtain  $\hat{n}_i$ , the estimated number of lethals carried by tree  $i$ . More precise values could be obtained by solving equation (3) numerically but this graphical procedure is adequate for most purposes. To produce Figure 1 we assumed that the number of fertilizations follows a Poisson distribution, which is approximated by the fertilization data of Koski (1971). The relationship between  $Q_i$  and  $n_i$  was then

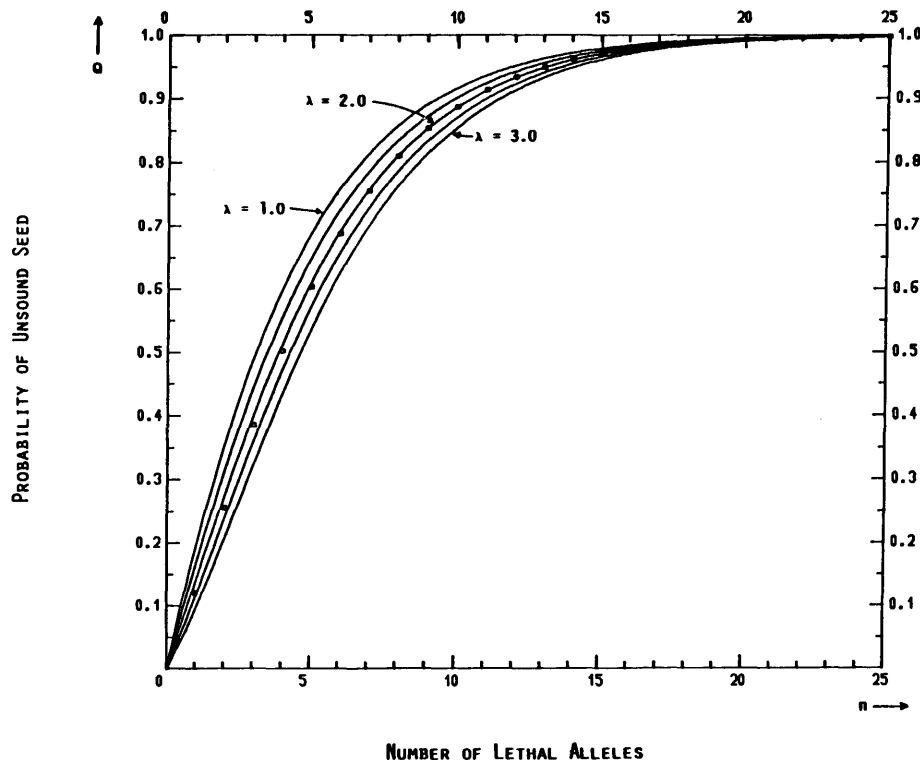


Figure 1. — Probability of unsound seed as a function of number of lethal alleles, when the number of fertilizations  $F$  has a Poisson distribution with parameter  $\lambda$ . From left to right, the curves correspond to respective  $\lambda$ -values 1.0, 1.5, 2.0, 2.5 and 3.0. The small squares correspond to the case where  $P(F = f)$ ,  $f = 0, 1, \dots, 5$  are, respectively, 0.0852, 0.1407, 0.4704, 0.2481, 0.0519 and 0.0037, reported in BISHIR-PEPPER (1977) for Scotch Pine, and having mean number of fertilizations equal to 2.05. The Poisson curve for  $\lambda = 2$  provides a good approximation to the estimates of  $n_i$  corresponding to this distribution of  $F$ .

plotted for several different values of the Poisson parameter  $\lambda$ , which represents the mean number of fertilizations. Also included for comparison are points associated with a graph of  $Q_i$  vs.  $n_i$  using an observed distribution of the number of fertilizations taken from BISHIR and PEPPER (1977), following SARVAS (1968). It is apparent that the Poisson gives an adequate approximation.

### Some Applications of the Model

The model may be applied to observed proportions of unfilled seeds obtained by selfing and crossing a selected sample of conifers. Table 1 lists percentages of unfilled seeds observed in a complete diallel cross of five sample trees from a natural stand of *Pinus virginiana* (BRAMLETT and PEPPER, 1974). Each entry is based on observation of more than 400 seeds.

Table 2 shows least squares estimates of  $b_i$  and  $Q_i$ , and the number  $n_i$  of lethals, for these five trees. For comparison, the last two columns contain estimates of numbers of lethals obtained using the BISHIR-PEPPER (1977) method (our model without the  $b_i$ ), and the 'relative self fertility' approach of SORENSEN (1969), following MORTON *et al.* (1956). While the  $b_i$  give important additional information about the sample trees, their inclusion in the model produces relatively little change in estimates of numbers of lethals. We conclude that previously reported levels of lethal alleles are accurate, deviating from the true values by probably no more than 10%.

Unfortunately, the columns are not strictly comparable. The Bishir-Pepper estimates arise from use of an observed probability distribution, having a mean of 1.7, for number of fertilizations per ovule in pine. For the present model, interpolation in Figure 1 was used to obtain estimates corresponding to a mean of 1.7 fertilizations. However, Figure 1 is based on Poisson distributions which, according to our calculations, give estimates averaging from 0.3 to 0.5 higher than the distribution used in Bishir-Pepper. The Sorensen-Morton estimates are based on still another approximation, described in the discussion section below.

Tables 3 and 4 contain similar data and parameter estimates for six sample trees from a *Pinus taeda* seed orchard. Only for tree # 3 is the estimated number of lethals significantly reduced by including  $b_i$ . Otherwise, reductions are less than one lethal per tree.

### Discussion

The model presented here allows estimation of maternal environmental effects as well as numbers of lethal alleles for individual trees. As is evident from equation (6), selfed

Table 2. — Parameter Estimates Using the Data in Table 1.

Tree #	b-value	Q-value	Estimated no. of lethals		
			Present model	Bishir-Pepper	Sorensen-Morton
1	0.223	0.910	10.5	11	9.6
2	0.068	0.989	18.1	>17	18.0
3	0.105	0.922	11.0	11	10.2
4	0.045	0.644	5.1	4.9	4.1
5	0.053	0.652	5.2	5.0	4.2

Table 3. — Percent unsound seed in a diallel cross from a *Pinus taeda* seed orchard. (Adapted from BRAMLETT, D. L. — personal communication)

Tree # (Female)	Tree # (Male)					
	1	2	3	4	5	6
1	95	35	33	17	20	38
2	15	97	28	20	25	33
3	--	--	82	67	64	60
4	34	19	19	82	24	24
5	18	23	18	16	91	16
6	33	13	9	15	21	95

Table 4. — Parameter estimates using the data in Table 3.

Tree #	b-value	Q-value	Estimated no. of lethals		
			Present Model	Bishir-Pepper	Sorensen-Morton
1	0.288	0.930	11.4	12	10.6
2	0.240	0.961	13.6	14	13.0
3	0.637	0.504	3.7	7.4	2.8
4	0.240	0.763	6.8	7.4	5.8
5	0.182	0.890	9.8	10	8.8
6	0.188	0.938	11.9	12	11.1

data alone are not sufficient to estimate all parameters; outcross data are required for estimation of the  $b_i$ , after which the  $\hat{n}_i$  are computed using the  $\hat{Q}_i$  values obtained from equation (7).

Equation (7) itself is closely related to 'relative self-fertility',  $R$ , used by SORENSEN (1969) to estimate the number of 'embryonic lethal equivalents',  $2B$ . He defines  $R$  as "the number of filled seeds per 100 round seeds following selfing as a proportion of the number of filled seeds per 100 round seeds following outcrossing" (SORENSEN, 1969, p. 392). In our notation,  $R$  represents the ratio  $(1-s_i)/(1-c_{ij})$ . The assumption  $Q_{ij} = 0$ , utilized above, means that  $1-c_{ij} = 1-b_i$ . Also,

$$1-s_i = 1-b_i-(1-b_i)Q_i = (1-b_i)(1-Q_i).$$

Hence

$$R = (1-b_i)(1-Q_i)/(1-b_i) = 1-Q_i,$$

the filled seed proportion accruing from absence of lethal effect, after adjustment for maternal environmental effects. At this point, the SORENSEN-MORTON approach is equivalent to our equation (7). The differences, in Tables 2 and 4, between estimates of  $n_i$  using our model and those obtained from  $R$  derive from Sorensen's use of the approximate formula

$$2B = -4 \ln R = -4 \ln(1-Q_i)$$

to compute  $\hat{n}_i$ .

In addition to the information already obtained from estimates of mean effects, the variability in the estimated numbers of lethals is informative. As noted above, the total number  $M$  of loci at which lethals may occur is at least  $10^4$  (NAMKOONG and BISHIR, 1987). Denote by  $q$  the average proportion of these loci at which lethals actually are pre-

sent. Alternatively,  $q$  is the probability a particular locus contains a lethal allele. If lethals are distributed uniformly and independently among loci, the number of lethals  $n_i$  carried by an individual tree should follow a binomial distribution having parameters  $M$  and  $q$ . The variance,  $Mq(1-q)$ , of this distribution cannot exceed the expected value  $Mq$ , although, since  $1-q$  is close to one, the variance and mean may be nearly identical. A good estimate of  $Mq$  is the average of the estimated values of the  $n_i$ , typically around 10. To obtain the total variance associated with the  $\hat{n}_i$ , we must add the variance of  $\hat{n}_i$  around  $n_i$  to that of  $n_i$  itself. It can be shown that this added variance is relatively small, about 0.6, so the total variance of the  $\hat{n}_i$  is around 11. The value 0.6 is obtained by starting with the approximate variance of  $\hat{Q}_i$  obtained previously. Then, assuming the curves in *Figure 1* are exponential in form, we compute the variance of  $\hat{n}_i$  from that of  $\hat{Q}_i$ . The distribution of the  $\hat{n}_i$  is probably skewed, but if the model assumptions are accurate we may conclude that about 95% of conifers carry between 4 and 16 lethals, inclusive.

Carrying the analysis a step further, we can compute the variance of the sample variance  $s^2$  of the  $\hat{n}_i$  obtained from a sample of  $t$  trees. Following WILKS (1962) we find  $\text{Var}(s^2) = 2\sigma^4/(t-1)$  when the underlying observations are normally distributed (a good approximation to our binomial distribution of the  $n_i$ ). Thus if model assumptions hold, observed sample variances should have a mean value around  $\sigma^2 = 11$  and variance given by the above formula. The standard deviation of  $s^2$  is thus about 7 when  $t = 6$ , dropping to 2.7 when  $t = 35$  and 1.75 when  $t = 80$ .

It is of interest in this regard to examine variances observed in single stands and old fields in comparison with those observed in mixed or widely dispersed stands. A study by FRANKLIN (1972) on *P. taeda* from a single even-aged stand shows a mean of 8.5 and a variance of 6.6. Similar figures were obtained for *Picea glauca* by FOWLER and PARK (1983) where an old-field stand, denoted TAY, can be estimated as having a mean of 12.9 and a variance of 6.5. In contrast, the data on *Pinus virginiana* (Tables 1 and 2) show a sample mean of 9.9 lethals carried and a sample variance of 30.0. Here the sample variance exceeds expected chance fluctuations. It is apparent that the  $n_i$  associated with trees 4 and 5 are aberrant and it turns out these trees were located close to one another while the others were widely scattered over the stand area. Similarly, the data on *Pinus taeda* (Tables 3 and 4) give a sample mean of 9.5 and sample variance 13.8. These observations were drawn from a seed orchard which was probably established over a period of a few years from heterogeneous pollen and seed sources (BRAMLETT, D. L., personal communication).

From a mixed age stand, denoted AFES, FOWLER and PARK (1983) provide data on two different samples, the first having mean 8 and variance 17.6, the second mean 9.1 and variance 9.6. On the same AFES area, the mean and variance for *Larix laricina* in four subpopulations can be estimated from the data of PARK and FOWLER (1982) to be 12.3 and 17.2 while *Picea mariana* of mixed ages yield a mean of 6.6 and a variance of 20.2. We further note that the 35 *Pseudotsuga menziesii* trees sampled by SORENSEN (1969) had mean 11.2 and variance 32.0, and were from five different areas. Similarly, the 80 *Pinus sylvestris* studied by KOSKI (1971), with mean and variance of 9.4 and 18.0, were also distributed over a wide geographic area. Observed sample variances from mixed or dispersed stands thus tend to exceed the associated means, often by considerably more than two standard deviations.

It seems clear from these observations of large variance that lethal alleles are not uniformly distributed in forests. Since, as observed in NAMKOONG and BISHIR (1987), it is the rate of mutation to lethal form that determines the number of recessive lethals carried in a population, we suspect that mutation rates may have varied over different forest locations. However, it is also possible that other factors such as localized bottlenecks on selection could have affected the lethal load and generated the variation we presently observe in conifer forests.

Variation apparently also exists from ramet to ramet of the same tree. As indicated above, the variance of individual  $u_{ij}$  in Tables 1 and 3 should not exceed  $1/1600$ . The accompanying standard deviation is at most  $1/40 = 0.025$ . Hence at least 95% of the proportions associated with outcross observations should lie within  $\pm 0.05$  of the true  $b$ -value. For a particular row of the table, i.e., a particular female tree, two outcross proportions should differ by more than 0.1 only rarely. In the seed originating from the natural stand of *Pinus virginiana* (Table 1), differences exceeding 0.1 occur only in row 3. However, for the different ramets used in the *Pinus taeda* seed orchard (Table 3), larger differences occur in every row except row 5 and the deficient row 3. Evidently, ramet effects introduce more variation into sound seed yield than is expected from sampling different branches of the same standing tree.

Another view of the same phenomenon is obtained by looking at the difference between proportions  $u_{ij}$  and  $u_{ji}$ , adjusted for the estimated difference in  $b_i$  and  $b_j$ . After this adjustment, the observed  $u$ 's should be roughly equal, as the genetic makeup of the parents is the same in both cases. However, in Table 3 this is not the case for tree pairs 1 and 2, 1 and 4, and 2 and 6. For this sample it seems likely that maternal environmental effects, as measured by the  $b_i$ , include variation in ramet environment.

The above discussion has implications for sampling methodology. Accuracy of estimates coming from the model depends on the validity of the assumption that the  $Q_{ij}$  are nearly zero. This is likely to be true for genetically unrelated trees. However, with closely related trees — for example, near neighbors — this may well not be the case. To obtain accurate estimates, one should choose widely separated trees.

If the degree of genetic relationship (e.g., the number of lethals in common loci) between two trees or among a group of neighboring trees is of interest, then non-neighbors should be included in the sample in order to get clean estimates of the  $b_i$ . Following this the  $n_{ij}$  for the neighboring trees can be estimated.

It is also necessary to include widely separated sample trees in order to check on variability from one forest location to another in the numbers of lethals that are carried. In this case it seems advantageous to sample several trees in each location in order to compare variability of estimates within locations with that between locations. Here a combination of the methods outlined in the two preceding paragraphs would be appropriate.

### Summary and Conclusions

In this paper we have introduced a model for unsound seeds in conifers which includes maternal environmental effects as well as effects due to recessive lethal alleles. Applied to typical data on proportions of unsound seed, the model yields parameter estimates which indicate that the probability of failure due to the maternal environment typically ranges from 0.05 to 0.3. Adjusted for maternal

effects, the data provide lower estimates of numbers of recessive lethals than heretofore obtained, but the reduction is slight, typically less than one lethal per tree.

The model and the estimates obtained indicate greater variability in proportions of unsound seed than would be expected from sampling error alone. Our results suggest first, that maternal effects may differ markedly from ramet to ramet of the same tree and second, that numbers of lethals that are carried differ from one forest location to another. In turn, these observations have implications for sampling methodology.

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## Short Note: Use of Kung's Method for Estimating Parental Effects in an Irregular Mating Design

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#### Abstract

Wood density parameters were determined by x-ray densitometry for a second generation progeny trial of *Pinus caribaea* var. *hondurensis* BARR. et GOLF.. The mating design from which the trial originates is very irregular and represents only 29% of all possible crosses between the parents involved. An enhanced version of the program developed by KUNG (1978) was used to calculate combining abilities.

*Key words:* *Pinus caribaea* var. *hondurensis*, combining abilities, wood density, incomplete partial diallel.

#### Zusammenfassung

Für eine Nachkommenschaftsprüfung mit *Pinus caribaea* var. *hondurensis* BARR. et GOLF. in zweiter Generation wurden Holzdichte-Parameter durch Röntgenstrahlen-Dichtmessung bestimmt. Der Kreuzungsplan aus dem der Versuch hervorging, ist sehr unregelmäßig und umfaßt nur 29% aller möglichen Kreuzungen zwischen den beteiligten Eltern. Eine vergrößerte Version des von KUNG (1978) entwickelten Programmes wurde zur Errechnung der Kombinationseignung benutzt.

#### Introduction

*Pinus caribaea* var. *hondurensis* BARR. et GOLF. was introduced to Queensland plantations in the 1950's and has been the subject of intensive breeding since that time (see NIKLES, 1973). One of the series of second generation progeny trials was intended to be an incomplete partial diallel among 19

parents. Biological and operational constraints reduced representation to 53 full sib families, the product of matings between 13 female and 14 male parents. The irregular and incomplete mating design of this trial, in which only 29% of all possible crosses are represented, lends itself to use of the method developed by KUNG (1978). This approach is valuable because it circumvents problems associated with the calculation of heritabilities for such data (SIMMONDS, 1979).

#### Experimental materials and assessment procedures

Experiment 507/2A Genetics was planted in March 1973 near Cardwell, Australia (18°11'S, 146°00'E). It was duplicated on well and poorly drained sites. On each site, eight blocks were established. Each block comprised six plots, each of which contained one representative of each family. Four fertilizer treatments were applied at each site. In July, 1983, 8 mm increment cores were collected, bark to bark, at breast height from two randomly selected representatives of each family in the two most complete fertilizer treatments common to both sites. Thus, a total of eight cores were sampled for each family.

At the Oxford Forestry Institute, cores were prepared for and assessed by the x-ray densitometry techniques described by HUGHES and SARDINHA (1975). For each radius, mean weighted radius density,  $\bar{p}$ , was determined as (i)

$$\bar{p} = \frac{\sum a_i p_i}{\sum a_i} \text{ and within radius variability, } \bar{v}, \text{ as (ii)}$$

$$\bar{v} = \sqrt{\frac{\sum a_i (p_i - \bar{p})^2}{\sum a_i - 1}},$$

where  $p_i$  = density at the  $i$ th densitometer step;

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