clones and light intensities in rates of net and gross photosynthesis, but not in rates of dark respiration; (2) differences in rates of photosynthesis among light intensities were caused by differences in the uptake of CO, per unit of photosynthetic tissue (photosynthetic efficiency); (3) differences among clones in net and gross photosynthesis were related to differences in plant size and photosynthetic efficiency, but more closely to photosynthetic efficiency; (4) differences existed among clones in early first-year growth and distribution of assimilate; (5) differences among light intensities, with light saturation at approximately 3,000 to 5,000 foot-candles, indicate that low survival and growth of field-planted stem cuttings probably are not related to the effect of light intensity on pihotosynthesis when the seedlings receive X to ½ of full sunlight; (6) differences among clones in rates of photosynthesis provide an additional character that may be used as a basis for selection in tree-improvement programs; and (7) differences among clones from stem cuttings in early first-year growth and distribution of assimilate give strong evidence for the existence of metabolically superior genotypes, the selection of which could be extremely beneficial in any tree improvement program.

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Linkage Between Marker Genes and Embryonic Lethal Factors May Cause Disturbed Segregation Ratios

By FRANK SORENSEN[1])

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Several recent papers have reported ratios of normal to deviant or marked seedlings after self-pollination of trees of coniferous species. The range of reported ratios is from 2.2 : 1 (SQUILLACE and KRAUS 1963) to 18.8 : 1 (FOWLER 1965 c), with many of them clustered around the 3 : 1 monohybrid segregation ratio, or somewhat higher at 4 to 10 : 1.

It has seldom been clear why the observed ratios often differed considerably from the expected 3 : 1 for marker traits, although several causes have been suggested. These have been:

1. Deviant seedlings difficult to identify, perhaps because mutant trait sensitive to environment.
2. Sampling error. Most selfed families are small and observed ratios such as 2.2 : 1 and 4 or 5 : 1 do not differ significantly from the expected 3 : 1 ratio.
3. More than one genetic factor may underlie the trait.
4. Embryo abortion after one fertilization per ovule. Many deviant seedlings are weak as seedlings. Therefore, it is not unreasonable to expect that they were weak as proembryos or embryos also and that a proportion may have succumbed before germination. In other words, the deleterious effect of the marker mutant may be pleiotropic as well as direct.
5. Pregermination selection after multiple fertilization. Multiple fertilivation has been verified in some coniferous species (SARVAS 1962, p. 94) and more than one embryo commences growth within a single gametophyte. If a deviant (homozygous recessive) embryo is competing with a normal embryo in this situation, the deviant embryo may be selected against. This has occurred in *Pinus resinosa* AIT. (FOWLER 1964).

Any of these reasons may be valid. However, there is a fifth possible cause of deviation from the monohybrid ratio – linkage between the marker and a gene which somehow prevents germination (an embryonic lethal gene). For example, if a tree is heterozygous for both a recessive marker gene (selectively neutral) and a recessive embryonic lethal gene, and if the two recessives are completely linked in coupling, a selfed progeny from this tree will contain no marked seedlings. Only normals would germinate. Another example: if the two genes are completely linked in repulsion, a selfed progeny will contain normal-to-deviant seedlings in the proportion of 2 : 1. Now, none of the aborting embryos would carry the marker gene as homozygous recessive; all deviant individuals would germinate. Of

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Table 1. — Effect of linkage between embryonic lethal factor and seedling marker gene on ratio of normal-to-marked seedlings resulting from selfing a tree carrying a recessive marker.

Recombination value	Marked seedlings	Normal : marked seedlings
	Percent	*Ratio*
	Genes in coupling	
0.50 (independent)	25	3:1
.40	21	3.7:1
.30	17	4.8:1
.20	12	7.3:1
.10	6.3	14.8:1
0 (completely linked)	0	∞
p	$\left(\frac{p(2-p)}{3}\right) 100$	$\frac{3-p(2-p)}{p(2-p)} : 1$[1]
	Genes in repulsion	
.50 (independent)	25	3:1
.40	28.0	2.6:1
.30	30.3	2.3:1
.20	32.0	2.1:1
.10	33.0	2.03:1
0 (completely linked)	33.3	2:1
p	$\left(\frac{1-p^2}{3}\right) 100$	$\frac{p^2+2}{1-p^2} : 1$

[1]) General formulae courtesy of Dr. A. E. Squillace, personal communication.

course, if the marker gene and the lethal gene segregate independently, normal-to-deviant seedlings would appear in the ratio of 3 : 1. *Table 1* shows the expected effect of linkage on the frequency of marked seedlings at several linkage intensities. *Figure 1* shows the effect through the range of linkage intensities.

How many embryonic lethal factors might the average forest tree carry? Initial work which we have done in coastal Douglas-fir *(Pseudotsuga menziesii* [Mirb.] Franco) indicates 9 to 10 segregating factors per modal individual. This number was obtained in the course of comparing set of sound seed after selfing and after controlled outcrossing with pollen from unrelated trees. It is a preliminary estimate based on 24 Douglas-fir trees in west-central Oregon. (The sample is presently being enlarged, and some trees are being tested a second time to increase the precision of the estimate.) Fowler (1965 b), assuming an average of 2 fertilizations per ovule in *Pinus banksiana,* has estimated that the mean minimum number of lethal alleles for which 5 tested trees were heterozygous was 15.

If one fertilization per ovule is assumed, a sound seed set of about 7 percent would result from the selfing of a tree carrying 9 to 10 independent lethal factors. (That is, such a tree might set about 2 sound seeds per cone on selfing versus an average of 28.5 sound seeds per cone when outcrossed to unrelated trees.) A seed set of about 25 percent would result when a tree carrying five recessive lethals is selfed. Self seed sets as low as 5 to 25 percent of outcross seed sets do not appear to be uncommon in several tree species (Fowler 1965 a and Squillace and Kraus 1963 list references pertaining to this figure).

It should be noted that the low seed set after selfing could be due either to reduced fertilization or to greater embryo mortality. However, embryo mortality appears to be the cause in Douglas-fir (Orr-Ewing 1957), *Picea glauca* (Mergen *et al.* 1965), *Pinus sylvestris* (Sarvas 1962), and several other conifers (Hagman 1965). (For discussion of this and other causes, some nongenetic, see Sarvas 1962, pp. 122—128). Therefore, I will assume that, in general, low seed set after self-pollination is due mainly to embryo mortality, and that this is probably conditioned by embryonic lethal factors carried in the parent as heterozygous recessives.

Given a certain frequency of recessive embryonic lethal factors, how often might one expect a distorted ratio for a marker gene to occur in a selfed progeny? Let us use Douglas-fir as an example and assume the modal tree carries 10 segregating recessive lethal factors. Let us also assume that each chromosome arm contains a group of genes 50 crossover units in extent (for some indication of the reasonableness of this figure, see Sturtevant and Beadle 1962, p. 102, and Snyder and David 1957, p. 164, where chromosome maps for *Drosophila melanogaster* and corn are given). Douglas-fir has 2 pairs of telocentric chromosomes and 11 pairs which have more or less median centromeres (Barner and Christiansen 1962), or 24 pairs of linked groups of genes approximately 50 crossover units in length. If a particular tree carries 10 independent lethal alleles, the probability that any marker would be linked to one of these factors, tightly or loosely, in coupling or repulsion, is about 0.35.

This probability has been calculated as the summation over the 24 linkage groups (arms) of the probability of the marker being in a specific linkage group (arm) times the probability of linkage on that arm with at least one lethal, or

$$\mathrm{Pr(linkage)} = \sum_{i} [\mathrm{Pr(marker\ on\ arm\ i)} \cdot \mathrm{Pr(linkage\ on\ arm\ i)}]$$

where,

$$\mathrm{Pr(marker\ on\ arm\ i)} = 1/24$$

$$\mathrm{Pr(linkage\ on\ arm\ i)} = 1 - \mathrm{Pr(no\ linkage)} = 1 - (23/24)n,$$

where,

$$n = \text{number lethals} = 10.$$

This, of course, is a simplified picture and complications may be mentioned which will affect the computations. The estimate of number of lethals may be inaccurate. The arms are surely not all equivalent to linkage groups of 50 crossover units. The distribution of arm lengths may be more or less independent of cytological lengths. However, the extent and direction of the last two effects are not known, so for the purpose of illustration in this paper the preliminary estimate of 10 lethals and the simplifying assumption of 50 crossover unit linkage groups have been used.

Considerable divergence from the normal/deviant ratio of 3 : 1 is seen when a selectively neutral marker gene and

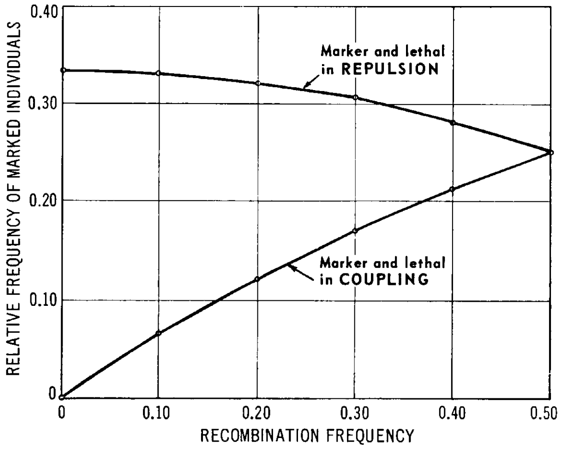

Figure 1. — Effect of linkage between embryonic lethal gene and marker gene on percent of marked individuals in a selfed progeny.

the lethal are separated by about 25 crossover units or less *(Figure 1)*. The probability that the single marker gene and 1 of the 10 lethals are in the same half-arm of a chromosome (there are 48 half-arms) is 0.19. If these assumptions are valid, then, among individuals which carry marker genes, about one in five would carry it within approximately 25 crossover units of a lethal gene. An additional one individual in five would carry the lethal and the marker more loosely linked. Thus, one would not have to deal with many recessive marker genes before he could expect some deviant ratios to occur because of this linkage. This, of course, applies only to species with a fairly high incidence of lethals. Where there are few, for example in *Pinus resinosa* (FOWLER 1965 a), it would be most improbable that linkage with a lethal would be the cause of a distorted segregation ratio of a marker.

In some cases, it may be desirable to know the cause of an abnormal ratio and/or genetic basis of a recessive trait which shows up in an unexpected proportion in some selfed progeny. Both embryo abortion and pregermination selection would usually (perhaps always) result in fewer deviant seedlings than expected, whereas in the case of linkage with a lethal either excesses or deficiencies could be expected. Hence, where there is a well-based ratio giving an excess of deviants, linkage should be suspected as the cause. Where excesses of normal seedlings are obtained, the cause may be elucidated by use of the technique of FOWLER (1964), by knowledge of the frequency of embryonic lethals, and in some rare cases by crossing two trees which both carry the same marker but not the same lethal. The last approach, perhaps largely unusable now, may gain utility with time as the inventory of marker-carrying individuals is built up in the more thoroughly studied species.

Summary

In this paper, an attempt has been made to estimate the frequency with which the observed segregation of marker genes may differ from the expected due to linkage with an embryonic lethal. It should be stressed that the estimate is based partially on unverified assumptions. However, the findings serve to point out that investigators working with segregation ratios in selfed progenies of forest trees can expect such linkage from time to time. A frequency of such linkage is estimated for coastal Douglas-fir.

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The Slash x Sand Pine Hybrid1)

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Interspecific hybridization has been recognized for several years as a potentially useful technique in forest tree improvement. Consequently, many organizations have incorporated some type of hybridization in their breeding programs, particularly when trying to develop material for use on problem sites. The successful cross of *Pinus elliottii* ENGELM. var. *elliottii* (slash pine) and *P. clausa* (CHAPM.) VASEY (sand pine) made in 1962 by the Union Camp Paper Corporation is the result of such an endeavor. Although difficulties in making the cross undoubtedly will restrict its commercial use, this hybrid is of interest for taxonomic study and for transferring characters between species by backcrossing. Of particular significance is the fact that it represents one of the few successful inter-Group crosses in the genus *Pinus.*

The hard pines of southeastern United States were arranged by SHAW (1914) into two Groups. *Pinus elliottii* (listed as *P. caribaea* by SHAW) was placed along with most of the other major pines of the region in the Group *Australes*, while *P. clausa* was placed in the *Insignes.* Later DUFFIELD (1952) regrouped the hard pines primarily on the basis of results obtained from hybridization studies. In this revision, all of the southern pines were grouped together except *P. clausa* and *P. virginiana.* They were placed with *P. banksiana* and *P. contorta* in a separate Group because of their morphological similarity.

Pinus elliottii and *P. clausa* occur sympatrically over extensive areas in central Florida, but they are easily identified because of striking differences in morphological features. For example, slash pine occurs as a medium- to large-sized tree generally of good form with long needles (12−25 cm.) and medium-sized cones (5−15 cm.) that open at maturity (SHAW, 1914; HARLOW and HARRAR, 1941). Sand pine, on the other hand, is commonly found as a small, low spreading tree of poor form with small needles (5−9 cm.) and cones (5−8 cm.) that are usually serotinous.

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