

SEGREGATION FOR CHLOROPHYLL DEFICIENCIES AND OTHER PHENODEVIANTS IN THE X_1 AND X_2 GENERATIONS OF IRRADIATED JACK PINE

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Readily identifiable phenotypes, such as cotyledonary chlorophyll deficiencies, whose genetic basis can be established, would be highly useful in forest genetics research as genetic markers. In the genus *Pinus* mutants with severe deficiencies, such as albinos, will die shortly after seed germination while those with less severe deficiencies will not. In either case, however, the symptoms appear in early stages of growth and hence these types of mutants are especially useful as gene markers.

Where the mutation is lethal, the gene or genes are carried in natural populations in heterozygous combinations. Selfing or cross-pollination with a heterozygous carrier is necessary, therefore, to identify individual trees with a heterozygous genotype for this characteristic. Controlled self-pollination studies have shown that the pines are self-fertile in varying degrees (see review by Squillace and Kraus 1963), so identification of genotypes heterozygous for this character is possible. Scoring of the trait, however, must be done in the early seedling stages.

Chlorophyll deficiencies in seedlings of conifers have been reported by numerous investigators including Johnson (1945), Johnson (1948), Eiche (1955), Langner (1959), Cram (1960), Barnes *et al.* (1962), Squillace and Kraus (1963), Fowler (1962, 1965a, 1965c), and Snyder *et al.* (1966). They have found limited use as genetic markers in such studies as the determination of natural self-fertilization frequency (Cram 1960, Squillace and Kraus 1963, Fowler 1965a, 1965c) and in pollen dispersion studies (Langner 1952). Other applications in forest genetics research where genetic markers are essential could well utilize chlorophyll deficiencies alone or in combination with other easily recognized juvenile phenotypes.

To be of value, however, the genetic basis or mode of inheritance of such markers must be known. Results of a number of studies in conifers show an apparent 3:1 or single recessive gene ratio of normal vs. chlorophyll-deficient seedlings (Johnson 1945, Johnson 1948, Cram 1960, Barnes *et al.* 1962). In these studies a limited number of parents were usually involved. In more recent studies, including a larger number of parent trees, the results show frequent deviations from the simple 3:1 ratio (Squillace and Kraus 1963, Fowler 1965a,

1965c, and Snyder *et al.* 1966). These authors suggest several factors that may influence the observed segregation ratios. Most important appear to be environmental effects limiting deviant seedling emergence and possible pregermination lethals associated with the chlorophyll-deficient character. Certainly, larger populations than have been reported in the results of some studies must also be considered as essential for accurate definition of segregation ratios.

This report shows the frequency of chlorophyll deficiencies in a relatively large population of jack pine (*Pinus banksiana* Lamb.) in the second generation following X-irradiation of seed, and in a control population. Presented are segregation ratios based on open-, self-pollinated, and backcrossed progenies. Radiation effects, other than those concerned with phenodeviants, will be reported elsewhere.²

Material and Methods

Jack pine seed collected in 1950 on the Chipewewa National Forest in Minnesota was irradiated on March 14, 1951, with X-rays at dosages of 1,000 *r* and 4,000 *r*.³ An unirradiated seedlot of the same origin was included as a control. The seed was sown in standard beds in the Hugo Sauer Nursery at Rhinelander, Wisconsin, on May 3, 1951. No seedlings survived after the first year in the nursery in the 4,000 *r* lot and only about one-fourth remained in the 1,000 *r* lot. Complete results of performance in the nursery will be reported elsewhere.²

In the spring of 1954, 200 seedlings from the control lot and 200 from the 1,000 *r* lot were planted on the Argonne Experimental Forest near Three Lakes, Wisconsin. Female strobili were evident beginning with the first year in the field, and the production of cones has steadily increased to the present.

During June and again in December 1961, 100 open-pollinated seeds were sown in sand in a greenhouse bench from: (1) 90 trees grown from

²Rudolph, T. D. *Effects of X-irradiation of seed on X_1 and X_2 generations in *Pinus banksiana* Lambert.* Ms. in preparation at Institute of Forest Genetics, Rhinelander, Wis.

³The seed used in this study was irradiated at Harvard University through the kind cooperation of Dr. Scott S. Pauley, at that time on the staff of the Maria Moors Cabot Foundation for Botanical Research at the University.

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the 1,000 r irradiated seed, (2) 45 subsamples from a bulk collection in the control plot, and (3) 45 subsamples from a bulk collection made in a natural stand in northern Wisconsin. Growing conditions in the greenhouse were the same as reported in another paper by Rudolph (1966) in these Proceedings.

Germination and early seedling development were observed periodically. The total number of deviant seedlings described and transplanted for further study from the two sowings were 324 from the individual trees grown from irradiated seed, 177 from the control plot collection, and 158 from the natural stand collection. From these preliminary trials, it was evident that we could further define the deviant types and possible differences in their frequencies due to X-irradiation of the seed from which the parents were grown only through study of both control-pollinated and open-pollinated individual tree progenies.

The controlled pollinations were made in 1962 and 1963. Ten isolation bags were employed on each of 24 trees in the 1,000 r plot in 1962, and on 86 trees in the 1,000 r plot and 21 trees in the control plot in 1963. The 24 trees included in the irradiated plot pollinated in 1962 were included in the 1963 pollination. The strobili in 8 bags on each tree were selfed, the strobili in 1 bag were pollinated with a mixture of Chippewa National Forest origin, and the strobili in 1 bag per tree were left unpollinated to serve as a control on the bagging technique. Pollination records and field labeling were maintained on an individual-bag and -branch basis to permit definition of possible radiation-induced chimeras on the basis of the seedling progenies.

Cones from the two years' pollinations were collected in September 1963 and 1964. The seed was extracted by dismembering each cone and was stored at 38°F. Beginning on March 18, 1965, the seed was sown in sand in a greenhouse bench. (For greenhouse conditions see second paper by Rudolph in these Proceedings.)

Germination counts were made after one month. Beginning at germination and during early seedling development, deviant seedlings were searched for frequently; and when the seedlings were 3 months old a final check was made. All unusual seedlings selected from the population, together with controls, were potted for further study.

Results and Discussion

Among the most easily recognized phenodeviants in the early seedling stages of the material in this study were plants showing chlorophyll deficiencies in their cotyledons. Although quantitative differences in chlorophyll deficiency were evident between and, rarely, within progenies, it

was felt that the differences could not be accurately classified as proposed by Gustafsson (1940). Furthermore, the expression of cotyledonary chlorophyll deficiencies is influenced by environmental conditions during seed germination and early seedling growth (Eiche 1955, Snyder *et al* 1966). All chlorophyll deficiencies were therefore considered as one type of deviant in the present study. Cross-breeding studies between individual trees heterozygous for the chlorophyll character are being continued, together with attempts to distinguish accurately between chlorophyll-deficient types among the pedigreed progenies.

Table 1 lists progenies showing more than 4 percent of the plants with this character. Chlorophyll deficiencies occurred at lower frequencies in 14 other selfed progenies. Of the 35 segregating progenies listed, 12, or more than one-third, showed an apparent segregation ratio of 15 normal: 1 mutant suggesting, in the case of the selfed progenies, heterozygosity for two recessive genes in the parent. Four of the 35 progenies segregated in a 3:1 ratio for chlorophyll deficiency in the cotyledons, indicating heterozygosity for one recessive gene in the parent. Nine others showed an apparent 7:1 ratio which does not result upon selfing either of the suggested genotypes. A 7:1 ratio could result from crossing a double heterozygote and a single heterozygote in a 2-gene model. This would be evident only in the backcrosses listed in table 1. However, only 2 of the 8 backcrosses listed show an apparent 7:1 segregation ratio.

Two other explanations are plausible. Perhaps the 7:1 ratio is a distorted 3:1 ratio resulting from a partial pre-germination lethal associated with the chlorophyll deficiency. On the other hand, the possibility cannot be discounted that the 7:1 ratio may actually result from an original 15:1 ratio distorted due to lethality independent of the chlorophyll deficiency, or in fact associated with the chlorophyll-sufficient character. This same type of phenomenon would explain the occurrence of 2:1 ratios in five of the progenies as being originally 3:1 ratios with a lethal associated with the chlorophyll character.

Some of the apparent 7:1, 2:1, and 1:1 ratios listed in table 1 also fit the simple 15:1 or 3:1 ratios, but with a low probability, in the chi-square goodness-of-fit test. This again suggests pre-germination lethals linked with the chlorophyll-deficiency factor in some cases and independent of it in others.

The presence of a relatively high proportion of chlorophyll-deficient seedlings in three of the open-pollinated progenies (N2, S42, and P43) suggests, on the basis of segregation ratios in their selfed progenies, that from 17 to 25 percent natural selfing occurred on these trees in the open-pollinated cones. These estimates agree quite well with

Table 1.--Segregation for cotyledonary chlorophyll deficiencies in open-pollinated, selfed, and back crossed^{1/} jack pine individual tree X₂ progenies

Tree number ^{2/}	Total no. of seedlings	No. of chlorophyll-deficient seedlings	Apparent ratio	P for chi-square goodness-of-fit test ^{3/}	Actual ratio
N2 Open-pollinated	235	12	--	--	18.58:1
N2 Self-pollinated	190	59	2:1	.60	2.22:1
N2 Backcross	34	6	7:1	.40	4.67:1
N8 Self-pollinated	11	1	15:1	.70	10.00:1
N12 Self-pollinated	31	5	7:1	.55	5.20:1
N15 Self-pollinated	219	11	15:1	.45	18.91:1
N35 Self-pollinated	19	1	15:1	.85	18.00:1
N35 Backcross	23	1	15:1	.70	22.00:1
N41 Self-pollinated	24	6	3:1	1.00	3.00:1
N46 Backcross ^{4/}	21	1	15:1	.80	20.00:1
N53 Self-pollinated	60	11	3:1	.20	4.45:1
N53 Backcross	29	2	15:1	.80	13.50:1
N62 Self-pollinated	65	5	15:1	.65	12.00:1
N81 Backcross ^{4/}	19	2	7:1	.80	8.50:1
N82 Self-pollinated	294	32	7:1	.40	8.19:1
N82 Backcross	159	8	15:1	.50	18.87:1
N90 Self-pollinated	20	1	15:1	.80	19.00:1
S16 Self-pollinated	8	3	2:1	.80	1.67:1
S18 Backcross ^{4/}	107	1	--	--	--
S27 Self-pollinated	15	1	15:1	.95	14.00:1
S42 Open-pollinated	235	17	--	--	12.82:1
S42 Self-pollinated	53	15	3:1	.60	2.53:1
S42 Backcross	40	9	3:1	.70	3.44:1
S47 Self-pollinated	46	2	15:1	.60	22.00:1
S48 Self-pollinated	37	11	2:1	.65	2.36:1
S66 Self-pollinated	14	8	1:1	.60	0.75:1
S89 Self-pollinated	14	2	7:1	.85	6.00:1
S100 Self-pollinated	8	1	7:1	1.00	7.00:1
P14 Self-pollinated	141	18	7:1	.90	6.83:1
P43 Open-pollinated	103	7	--	--	13.71:1
P43 Self-pollinated	89	30	2:1	.80	1.97:1
P51 Self-pollinated	180	66	2:1	.35	1.73:1
P87 Self-pollinated	85	9	7:1	.60	8.44:1
P88 Self-pollinated	244	12	15:1	.40	19.33:1
P92 Self-pollinated	112	10	7:1	.25	10.20:1

1/ Seedlings resulting from open-pollinated seed were backcrossed with the female parent.

2/ N and S trees were grown from 1,000 r X-rayed seed. P trees are from non-irradiated control seed.

3/ P values rounded off to nearest 0.05.

4/ Trees N46, N81, and S18 had no chlorophyll-deficient seedlings in the open-pollinated or selfed progenies.

the frequency of natural selfing reported by Fowler (1965a). Nevertheless, the estimates may be somewhat high for two reasons. First, they do not take into account chlorophyll deficiencies that may

have resulted from crossing with other heterozygous carriers. However, Snyder *et al* (1966) have found evidence from intercrossing 18 carriers that only 2 possessed a common gene for chlorophyll

deficiency, which suggests that the same phenotypes are produced by several non-alleles. Thus, the estimates of natural selfing presented here probably would not be greatly reduced by taking into account crossing among carriers. Second, since two of the three progenies under examination (N2 and P43) show a 2:1 segregation in their selfed progenies, possibly a lethal associated with the dominant chlorophyll factor is also tending to increase the proportion of chlorophyll deficiencies appearing in the open-pollinated progenies; if so, it would increase the apparent frequency of natural selfing.

The appearance of chlorophyll deficiencies in seedling progeny from backcrosses to female parents not showing chlorophyll deficiencies in their own selfed progeny (N46, N81, and S18) adds further to the uncertainty of the genetic basis for the chlorophyll character. From the response of the backcross progeny these female parents must be carriers of the recessive gene or genes for chlorophyll, but none of the homozygotes appear in the selfed progenies. Apparently all homozygotes for chlorophyll deficiency will die prior to germination when present in a largely homozygous genotype, but at least a few are viable in a more heterozygous genetic background. The possibility of cytoplasmic inheritance also cannot be excluded, and undoubtedly other explanations of this behavior could be offered. Indeed, possibly none of the explanations offered above could fully explain the extremely rare occurrence of chlorophyll deficiencies in the 14 selfed progenies not included in table 1.

No apparent differences in the frequency of trees carrying recessives for chlorophyll deficiencies were noted between the trees grown from irradiated seed and those from the control plot. In both groups, approximately one-fourth of the trees were found to be carriers of the recessive genes segregating in the ratios shown in table 1. This suggests that 1,000 r of ionizing radiation applied to seed did not increase the frequency of chlorophyll deficiencies in the second generation. However, since the trees from irradiated seed that were studied represent only a small part of the original population as a result of radiation-related mortality, it is also possible that the trees observed producing progenies segregating for this character represent only a small portion of those initially present. The high frequency of the recessive genes in this population does suggest a relatively high spontaneous mutation rate to the recessive condition at several loci. Squillace and Kraus (1963) present results which suggest a similarly high mutation rate for chlorophyll deficiency in *Pinus elliotii* var. *elliotii*. These authors also suggest that the frequency of the recessive genes may be influenced by other factors such as selection favoring heterozygotes over both homozygotes. Further study is needed to define the mutation rate

or other factors responsible for the apparent high frequency of the recessive chlorophyll character.

Numerous other phenodeviants were found among the seedling progenies grown. Some of those readily recognized and occurring in significant frequencies are listed in table 2. On the basis of their frequencies in open-pollinated progenies and segregation upon selfing, a frequency of about 10 percent natural selfing is indicated. Of special interest are the chlorophyll-deficient seedlings that appear to regain a normal green color following the cotyledon stage. Among other things, this suggests that some mature trees may in fact be homozygous recessive for the chlorophyll characteristics manifested at the cotyledon stage. Fowler (1965b) has reported on a *Pinus resinosa* tree that may have this genotype. The reverse situation found in three progenies in which the cotyledons are green and the primary and secondary leaves initially deficient in chlorophyll presents another interesting phenomenon. This characteristic may be similar to the virescent type in *Pinus elliotii* reported by Kraus and Squillace (1964). Both types of seedlings survive at the end of 6 months in the greenhouse, but under natural conditions survival might be greatly reduced.

Two types of deviants which the author has not previously observed in large, non-irradiated populations and which may be radiation-induced mutants were found in Trees S30 and S91 (table 2). Both are apparently distinct mutants and are easily recognized. In the S30 progeny the mutants can be distinguished as soon as the seedlings germinate. Those in the S91 progeny become readily apparent with the development of primary leaves. These and other mutants isolated will be grown for further study.

Although no definite chimeras on the X_1 trees were identified from the X_2 progenies, several were suggested by differences in progenies from different parts of the individual X_1 trees. Larger control-pollinated progenies will be grown from parts of the trees showing these apparent differences to more accurately define the type and extent of the chimeras if present.

Summary and Conclusions

Approximately one-fourth of the trees both in the population grown from irradiated seed and in a control population were found to be carriers of genes recessive for chlorophyll deficiencies. Although this suggests that 1,000 r of ionizing radiation applied to seed had no effect on the frequency of chlorophyll deficiencies in the second generation, high early mortality in the irradiated population may obscure possible differences. Several segregating ratios for this characteristic were found. Possible explanations for the deviations from normal Mendelian ratios are presented. On

Table 2.--Segregation for deviant characteristics other than cotyledonary chlorophyll deficiencies in open- and self-pollinated jack pine individual tree X₂ progenies

Tree no.	Type of pollination	Description of mutant	No. of seedlings	Ratio normal: mutant	Probable genotype of parent
N40	selfed	Cotyledons normal green; primary leaves yellow	52	3.0:1	Heterozygous for one recessive gene
N53	selfed	do.	60	14.0:1	Heterozygous for two recessive genes
N62	selfed	do.	65	64.0:1	Heterozygous for three recessive genes
N12	selfed	Cotyledons pale yellow; primary leaves green	31	9.3:1)	Unknown; possibly heterozygous for one recessive gene with associated lethal. Apparent 9.1% natural selfing
N12	O.P.	do.	115	114.0:1)	
N82	selfed	do.	294	72.5:1	Heterozygous for three recessive genes
S66	selfed	do.	14	2.5:1	Heterozygous for one recessive gene
S30	selfed	Thick, twisted, light-green hypocotyl; twisted thick cotyledons	131	5.6:1)	Heterozygous for one recessive gene with associated lethal. Apparent 12.3% natural selfing
S30	O.P.	do.	272	53.1:1)	
S47	selfed	Bright, yellow-green hypocotyl	46	8.2:1	Heterozygous for one recessive gene with associated lethal
S91	selfed	Primary leaves very short, thick, and bluish-green. Epicotyl dwarfed	357	5.6:1	Heterozygous for one recessive gene with associated lethal

the basis of the frequency of chlorophyll deficiencies in open-pollinated progenies as compared to ratios in selfed progenies, the proportion of natural selfing was found to be between 17 and 25 in the trees studied. Computed on the basis of other mutants, about 10 percent natural selfing was indicated. This percentage may be reduced by considering possible cross-pollination with heterozygous carriers of the same alleles.

In addition to the chlorophyll deficiencies, numerous other phenodeviants were noted and de-

scribed. Segregation ratios and suggested genotypes ranging from one to three pairs of genes are presented. However, it is clear from the results that the mode of inheritance of potential genetic markers, such as chlorophyll deficiencies in the cotyledon stage, is not as easily definable as was previously believed on the basis of results with one or a few progenies.

The occurrence of two types of mutants for hypocotyl, cotyledon, and primary leaf characters

possibly induced by the X-irradiation of seed is described.

The presence of chimeras in the X₁ trees, although not definitely established, is suggested in several cases by differences between progenies from different parts of the same tree. Larger progenies than available in the present study are necessary to define possible radiation-induced chimeras.

Studies of the genetic basis for the phenodeviants observed in these populations are being continued.

From the results presented in this study it is apparent that much information on the genetic constitution of individual trees and populations may be gained by studying even limited numbers of progeny from self-pollinations. However, to accurately define the genetic behavior in successive generations and the possible influence of seed X-irradiation on this behavior, extremely detailed study of large progenies is required.

Literature Cited

- Barnes, B. V.; R. T. Bingham; and A. E. Squillace. 1962. Selective fertilization in *Pinus monticola*. II. Results of additional tests. *Silvae Genet.* 11: 103-111.
- Cram, W. H. 1960. Shelterbelt tree breeding. Proc. 7th Meeting Comm. Forest Tree Breeding Can. Part II, pp. D 1-5.
- Eiche, V. 1955. Spontaneous chlorophyll mutations in Scots pine (*Pinus silvestris* L.) Medd. Stat. Skogsför. 45(13), 69 pp.
- Fowler, D. P. 1962. Initial studies indicate *Pinus resinosa* little affected by selfing. 9th Northeast. Forest Tree Impr. Conf. Proc.: 3-8.
- Fowler, D. P. 1965a. Natural self-fertilization in three jack pines and its implications in seed orchard management. *Forest Sci.* 11: 55-58.
- Fowler, D. P. 1965b. Effects of inbreeding in red pine, *Pinus resinosa* Ait. III. Factors affecting natural selfing. *Silvae Genet.* 14: 37-46.
- Fowler, D. P. 1965c. Effects of inbreeding in red pine. *Pinus resinosa* Ait. IV. Comparison with other Northeastern *Pinus* species. *Silvae Genet.* 14: 76-81.
- Gustafsson, A. 1940. The mutation system of the chlorophyll apparatus. *Lunds Univ. Arsskr. N. F. Avd. 2.* 36: 1-40.
- Johnson, A. G. 1948. Albinism in the Austrian pine. *J. Hered.* 39: 9-10.
- Johnson, L. P. V. 1945. Reduced vigor, chlorophyll deficiency, and other effects of self-fertilization in *Pinus*. *Can. J. Res.* 23C: 145-149.
- Kraus, J. F., and A. E. Squillace. 1964. Inheritance of yellow oleoresin and virescent foliage in slash pine. *Silvae Genet.* 13: 114-116.
- Langner, W. 1952. Eine Mendelspaltung bei Aurea-Formen von *Picea abies* (L.) Karst. als Mittel zur Klärung der Befruchtungsverhältnisse im Walde. *Z. Forstgenetik* 2: 49-51.
- Langner, W. 1959. Selbstfertilität und Inzucht bei *Picea omorika* (Pancic) Purkyne. *Silvae Genet.* 8: 84-93.
- Rudolph, T. D. 1966. Stimulation of earlier flowering and seed production in jack pine seedlings through greenhouse and nursery culture. *Forest Genet. Workshop Proc.* 1965 (this issue).
- Snyder, E. B.; A. E. Squillace; and J. M. Hamaker. 1966. Pigment inheritance in slash pine seedlings. 8th Forest Tree Impr. Conf. Proc. 1965: 77-85.
- Squillace, A. E., and J. F. Kraus. 1963. The degree of natural selfing in slash pine as estimated from albino frequencies. *Silvae Genet.* 12: 46-50.