

MAIZE GENETICS COÖPERATION

NEWS LETTER

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I. REPORTS FROM COÖPERATORS

Brookhaven National Laboratory *
Upton, Long Island, N.Y.

1. A chemical test for seed viability.

Ionizing radiation has a lethal effect upon seeds if given in sufficient dose. In every experiment involving killing of seeds, it is usually necessary to test the germination of a certain number of seeds. However, viability can be determined much more quickly by using a chemical, 2-3-5-triphenyltetrazolium chloride, commercially known as TPTZ. This is not a new compound, having been first prepared by Pechman and Runge in 1894. However, the application of TPTZ to testing seed viability is fairly recent, dating from George Lakon's work in Germany in 1942. Prior to 1942, Lakon analyzed TPTZ and found that it was a non-toxic, water soluble compound which, when reduced, formed triphenylformazan, a red precipitate, insoluble in water, but soluble in alcohol. He also noted that, when in solution, the chemical was sensitive to light. In his studies of seed viability, Lakon used seeds of barley, wheat, rye, oats, and corn. In corn, he observed a direct relationship between the regions of the embryo in which the red precipitate was formed and the organs of the seedling which would develop. Recently, many modifications of Lakon's method have been used on both animal and plant tissues.

In general steps in testing seed viability are: (1) soak the seeds in tap water; (2) bisect each seed medianly through the embryo; (3) soak one-half of each seed in TPTZ. The presoaking of the seeds is required only to facilitate the sectioning, as the initiation of germination is not necessary for the test. Presoaking times of one to eighteen hours were tried and it was found that, although the seeds which had been soaked for longer periods of time were easier to bisect, in general, comparable results were obtained when using seeds which had been presoaked for six to eighteen hours. All of the seeds were presoaked at room temperature (23-24°C.)

After presoaking, each kernel is cut longitudinally so that the embryo is bisected medianly through its entire length and one-half of each kernel is submerged immediately in TPTZ. Prolonged contact of the cut seed with the air and of the TPTZ to the light should be avoided. The TPTZ may be contained in small dishes, similar to Petri dishes, which have been painted on the outside with aluminum or black paint or in similar unpainted dishes which can be placed in the dark. Tests of whole, uncut seeds showed that the embryo of the seed contained some of the red precipitate, indicating that the TPTZ molecules are able to penetrate the pericarp. Treating whole seeds has a limited and questionable use, since only seeds with colorless pericarp could be used and it is impossible to identify which regions

* All research reported in this contribution was carried out under the auspices of the Atomic Energy Commission.

of the embryo contain the precipitate. Whole seeds after treatment of this type were planted in the greenhouse, but few of them produced seedlings. Further work on this is planned.

In the final step of the procedure, seeds were soaked in solutions of TPTZ varying in concentration from 0.0001% to 2.0%, in pH level from 2.0 to 11.0, and in length of time soaking from 15 minutes to 4 hours at room temperature and then examined. It was observed that accurate, reproducible results could be obtained under a wide range of conditions. The factors, pH, concentration, and length of time in TPTZ, are closely related in the effect upon the formation of the red precipitate. Solutions with pH levels of 4.0 to 10.0 and concentrations of 0.05% to 1.0% consistently formed precipitates in the viable embryos; the solutions with the lowest concentrations and lowest pH levels requiring the longest reaction time. Optimum conditions for the reaction were observed when the seeds were soaked in solutions of TPTZ ranging in concentration from 0.05% to 0.25% and pH levels from 6.0 to 9.0 for periods of time varying from one-half to one and one-half hours. The kernels which indicate viability possessed an embryo which was stained throughout, or at least stained in the regions of the coleoptile, plumule, secondary radicles, transition region, and scutellum.

The general conclusions drawn from this preliminary study are that the procedures used to test seed viability in corn with 2-3-5 triphenyltetrazolium chloride are not complicated or subject to rigid requirements of observation or chemical preparation.

Mary L. Koester

2. Photoperiodic effect on corn grass (CG/+).

In last year's News Letter, it was reported that Corn Grass plants show a remarkable response to length of day. These results were repeated in 1949. Plants grown from the October 1 planting produced few tillers, were upright in growth and produced tassels that shed good pollen, whereas the September 15 sowing produced typical Corn Grass plants with many tillers and no tassels. All plants were grown with no supplemental light. The change in type of growth took place earlier in 1949 (October 1) than in 1948 (November 1) although in the October 15, 1948 planting there was a more or less intermediate type. In 1949 plants were all crosses of Mangelsdorf's multiple tester and Corn Grass, while those in 1948 were from open pollinated Corn Grass pollinated by normal corn.

In the 1948 fall crop, the tall type of plant persisted for only two sowings. November 1 and 15 and plants from the two December sowings produced almost normal Corn Grass with the exception that a few tassels were produced. The January 1 sowings resulted in completed normal Corn Grass plants with no tassels.

Perhaps modifying genes have been introduced into the Corn Grass plants causing some tassel formation in the field in the summer of 1949. Thus it was possible to make a considerable number of con-

trolled crosses more easily with different ⁱⁿleakage testers and it should be possible to establish linkage with this gene fairly soon. Pollen of Corn Grass was put on Teopod ears to determine if these two dominant genes are allelic. Corn Grass has some of the characteristics of Teopod but is much more striking and shows a greater difference from normal corn than Teopod.

W. Ralph Singleton

3. No stimulation from X-rays or gamma rays.

Various workers have reported at different times preliminary data showing stimulation of slight amounts of radiation. Most, if not all, of these reports are based on limited data and, so far as we are aware, no extensive experiments have corroborated these preliminary indications of stimulation.

Last summer an experiment was planned to determine any stimulating effects of X-rays and gamma rays. The X-ray used was set at 160 KV and 10 m.a. Doses given were 250, 500, 1000 and 2000 r. Comparable doses of gamma rays were given by placing the seeds (in petri dishes) at a distance of approximately 1/2 meter from a 16 curie source of Cobalt⁶⁰. Four different 4 x 4 latin squares were planted with seed given the four different dosages of X-rays and gamma rays. In two latin squares standard early field corn was grown. In the other two short hybrids with the reduced gene (rd) were grown. These were used because it was thought small differences in height might be more readily detected. In all of the latin squares 4 row plots were planted, using a split-plot technique whereby two rows were checks with no treatment and for two rows the seed was radiated.

Height measurements were made at weekly intervals on one of the four replications of each latin square and total height was obtained on the mature plants of all plots. There was no significant difference in height of any of the 4 X-ray treatments or the 4 gamma ray treatments in comparison with the controls. Likewise the yields showed no significant difference for any treatment. Experiments will be run in 1950 using doses of 2000, 4000, 8000 and 16,000 r of both X- and gamma rays. On the basis of present information it seems unlikely that a stimulating effect will be found. Certainly none was found using doses of 250, 500, 1000 and 2000 r of either X-rays or gamma rays. Neither was a depressing effect formed for any of the dosages used.

W. Ralph Singleton

4. Segregation for sucrose storage in corn stalks.

In last year's News Letter (No. 23) we reported refractometer readings for segregating progenies between C103 and T1. In 1949 readings were made on segregating progenies of crosses of C103 with the following inbreds: C 22(su), C102, R 4, Hy, 38-11, Os420, I 159, Oh 40B, CI 7, DT 21, I 205, WF9, and 1188 a rd field corn line. The

C102•103 F₂ progeny showed the narrowest range of refractometer readings from 10.75 to 16.75 in one progeny, while in a second progeny the range was from 6.25 to 16.75 with an indication of a bi-modal curve for this progeny. At least three of the progenies C103•38-11, C103•WF and C103•Hy gave distinctly bi-modal curves with approximately 25% of the readings centered around a low mode 3.25 for 38-11, 6.25 for WF and 6.25 for Hy with the respective high modes of 11.5 for 38-11, 10.75 for WF and Hy. These data indicate that true low sucrose behaves as a recessive. Readings will be made on the F₃ generation this summer.

Inbreds show marked differences in the way they maintain total solids in the stalks as the grain is filling. Two weeks after pollination WF has almost as high a refractometer reading as C103 (more than 12%) but 50 days later the percentage for WF9 has dropped to about 4%, while 103 has gone up to 15% and then back to 12. Inbred 38-11 shows a similar pattern to C103, but in a lower range, going from 6-1/2% to 8-1/2% and then falling to 4% as the grain matured. C102 showed considerable variation from plant to plant, but in a lower range than C103. C102 is a Lancaster Surecrop line as is C103.

The genetic picture for sucrose storage is still obscured by other factors that affect amount of sucrose present in the stalk when the grain is mature. One of these factors is the size of ear produced. An incompletely filled ear will have a higher reading than one completely filled, although high readings have been obtained on some plants having well filled ears.

Time of sampling is another factor influencing solids present in stalk juice since some lines lose solids very fast as they mature while for others such as C103 the solids present remain fairly constant.

Possibly amount of sunlight a plant receives affects total solids stored. In 1948 at Mt. Carmel, Connecticut, there was a tendency for end plants on the south end of the row to have a higher reading than plants in the row. It is also probable that soil fertility will have some influence.

All of these variable factors must be controlled as well as possible before accurate genetic analysis can be made.

W. Ralph Singleton and Robert Van Reen

5. Sucrose storage in corn stalks.

Several lines of corn have been investigated during the past year to determine the extent of sucrose storage in the stalk after maturation of the ear. Selfed single ear progenies of C103, Ind. 38-11-2A, Ind. 38-11-CtSlCtS3, and T1 inbreds were grown and the stalk press-juice analyzed for reducing sugars and sucrose by the method of Shaffer-Somogyi. Along with the chemical analyses, the percentage of total solids in the press-juice was obtained using a Bausch and Lomb hand refractometer. The refractometer readings were compared with the sucrose content and a correlation coefficient of 0.79 calculated. Since there is such a high

correlation it is possible to obtain a fairly accurate estimate of sucrose production and storage by using the hand refractometer. By so doing it is possible to analyze many more plants than would be feasible chemically.

The mean refractometer readings found for the lines investigated are given in Table 1.

The results for the C103 plants are not markedly different from the plants analyzed at New Haven (10.8 to 15.3 for 32 plants) and at Yaphank ($10.0 \pm .67$ to $15.1 \pm .29$) reported by Singleton in last year's News Letter. Row 640 which was a greenhouse progeny of 610 gave refractometer readings comparable to those of 610.

The chemical analysis of the stalk press-juice of the T1 line substantiated earlier findings that the sugars present in this line are mainly reducing sugars and not sucrose. An analysis of 20 samples of T1 showed reducing sugars and sucrose contents of 3.5% and 1.4% respectively. This gives a value of 2.5 for the ratio of reducing sugars to sucrose. The chemical analysis of 22 samples of C103 (Row 610) showed reducing sugars and sucrose contents of 1.3% and 6.8% respectively or a ratio value of 0.2. Further studies will be conducted to determine the cause of this pronounced difference.

Other studies concerning sucrose formation and translocation are being planned utilizing C^{14} as a tracer. One problem of interest concerns the ability of the plant to translocate sucrose from the leaf to the stalk against an apparent concentration gradient.

Table 1. Mean refractometer readings of mature field corn (Su Su) inbreds

Row	Pedigree	No. of plants	Mean refractometer reading % total solids
609	C103	25	8.3 ± 0.50
610	C103	25	12.2 ± 0.44
640	C103 (progeny of 610)	20	11.3 ± 0.84
641	C103	20	12.0 ± 1.00
658	C103	23	10.7 ± 0.50
692	C103	22	14.0 ± 0.58
693	C103	22	15.5 ± 1.10
638	Ind. 38-11-2A	21	4.9 ± 0.59
639	Ind. 38-11CtS1CtS3	20	4.8 ± 0.47
659	TIHP	20	6.9 ± 0.51

Robert Van Reen

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Mutable alleles at the R locus.

Further study of the unstable alleles at the R locus, in chromosome X, has permitted several conclusions regarding the genetic behavior and nature of these mutants. As indicated in the previous report (Maize Genetics Coop. News Letter 23: 1949), these mutable loci are of independent, spontaneous origin, and all derive from the same parental r^{ch} gene.

Phenotypic observations, in conjunction with genetic transmission tests, further strengthen the proposed hypothesis that the timing and frequency of reverse mutation is under genic control. Moreover, it is believed that the control is a function of the altered state of the locus itself.

The genetic behavior of the unstable alleles is perhaps best demonstrated by a series of single anther pollinations from individual sectorial plants. Particularly instructive are those progenies from plants representing infrequent, but early mutational changes. The type cross employed was R^r/r^{ch}-mut x r^g/r^g.

Progeny tests of the pollen taken from fully purple anthers within the sector yield, for the most part, in the colorless seed category, plants which are effectively identical to the unmutated parental r^{ch} allele in phenotype. This suggests, clearly, that the mutational change occurred sufficiently early in development as to affect a majority, and in many cases, all of the gametes within the sampled area. Since in this, and in all other instances, sibs derived from the colored seed failed to exhibit an altered phenotype, it is concluded that the mutational changes specifically involve only one member of the allelic pair; namely, the newly established unstable r^{ch} locus.

In the case of our ^{*}cross tests of single green anthers from the same tassel the resultant progeny from colorless seed is largely composed of green plants, phenotypically equivalent to r^g, the bottom member of the series. However, in such crosses an occasional plant may be found which shows a few mutations very late in development. Such late mutations are observable only in the anther wall. Single anther tests from such areas give progeny which is about the same as that derived from wholly green anthers.

Testing a gametic sample from an area representing infrequent, but comparatively early mutation gave somewhat unexpected results. In the progeny of this test may be found a variety of sectorials, differing in both time and frequency of mutation, in addition to a range of stabilized intermediates, full r^{ch} reversions, and plants indistinguishable from r^g.

The recovered intermediate and full r^{ch} alleles are quite stable, but in several cultures an occasional sectorial plant has been observed. While in these particular cases it was not possible to rule out pollen contamination, other evidence makes this seem improbable. A number of ears, carrying P_1 , derived from recovered full r^{ch} reversions showed sectors for late loss of pericarp color. Since these sectorial pericarp-seeds have given rise to variegated plants, the findings would seem to indicate that the full r^{ch} alleles recovered from reverse mutation of the mutable locus are not nearly as stable as the original parental gene.

Seymour Fogel

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1. Chromosomal rearrangements from exposure to radiation.

The analyses of chromosomal rearrangements resulting from the exposure of corn seeds to the bomb explosion at Bikini and the control X-ray treatments have been completed.

Among all of the chromosome aberrations analyzed in connection with these studies, 1176 were in translocations, 57 were inversions, 24 were deletions, and a few were trisomic pieces. Except for the deletions, an effort is being made by Dr. Anderson and his associates to maintain all in suitable standard stocks.

The breaks produced by the various dosages and sources of radiation are not distributed at random, among the 10 chromosomes, the 20 chromosome arms, or the different sections of the chromosome arms.

The non-random distribution of the Bikini-treated material was different from that of the X-ray treated controls.

Chromosome arms with mixed heavily and lightly staining areas have more than the expected number of breaks regardless of the radiation treatment.

Chromosome arms with a heavily staining distal area have less than the expected number of breaks following all types of radiation exposures.

Chromosome arms staining heavily adjacent to the fibre attachment had more breaks than expected when treated with X-ray, and fewer breaks than expected when exposed to the Bikini radiations.

Chromosome arms staining lightly throughout their length had fewer breaks than expected when treated with X-ray and more than expected when exposed to the Bikini radiations.

The distribution of breaks along the chromosomes that have mixed heavily and slightly staining areas differs distinctly from that of chromosomes with a heavily staining distal region. X-rays produced more breaks in the middle sections of the chromosomes and less in the distal sections than were produced by the Bikini exposure.

In general, the Bikini exposure produced more breaks in the lighter staining distal sections of the chromosome arms than the higher X-ray exposures.

Among the whole population of translocations there is a tendency for breaks the same distance from the fibre attachment to be associated more frequently than expected.

A. E. Longley

2. Tryptophane, auxin, and niacin interrelations in the corn kernel.

Corn is an unusually rich source of the plant growth hormone, indoleacetic acid (IAA), and at the same time deficient in tryptophane and niacin. It seemed possible that one of the reasons for tryptophane deficiency in corn was not because corn grains fail to form the amino acid, but rather that the amino acid is formed and then enzymatically converted to IAA. A study of the free auxin¹, auxin complex², tryptophane, and niacin relationships has been completed on two strains (CC5/L317 and suKYS) of corn differing widely in the amount of auxin found in the kernels.

¹ Free auxin is considered to be that auxin extracted with either at 0°C.

² Auxin complex has been used in this work as that auxin released from the corn kernel under alkaline conditions, and may not represent the absolute total auxin. Work is now in progress to identify this substance. At present it appears to be a small molecule with a molecular weight around 500 units.

- a. The auxin complex, free auxin, and niacin were shown to reach a peak content during the milk stage, and that individual values for free auxin, niacin, nitrogen, and tryptophane contents were nearly the same when compared at the corresponding stages between the two strains. In the case of suKYS, as much as forty per cent of the tryptophane may have been directed into IAA production.
- b. A tryptophane converting enzyme was demonstrated in the kernel, and was found to reach a peak of activity correlated to the production of free auxin and auxin complex.
- c. The tryptophane converting enzyme had three times as much activity in the high auxin strain which seems to be reflected in the fact that this strain also contains three times as much auxin as the low one.

Comparison of the CC5/L317 and suKYS strains of corn for tryptophane, free auxin, auxin complex, niacin, nitrogen, and tryptophane converting enzyme activity. All values are expressed in micrograms per kernel, or in the case of enzyme conversion are expressed in activity units per kernel.

Days after polli- nation	Free auxin		Auxin complex		Niacin		Total tryptophane		Total nitrogen		Activity of tryptophane converting enzyme	
	CC5/L317	<u>su</u> KYS	CC5/L317	<u>su</u> KYS	CC5/L317	<u>su</u> KYS	CC5/L317	<u>su</u> KYS	CC5/L317	<u>su</u> KYS	CC5/L317	<u>su</u> KYS
0	0	0	.005	.007	.06	.04	2.1	1.9	30	27	0.3	0.6
4	0	0	.002	.003	.15	.15	4.6	5.1	87	94	1.2	2.7
8	0	0	.02	.05	.34	.23	8.8	11.0	170	168	9.7	19.2
12	.02	.003	.31	.89	1.19	.66	18.0	8.4	407	273	65.0	47.0
16	.22	.013	3.41	4.95	2.52	1.64	30.0	25.3	700	443	95.5	93.7
20	.51	.07	8.15	17.0	3.54	2.87	47.0	40.5	964	763	355.0	700.0
24	.15	.48	9.62	26.8	3.95	5.17	58.0	66.7	1160	1170	330.0	1050.0
28	.14	.55	6.90	34.8	3.86	5.80	72.0	78.6	1740	1480	139.0	348.0
32	.11	.48	2.96	32.2	6.34	4.84	81.0	80.1	2180	1820	89.0	52.0
36	.07	--	1.80	--	5.10	--	--	--	2160	--	--	--
39	.04	--	.69	--	4.55	--	--	--	2530	--	--	--

- d. It has been hypothesized, on the basis of the indole balance between the two strains, that high IAA content is a reflection of the potential for the plant to manufacture tryptophane and its precursors, rather than of the tryptophane content at any stage. This balance was determined by summation of tryptophane, auxin complex, and niacin values at the respective stages.
- e. This balance of the indole nucleus further shows that the discrepancies in the later stages were mainly due to the higher production of auxin complex in suKYS.
- f. Apparently the auxin complex does not give rise to the free auxin, but rather that the reverse may be the actual situation. Thus, the auxin complex may be a "postcursor" rather than a "precursor" of indoleacetic acid. It would appear, therefore, that tryptophane is converted to free auxin by an enzyme system, and this free auxin is in turn transformed into the auxin complex.

M. L. Stehsel and S. G. Wildman

3. Tryptophane, niacin and indoleacetic acid in endosperm mutants.

Assays of the tryptophane, niacin and indoleacetic acid (heteroauxin) levels in several endosperm mutants have been carried out. Degermed kernels from segregating ears were used for assay. The "normal kernels" were a mixture of genotypes. Hence, if any endosperm gene was incompletely recessive in its biochemical effect, then the differences in the table may not represent maximal ones. At least three samples of each character except mn were assayed. Average values are cited in the accompanying table. Three inbreds are listed for comparison. Brittle-1, shrunken-1, and shrunken-2 therefore are similar to sugary-1 in their effect on tryptophane, niacin and indoleacetic acid levels. Possible bases for these differences are being studied ontogenetically.

<u>Kernel type</u>	<u>Tryptophane</u> micrograms /gm	<u>Niacin</u> micrograms /gm	<u>Indoleacetic acid</u> micrograms /gm
wx	249	40	43
normal	338	33	41
su	521	50	25
normal	395	27	12
bt ₁	888	108	61
normal	479	21	24
sh ₁	420	51	29
normal	281	32	19
sh ₂	1037	92	36
normal	420	26	9
mn	382	28	7
normal	238	25	16
<u>Inbreds</u>			
L317	175	11	8
CC5	190	34	6
WF9	376	25	68

H. J. Teas and Anna C. Newton

4. Tryptophane and niacin in sugary endosperms.

Endosperms from TB-4a x su assayed for tryptophane and niacin showed the same relationship as su and Su on segregating ears or in comparable lines, i.e., both substances were present in greater amounts in su (deficient) seeds than in phenotypically Su (hyperplid) seeds. Thus in the case of the su gene the quantity of both substances in the endosperm is a function of the genetic constitution of the endosperm itself. The embryo genotype apparently plays no part in the quantity of tryptophane or niacin storage by the endosperm.

H. J. Teas and Anna C. Newton

5. Tryptophane and niacin assays.

Assay of tryptophane by a chemical method and by the use of Lactobacillus arabinosus and Streptococcus faecalis gave very similar results (82, 80, and 79 micrograms/sample) for a pure protein sample using enzyme hydrolysis; however on corn samples the values obtained with L. arabinosus were higher than those with S. faecalis. Eight

developmental stages of su KYS averaged 17% higher with L. arabinosus and a corresponding series of KYS averaged 18% higher. This points to the presence in corn of substances that assay as tryptophane with the less exacting organism but are not tryptophane.

Anna C. Newton and H. J. Teas

6. A blue-fluorescent seedling and anther character.

Ultraviolet light examination of an extensive series of Bikini progeny seedlings in 1948-49 revealed a family segregating for bright blue fluorescence (recessive), in place of the usual dull red color. The blue-fluorescent seedlings appear normal in daylight. Chromatographic adsorption columns run on acetone extracts of the blue-fluorescent seedlings showed that chlorophyll was present in approximately normal amounts though seedlings had failed to show characteristic reddish fluorescence because of the masking effect of the blue-fluorescent material. Paper strip chromatograms showed three main bands of blue-fluorescent material in the mutant that were absent or very faint in the normal seedlings. Eluates from each of the major bands were active as tryptophane substitutes for Lactobacillus arabinosus but not for Streptococcus faecalis (the former is known to utilize anthranilic acid or indole in place of tryptophane, but the latter requires tryptophane). One of the bands has been shown to be identical with anthranilic acid in adsorption spectrum, pH fluorescence curve, biological activity, and R_f values on chromatograms, although as yet insufficient material has been obtained for determination of melting points and preparation of derivatives. The two other major components can be degraded to anthranilic acid by relatively mild procedures. Anthers of both heterozygous plants and homozygous plants are blue-fluorescent and exhibit the same chromatographic bands as blue-fluorescent seedlings.

H. J. Teas and E. G. Anderson

7. Seedling leaf necrosis.

A type of seedling character has been found in our radiation studies which shows early necrosis of the leaves. The seedlings are green and normal looking for several days, usually long enough to have two leaves fully expanded. Then the leaves suddenly collapse and dry up. The seedlings remain alive and some may shoot out another leaf which quickly suffers the same fate. After a time the seedlings die. One such necrotic was associated with an induced translocation involving chromosomes 5 and 9. Eighty-five self ears were obtained from out-crosses to standards. These were distributed as follows:

Semisterile	segregating necrotic	40
Normal	normal	40
Semisterile	normal	3
Normal	segregating necrotic	2

This indicates 6 per cent crossing over between necrotic and the translocation but does not identify whether the gene is in chromosome 5 or 9. The break points in the two chromosomes are 5 S.25 and 9 S.25.

E. G. Anderson and H. J. Teas

8. Lethal seedling type.

A chlorophyll-free lethal seedling type with a light reddish brown color has been obtained four times in our radiation studies. One was induced by X-ray, one by the Bikini bomb radiation, while the other two may have been either induced or present in the stock. Inter-cross tests have shown the two definitely induced ones to be identical with one of the latter. The fourth one has not yet been intercrossed. The peculiar color plus the failure of the leaves to unfold under most cultural conditions give the seedlings an appearance reminiscent of the root-parasite Orobanche.

E. E. Dale and E. G. Anderson

9. Viviparous mutants.

Work has been progressing on a series of viviparous (pre-mature germinating) mutants. These mutants can be grouped into three general classes with regard to the color of the endosperm and of the seedling:

- a. With yellow endosperm and green seedling. Eleven mutants of this class have been grown, including vp1 and vp4. One of these mutants has been located in the long arm of chromosome one by means of a test with translocation B-1a. Heterozygous viviparous plants pollinated by TB-1a pollen gave some seeds with large endosperms (hyperploid endosperm and deficient embryo) which were viviparous, and also some seeds with small endosperms (deficient endosperm and hyperploid embryo) which were dormant. Thus this viviparous resembles vp5 in that dormancy in both of these mutants is determined by the embryo.
- b. With pale yellow to white endosperm and albino seedling. Three mutants of this class have been grown. One of them (furnished by Dr. Sprague) has proven to be the same as vp5, which is located on the short arm of chromosome one. The third mutant is non-allelic to vp5 and as yet has not been located.
- c. With pink endosperm and albino seedling. Eight of these have been studied and all have proven to be identical with one which was furnished by Dr. Sprague. As yet the location of this mutant is unknown.

In conjunction with these studies corn embryos which have obtained a length of one mm. or greater (about 14 days after pollination) have been cultured on sterile nutrient media. On transferring to culture conditions these embryos proceed to grow at once into seed-

lings. This indicates that there is something missing under culture conditions which is responsible for maintaining dormancy in their natural environment. An attempt is being made to determine the cause of maintenance of dormancy in normal seed development.

D. S. Robertson

10. Tests for analyzing gene differences between inbreds.

A number of translocations marked with endosperm genes have been crossed recurrently to the inbred lines R4 and WF9. These conversions have been carried on chiefly by Dr. M. T. Jenkins at Beltsville and by Dr. Anderson at Cal Tech. In addition to the translocation lines, normal sugary and waxy lines have also been converted to R4 and WF9 backgrounds for use in testing.

To test for genes which differentiate the two inbred lines, many of the R4-converted translocations were crossed to WF9, and the WF9-converted translocations, to R4. The F₁'s involving wx were test crossed to wx R4 and to wx WF9, those involving sugary, to su R4 and to su WF9. In the waxy series 45 test-crosses involving 18 translocations have been made; in the sugary series 28 test crosses, involving 13 translocations. By growing the normal and waxy seeds of each test cross in adjacent cultures, one should detect as consistent differences between the two cultures any linked genes which differentiate the two inbred lines.

The testing or observation of these test crosses is being done as a cooperative effort to locate genes for the characteristic differences between these two inbreds. One set will be grown at Johnston, Iowa by Dr. Wm. L. Brown of the Pioneer Hi-Bred Corn Company. A duplicate set will be subjected to corn borer resistance tests at Toledo, Ohio by Mr. F. F. Dicke of the European Corn Borer Laboratory of the Bureau of Entomology and Plant Quarantine. A set will also be grown at Lincoln, Nebraska by Drs. E. F. Frolik and Rosalind Morris and another here at Cal Tech.

A number of similar crosses and test crosses have been made involving KYS, with emphasis on the analysis of genes located in the distal part of the long arm of chromosome 10. Several have also been made with L 289 in an attempt to locate genes responsible for the failure of this line to tolerate the climate of southern California.

These tests are the initial application of this technique for the analysis of gene differences between inbred lines. Further work is under way to extend the analyses to other inbreds.

E. G. Anderson and Earl B. Patterson

Charles F. Kettering Foundation
Yellow Springs, Ohio

1. Irradiation Experiment.

Two years ago there was made available through the National Cash Register Company, Dayton, Ohio, a 500 mg. Radium-Beryllium neutron source. The National Cash Register Company rented it from the Eldorado Mining and Refining Company, Ottawa, Canada for their annual demonstration lecture to the public. Fear of the exposure effects of the radiation source brought a decision not to utilize it as first intended. Primarily to keep it out of the way of harming anyone, the source was stored temporarily in the attic of the Antioch College Science Building.

Dr. Harry V. Knorr exposed the seeds to the source. The exposure was made so that there was 15 cm. of solid paraffin between the seeds and the source. This was to slow down the neutrons, produced by the radiant effect of radium on beryllium. The gamma (X-rays) radiation is derived from the radium and may be the sole cause of resultant mutations.

Exposure

The following seeds were irradiated for 15 hours by the above slow neutron source. Radiation equivalent to 432 R units due to gamma rays plus neutron radiation with flux of 100,000 per second to the area.

1. 110 Sweet corn kernels, Country Gentleman, Burpee 337.
2. 110 Soybean seeds, Lincoln variety.
3. Jimson weed seeds.
4. Ruby Red Petunia seeds.
5. Salmon Pink Petunia seeds.

The following seeds were irradiated for one hour by the slow neutron source. Radiation equivalent to 28.88 R units due to gamma rays plus neutron radiation with flux of 100,000 per second to the area.

6. 110 Sweet corn kernels, Country Gentleman, Burpee 337.
7. 110 Soybean seeds, Lincoln variety.
8. Jimson weed seeds.
9. Admiral Petunia seeds.
10. King Henry Petunia seeds.

The following seeds were irradiated for 15 minutes by the slow neutron source. Radiation equivalent to 7.22 R units due to gamma rays plus neutron radiation with flux of 100,000 per second to the area.

11. 110 Sweet corn kernels, Country Gentleman, Burpee 337.
12. 110 Soybean seeds, Lincoln variety.
13. Jimson weed seeds.
14. White Perfection Petunia seeds.

F₁ generation

1 and 2 were grown in the field the summer of 1948. Plants were phenotypically normal. Rabbits ate all the soybeans of 2. 6 and 11 were grown in the field the summer of 1949. They too produced phenotypically normal plants. Unfortunately there were no controls set aside. 1, 6, and 11 were self-pollinated to produce F₂.

F₂ generation

Ten seeds of each F₁ from treatment 1 were sown in the greenhouse and seedlings observed for variation. There were 19 different F₁ progenies in this treatment. All showed some variation. The frequency of the different variations is as follows:

Runty fine stripe	7
Fine stripe	2
Fine stripe frayed leaves ..	1
Lethal yellow	2
Albino	1
Runty or small	5
Frayed leaves	2
Wavy leaves	1
Oily or greasy green	2
Narrow leaves	1

Some progenies showed more than one type of variation, thus accounting for the 24 variants among the 19 F₁ lines in this treatment. F₃ progeny showed wavy leaves variant not to be inherited, but both oily or greasy green variants, the albino variant, and both lethal yellow variants to be transmissible by inheritance, and therefore to be bona fide mutants. Other variants have not been progeny tested to determine their status as hereditary characteristics (mutants).

Twenty seeds of each F₁ from treatments 6 and 11 were sown in greenhouse and seedlings observed for variation.

Out of 23 different F₁ progenies from treatment 6 three showed the following variations (one each):

Lethal yellow
Albino
Fine stripe

None of these have been tested to determine their status as hereditary characteristics.

Out of 31 different F₁ progenies from treatment 11, five showed the following variations (one each):

Albino
Yellow-green lethal
Wavy leaved
Fine stripe
Oily or greasy green

None of these have been tested to determine their status as hereditary characteristics.

Should any one be interested in these corn variants or in irradiated soybean seeds, Jimson weed seeds, and petunia seeds not yet processed, I shall be glad to furnish any upon request.

2. Miscellaneous observations.

In addition I would like to report that:

- a. Branched ear, reported last year, is perhaps "ramosa" since the tassel is now known to be affected, too.
- b. In cloudy weather "zebra" shows up poorly. The light stripes appear to be the result of chlorophyll destruction, which is more rapid the greater the light intensity.
- c. "Embryoless" or germless (gm) is linked with albino (w) with 12% crossingover on the basis of pedigree 482(7).

Record: 84 embryoless out of 358
274 normal --- 246 green + 20 albino

This albino is yet unidentified as to location and originated as a mutant from Cornell 45-61(6)(x).

- d. A yellow seedling (luteus) on chromosome 6 closely linked with pigmy: 4811 45 = 58 green + 39 yellow + pigmy out of 150 seeds sown. (Pigmy does not reduce the expected 1/4 tall yellows.) This yellow seedling is linked with W₁ (locus 61 on chromosome 6) with about 10% crossingover: 4811 10 = 87 green + 7 yellow + 41 white + pigmy out of 200 seeds. Assuming:

WWLL	WWll	wWLL	wWll
green	yellow	albino	albino
87	7	7	34
r	s	s	r
5.2	.6	.6	5.2

$$\text{Linkage} = \frac{\text{cross overs}}{\text{total}} = \frac{1.2}{11.6} = 10\%$$

H. C. Eyster

Connecticut Agricultural Experiment Station
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1. Chromogenes and plasmagenes.

Since pollen sterility and chlorophyll deficient maize is conditioned by both nuclear and cytoplasmic factors it is necessary to distinguish between these two types of determiners. The terms "chromogenes" and "plasmagenes" are proposed.

Both the plasmagenes and chromogenes for pollen sterility have been combined in the same plants and are apparently independent in their action. However, there are numerous chromogenes that can restore pollen production partially when in combination with the plasmagene. In a few cases pollen production can be restored completely.

Inbreds that are commonly used in the commercial production of both field corn and sweet corn are segregating for these chromogenes that restore fertility. Therefore, these long inbred lines are not homozygous for these determiners that normally have no expression and have not been selected for or against. This is further evidence for enforced heterozygosity.

D. F. Jones

2. Who put the first bags on corn plants?

In his experiments on the effects of cross and self-fertilization Darwin covered his plants with cotton netting, but I can not find any statement that he used bags of any kind. His corn plants were isolated by putting single plants without covering in different parts of a greenhouse and allowing pollination to take place naturally. The cross-pollinations with all of his plants was brought about by natural means.

In the Michigan Board of Agriculture Report for 1881, W. J. Beal states that paper sacks were put on corn plants for the purpose of making cross pollinations.

According to P. G. Holden, Beal and his assistants about 1885, in their experiments at Michigan Agricultural Experiment Station, used both paper and cloth bags. The cloth bags were dipped in oil thinned with turpentine and dried before using to make them waterproof. To eliminate the inconvenience, or risk, in removing the sacks from the tassels and ear shoots they inserted a blow gun into the tassel bag to suck up the pollen and then into the sack over the ear shoot to blow the pollen on to the silks.

McCluer, in his experiments with corn at the Illinois Experiment Station, published in 1892, used cloth bags. Pollen was gathered on a sheet of smooth paper and this was rolled into a funnel to facilitate the application of the pollen to the silks. When the pollina-

tions were made an umbrella was held over the ear shoots to protect them from drifting pollen. Morrow and Gardner at the same Station, in a report one year later, put cloth bags on the ears and paper bags on the tassels. Collins and Kempton in 1912 tried paper tubes extending from the tassel to the ear on the same plants. Roberts at the Kansas Station used this same type of tube to make crosses between plants growing in adjacent rows. Jenkins devised the bottle method in 1922. Excised tassels were kept in a functioning condition by inserting the stems in water in a bottle wired to the stalk below the ear. This method is now extensively employed in the drier sections.

Paper bags on both tassels and ears were tied with string at first. Roberts at the Kansas Station in 1911 fastened paper bags on the ears and tassels with large pins. Coiled wires have been used by Collins and Kempton to permit expansion of the ear shoots. Paper clips were first tried about 1922. Later, wire stapling clippers have been used extensively.

Roberts resorted to an insect powder blow gun to apply pollen in 1911. Merle Coulter in 1919 devised a glass thistle tube blow gun that could be easily sterilized in alcohol.

Small glassine bags to cover the ear shoots replaced the larger manila bags in 1922, or before.

Early corn pollinators worked in pairs and went to a great deal of trouble to sterilize their hands in alcohol before each pollination. When it was found that most of the contamination comes from insects traveling up the stalks and from pollen drifting in the air, these elaborate precautions were discontinued. Pollination was further simplified by cutting back the silks to a short stub. At the Connecticut Station a knife sterilized in alcohol was used at first. Later it was found that a sterile knife was not necessary if the shoot was cut back below the tip of the ear shoot.

Much of the insect and wind contamination can be avoided by using an ear bag small enough to fit tightly around the ear shoot, or by folding one of the lower corners and tucking this in between the ear shoot and the stalk. Pollen is applied after tearing off the top of the glassine bag without removing it. The bag is folded over afterwards. The tassel bag, or a similar one, is placed over the ear shoot bag after pollination and fastened with a paper clip or wire staple.

D. F. Jones

3. Suppressor genes concerned with chloroplast biosynthesis.

A method has been devised for the demonstration and isolation of suppressor mutations through the use of a pleiotropic gene. The pleiotropic gene alters the endosperm color (from normal yellow to light yellow) and plant color (from normal green to albino) when present in the homozygous recessive state. A suppressor mutation (defined as "a sudden change occurring at a particular chromosomal locus such that a gene is formed capable of nullifying the effect of a particular

major gene") which affects the plant color without affecting the endosperm color can be detected and isolated in the presence of the pleiotropic gene by growing light endosperm seeds. An example will serve to clarify the use of this method. The widely used inbred, Ohio 28, was crossed with a tester stock which was heterozygous for the pleiotropic gene. Six plants from F₁ progeny of this cross were selfed and one ear which segregated 390 dark to 110 light kernels (a satisfactory 3:1 segregation) was harvested. Forty seeds of both the dark and light types were germinated in seedling flats under greenhouse conditions. Color classifications of these plants are summarized in table 1.

Table 1

<u>Plant Color</u>	<u>Endosperm Color</u>		Expected (on basis of incompletely dominant suppressor gene mutant)
	<u>Dark</u>	<u>Light</u>	
Normal green	40	0	
Light green	0	12	9.75
Very light yellow green	0	16	19.50
Albino	0	11	9.75
% Germination	100	98	
			Chi square = 1.445
			D.F. = 2
			P = 30 - 50%

The good fit indicates that an incompletely dominant suppressor gene mutant is segregating in this progeny. Since the tester stock has no such gene, it is evident that the inbred Ohio 28 is carrying a suppressor gene mutant and is presumably homozygous for the dominant allele.

In a similar manner several other inbreds have been tested and classified as shown in Table II.

Of the 25 individual inbreds tested 11 (or 44%) have segregating progeny indicative of suppressor mutations. If the frequent presence of suppressor genes compensating for the deleterious action of this major gene's recessive allele is found to be rather typical the evolutionary significance of suppressors is obvious. One line, Connecticut C106, seems to have two suppressor genes which are not well fixed. In this line 15:1 as well as 3:1 segregations have been found in light seed progeny. The other inbreds seem to be fixed (homozygous) for the suppressor genes. However, it has not been possible to grow extra progeny of all these F₂'s.

Work is in progress to ascertain the linkage relationship of the pleiotropic gene and to utilize the various mutant forms in studies of chloroplast biosynthesis. Small amounts of seed are available upon request.

Table 2. Suppressor gene classification.

Inbred	Has none	Has one incompletely dominant		Has one completely dominant
Ohio 56A	X			
West Va. W1455		X		
Wisc. W16	X			
Id. 50	X			
Conn. 106		X ?	and	X
South Dakota 105	X			
Ind. P8		X		
Conn. 103				X
Conn. 14	X			
Conn. 102	X			
Ill. Hy 2	X			
Ohio 56-1				X
Ind. Hs 8	X			
Kansas K155	X			
Ohio 61		X		
Ind. DC 6				X
Iowa LK				X
Minn. A25	X			
Iowa I198		X	or	X
Rogers Flint		X		
Wisc. W23	X			
Iowa T1				X
Iowa Os 420	X			
Minn. A158	X			
USDA C.I. 4-8	X			

H. L. Everett

4. A small growing room has been constructed as a tool to study the genetics of photoperiodism in corn. The length of day, temperature, humidity and mineral nutrition are all rigidly controlled in four individual chambers. Two single genes that condition corn to a short photoperiod are being studied.

W. C. Galinat

5. The relation of photoperiod to tassel proliferation in a short-photoperiod line of sweet corn has been investigated. An extended number of short-photoperiodic cycles seems to be essential to normal floral development. This sweet corn line contains the indeterminate growth gene (*id*) reported by W. R. Singleton (1946).

A study has been made of the morphology of the teratological structures resulting from proliferation. The tassels bear propagules which may be asexually propagated and which will then grow to maturity. The propagules result from a rachilla elongation between the two

paleas in the spikelets. The glumes were found to be leaf blades, the lemmas represent leaf sheaths and they will develop into liguled leaves. The palea is a homologue of the prophyllum since vegetative shoots come from paleas in upper lemma-leaves on the extended rachilla. The ear and its shank represent a reduced axillary branch since the ear shoots elongate into axillary branches. Three lodicules were found in the proliferated corn florets.

W. C. Galinat

6. A method has been worked out to obtain pollen from vestigial glume tassels. The glumeless ear characteristic, that is associated with the glumeless tassel, is a quality characteristic for use in sweet corn hybrids. The vestigial glume (Vg) gene is the one reported by G. F. Sprague, (1939). The Vg plants are mechanically male sterile since the naked anthers blast under ordinary field conditions. The pollination technique involves the use of an open polyethylene cylinder over the developing tassel so that a glumed condition is simulated. In this moist atmosphere the anthers mature but fail to dehisce. The mature anthers are shaken off the tassel into a paper bag. Next they are ground up to loosen the pollen and finally, after drying, good pollen is separated from ground anthers and immature pollen by use of a small sieve. Now the good pollen is ready to be used for field pollinations either in small glassine bags or in duster blow guns commonly used in corn pollinating. It is necessary to make line crosses so that there is a segregation for Vg plants since normal plants are necessary to pollinate the glumeless ears.

Since the glumeless lines are mechanically male sterile, they may be used in such a way as to eliminate the detasseling step in the making of hybrid corn seed.

W. C. Galinat

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1. Locating genetic factors for kernel-row number by use of translocations.

This study was undertaken to obtain experimental evidence on the number and location of genes affecting kernel-row number in maize.

Four different twelve-rowed maize inbreds were crossed to a series of eight-rowed lines, each of which was heterozygous for one of various well-distributed translocations. Some of the semisterile segregates (translocation heterozygotes) in the F_1 generation were backcrossed to their twelve-rowed parent and an equal number was crossed to the normal eight-rowed inbred R. The progeny of the F_1 's backcrossed to the twelve-rowed parent and those crossed to the eight-rowed inbred were grown and classified for row number and semisterility.

Analyses of variance, based on the means for kernel-row number in the normal and semisterile segregates of each culture, were calculated for each group of segregates having a common twelve-row parent. Significant or highly significant differences between replications were found in each of the analyses. Thus, it was concluded that environment may play a greater role in affecting the expression of the number of kernel rows of the maize plant than was formerly believed.

Each analysis of variance showed that there were highly significant differences in the mean kernel-row number of the lines derived from the various eight-row translocation lines. It was concluded that lines of the same eight-row phenotype may be of different row-number genotypes.

As was expected, the progeny of the lines which had been backcrossed to the twelve-rowed parent had a higher mean kernel-row number than the progeny of the lines crossed to the eight-rowed inbred.

The frequency distributions for number of kernel rows of the semisterile plants were compared with those of their normal sibs in an attempt to find evidence of linkage between genes for kernel-row number and the translocation loci.

Summarizing the linkage evidence and interpretations on dominance the following conclusions were drawn concerning the genes that differentiate the eight- from the twelve-rowed condition in each of the twelve-rowed lines.

The twelve-rowed line 39 differs genetically from the eight-rowed condition by the following genes:

- a. Two or more genes lacking dominance, or two or more groups of dominant + and - genes closely linked in the repulsion phase. One of these genes or groups of genes is located near the P locus in chromosome 1. The other gene or group of genes is located in the long arm of chromosome 1 or the long arm of chromosome 7, or both.
- b. Two or more dominant genes for increasing kernel-row number in the twelve-rowed parent. One gene or group of genes is located in chromosome 3 near the ts₄ locus. The other gene or group of genes is located in the short arm of chromosome 6 or in the long arm of chromosome 10, or both.

The twelve-rowed line 2 differs genetically from the eight-rowed condition by the following genes:

- a. A gene or genes lacking dominance or a group of dominant + and - genes closely linked in the repulsion phase, located in chromosome 6 near the Y locus.
- b. A dominant gene or genes for decreasing row number in the eight-rowed parent, located in chromosome 9 near the wx locus.

The twelve-rowed line II differs genetically from the eight-rowed condition by a gene or genes lacking dominance or by a group of dominant + and - genes linked in the repulsion phase, located in the long arm of chromosome 4 or the long arm of chromosome 6, or both.

The twelve-rowed line 4 differs genetically from the eight-rowed condition by the following genes: two or more genes lacking dominance, or two or more groups of dominant + and - genes closely linked in the repulsion phase. One gene or group of genes is located in chromosome 6 near the Y locus. The other gene or group of genes is located in the short arm of chromosome 2 or the long arm of chromosome 4 or both.

Other genes influencing kernel-row number may well have gone undetected, since in these experiments all regions of the chromosomes were not adequately tested.

T. J. Mann

2. Inhibition of growth in maize embryos by canavanine and its reversal.

The natural amino acid canavanine, a structural analogue of arginine, is known to be a strong inhibitor of growth in *Neurospora* and certain other organisms. Experiments were conducted in 1949 to determine the effects of canavanine on growth of maize seedlings grown from excised embryos cultured in vitro. The results parallel and extend those observed in other organisms; i.e., canavanine indeed inhibits the growth of such embryos and this inhibition can be alleviated by the addition of appropriate amounts of arginine or certain other amino acids. These results seem to have significant implications for our understanding of the genetics and biochemistry of maize. A paper reporting these results has been submitted for publication in a botanical journal, and the following is a summary of the paper.

A number of inbred strains of maize were self- or cross-pollinated and the immature embryos were excised through aseptic techniques at time periods ranging from 18 to 40 days after pollination. These embryos were placed on the surface of artificial culture media contained in 18 x 150 mm. test tubes, and then were incubated at 30°C in a dark chamber either for 10 or for 15 days. The seedlings were then removed, dried at 80°C for 48 hours, and weighed.

It was found that canavanine added at the rate of 20 mg. per liter to the basic culture medium effectively inhibited growth in all lines tested. This inhibition was greatly reduced in those cultures to which arginine was added in concentrations of M/5000 or M/2500. Genetically different strains show variation in their sensitivity to canavanine and also in the degree of reversal of the inhibition when arginine is added. In some cases, there is indication that hybrid embryos are less sensitive than are their component inbreds and that in the hybrids reversal of the inhibition by adding arginine is more complete. Age of the embryo when excised also seems to be a factor in sensitivity.

Table 1.

Effect of amino acids on canavanine inhibition of hybrid maize embryos. Strain 38-11 x CI 21 embryos were excised 24 days after pollination; those of CI 21 x R 20 at 21 days age.

The values represent mean dry weights in mg. of growth of seedlings in 15 days.

Each mean value is based on four replications.

Supplement	Concentration	Growth of 38-11 x CI 21	% of control	Growth of CI 21 x R 20	% of control
None (control)		22.8 ± 5.0		22.0 ± 2.7	
Canavanine	20 mg/l.	1.9 ± 0.4	8.3	1.4 ± 0.2	6.4
Canavanine + Arginine	M/5000	21.0 ± 4.0	92.1	13.8 ± 1.5	62.7
	M/2500	-----		15.8 ± 1.2	71.8
Canavanine + Lysine	M/5000	8.0 ± 1.9	35.1	2.4 ± 0.4	10.9
	M/2500	11.3 ± 1.9	49.6	1.8 ± 0.1	8.2
Canavanine + Glutamic acid	M/5000	8.8 ± 1.2	38.6	10.2 ± 1.7	46.4
	M/2500	17.0 ± 1.8	74.6	12.9 ± 1.3	58.6
Canavanine + Citrulline	M/5000	12.3 ± 1.7	53.9	8.7 ± 2.6	39.5
	M/2500	13.5 ± 1.6	59.2	10.7 ± 0.7	48.6
Canavanine + Ornithine	M/5000	10.8 ± 0.8	47.4	10.5 ± 1.9	47.7
	M/2500	15.4 ± 1.2	67.5	13.2 ± 1.9	60.0
Canavanine + Methionine	M/5000	4.8 ± 0.3	21.0	-----	
	M/2500	5.2 ± 1.2	22.8	-----	

Besides arginine, the amino acids citrulline, ornithine, glutamic acid, lysine, and, to a much lesser extent, methionine are effective in counteracting canavanine inhibition of these embryos. This is shown in the accompanying table. Other amino acids tested were without appreciable effect. These included leucine, isoleucine, valine, threonine, tyrosine, proline, histidine, and glycine. The partial reversal of the inhibition by ornithine and by citrulline might lead to the interpretation that these compounds are precursors of arginine in maize, as has been shown to be the case in the mammalian liver and in *Neurospora*. The effectiveness of glutamic acid in relieving canavanine inhibition may be related to the fact that glutamic seems to be a precursor in the biosynthesis of arginine in certain microorganisms. The effect of lysine in relief of the inhibition parallels results obtained in *Neurospora* and *Avena* but the basis of this effect is still obscure.

Experiments were conducted to determine the effect of light, temperature, pH, and sucrose concentration of the medium on the inhibition. None of these factors significantly altered the response of 22-day old embryos of Inbred K64 to the inhibition or to its reversal by arginine.

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1. Blotched leaf.

In June, 1944, a supply of seed of Hawaiian Yellow Field Corn was received from A. J. Mangelsdorf, Hawaii Sugar Planters Association. It was derived from crosses between many tropical and subtropical corns and was extremely variable. Several inbred lines were isolated from it and in these appeared plants showing protogyny, adherence and blotched leaves; the last-named is discussed here.

Blotching appeared just before tasseling, at about 60 days; the spots were yellow, numerous, evenly scattered over the leaf and later became coalescent. They were not notably elongate; nor were they ever few in number and large in size with a necrotic centre, as described by Emerson (Cornell Univ. Agr. Exp. Sta. Mem. 70, 1923). The present gene will be referred to as Bl₃- bl₃.

There was some variation of expression in different cultures but recessivity was complete and classification easy.

Some families segregated also for light red vs. green plant colour (table 1).

Table 1. Segregations

Parentage	Red		Green	
	Normal	Blotched	Normal	Blotched
Red-normals selfed	62	15	18	18
Red-normal selfed	39	9	-	-
Red-blotched selfed	-	37	-	10
Green-blotched selfed	-	-	-	43
Red-normal x Green-blotched	16	6	2*	1*
Green-normal x Green-blotched	-	-	11	10

* Presumably plants with 'red' genotypes mis-scored.

Single-factor segregations are in agreement with expectation; but there is good evidence of coupling linkage ($X^2 = 15.03$; crossing over $31.2 \pm 5.4\%$ by the method of maximum likelihood). Which anthocyanin locus was concerned is not known, but it seems likely that it was R, which would place Bl₃ in the tenth chromosome (Rhoades and Rhoades, Genetics 24: 302, 1939).

Blotched plants seemed to be slightly less vigorous than normals. This impression was supported by measurements of weights of fresh cobs in several families (table 2).

Table 2. Mean weight (gm.) of fresh cobs in segregating families.

Family	Normal	Blotched	Blotched as % of normal
28	113	51	46
25	87	68	79
51	227	118	52
26	108	85	79
21	147	81	55
Means	125	82	66

Differences between families and between the genes were significant at the 1% level.

N. W. Simmonds

2. Sweet corn.

It is commonly said that sweet corn "does not grow" in the tropics. However, breeding projects in Porto Rico (Harper, Agric. Amer., 6: 74, 1946) in Cuba (del Valle) and, latterly, in Trinidad have all been successful in producing a fairly vigorous mass-bred sweet corn. The programmes have been of the obvious kind - crossing an imported sweet corn to locally adapted field corns, selfing or crossing, selection, and mass breeding. At the I.C.T.A., the sweet corn used was one which had been grown at the College Farm for 6 to 8 years and was notably poor in vigour. A museum plot of 30 to 40 plants was grown once or twice a year, 6 to 8 cobs were selected and the grain was shelled, bulked and stored until the next sowing. In at least one year the population was greatly reduced by bad seed and disease. It is assumed that no su pollen other than that from the plot itself would have been available and this, together with the necessary selection for su su su grains, must have constituted an isolation mechanism (but not necessarily an absolutely rigid one if an occasional Su su plant survived to flower). From this realization it was but a short step to the idea that, perhaps, existence as a small isolated population had led to inbreeding, loss of heterozygosis and consequent loss of vigour.

Loss of heterozygosis under a system of random mating is $\frac{1}{2N}$ per generation (Wright, Genetics 16: 97, 1931). If N is the population number, g the number of generations, p_0 initial heterozygosis, and p_g heterozygosis at generation g ,

$$p_g = p_0 \left(1 - \frac{1}{2N}\right)^g$$

whence table 3 is constructed.

Table 3. Heterozygosis - $\left(1 - \frac{1}{2N}\right)^g$

g	N	1	3	5	10	50
1		0.50	0.83	0.90	0.95	0.99
3		0.13	0.58	0.73	0.86	0.97
5		0.03	0.40	0.59	0.77	0.95
7		0.01	0.28	0.48	0.70	0.94
9		0.002	0.19	0.39	0.63	0.92

The problem is to determine the effective population number. It is not simply the number of plants in the plot for only a few of these are ovule parents; nor is it the number of cobs selected, for these represent only ovule parents and not pollen parents. The true value must lie somewhere between the two.

I am indebted to Professor Wright for the solution. In the following equations, N_t = total plants in the plot, N_o = ovule parents, N_f = female parents, N_m = male parents, and N_e = effective population

number. Case A assumes that self-fertilization occurs with the same average frequency as pollination by any other plant; case B assumes that self-fertilization is excluded; case C assumes separate sexes and has already been treated by Wright.

$$\text{Case A: } \frac{1}{2N_e} = \left(\frac{1}{8N_o} + \frac{3}{8N_t}\right) - \left(\frac{1}{8N_o} + \frac{1}{8N_t}\right)^2$$

$$\text{Case B: } \frac{1}{2N_e} = \left(\frac{1}{8N_o} + \frac{3}{8N_t}\right)\left(1 - \frac{1}{8N_o} - \frac{3}{8N_t}\right)$$

$$\text{Case C: } \frac{1}{2N_e} = \left(\frac{1}{8N_m} + \frac{1}{8N_f}\right)\left(1 - \frac{1}{8N_m} - \frac{1}{8N_f}\right)$$

Case A, it will be noted, is not appreciably different from Case B, itself probably a fair approximation to actual events in the field.

A	$N_o = 6, N_t = 30$	$\frac{N_e}{15.3}$	$N_o = 8, N_t = 40$	$\frac{N_e}{20.2}$
B	$N_o = 6, N_t = 30$	15.5	$N_o = 8, N_t = 40$	20.5
C	$N_f = 6, N_m = 30$	20.5	$N_f = 8, N_m = 40$	27.2

N_e for sweet corn at the College Farm must have been 15 to 20 and this leads to the expectation that $p_g = 0.7p_o$, assuming 12 generations. Actually it would be rather lower than this because, as noted above, in a few bad seasons, N_e must have been reduced far below 15 to 20. Probably, then p_g approached $0.5p_o$ and this is in accord with the general aspect of the material, bearing in mind the appearance of corn once-selfed, and knowing that quite vigorous sweet types can be bred.

These considerations have general application to small museum or maintenance plots of any outbred plant and it might be suggested that, subject to detailed study and test, the N_e of such populations should not be allowed to fall below 90 ($p_g = 0.9p_o, g = 100$).

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1. Male sterility.

Plantings of various white single, 3-way and double crosses continued the past two years showed a number of additional inbred lines as carrying the necessary factors to cause pollen sterility when used as the male parent on 33-16 or Ky 27. No white inbreds other than 33-16, Ky 27 and Mo 2RF were found to carry the cytoplasmic contributions for sterility except 33-16 made waxy. The yellow single cross M 1984 x M14, supplied by Dr. G. F. Sprague, was 100 per cent incompletely sterile whereas the reciprocal was normal. Five cytoplasmic male sterile yellow crosses supplied by Dr. D. F. Jones ranged from 40 to 100 per cent completely sterile.

Poor seed set has occurred in commercial hybrids which have exhibited only 20 per cent male sterile plants when it would seem the remaining plants should furnish sufficient pollen to produce good set. By keeping tassels bagged throughout the pollen shedding period and measuring the amounts of pollen produced it was found that pollen shedding plants in male-sterile hybrids produced only 67 per cent as much pollen as plants in non-sterile reciprocals.

Crosses made using 33-16 as the seed parent and backcrossing to 33-16 three times continued to exhibit male sterility, whereas when 33-16 was used as the pollen parent in making the original cross and backcrossing to 33-16 three times the plants have been normal, showing that 33-16 carries a cytoplasmic contribution for sterility.

L. M. Josephson

2. Barren stalks in Ky 203.

In the fall of 1949 a number of farmers fields of Ky 203 were reported to have a higher percentage of barren stalks. The trouble was reported late in the season after the majority of the corn was harvested so it was impossible to get an accurate estimate of the amount occurring but a few fields were observed. The trouble was reported from several locations in the State but most of it occurred in the river-bottom near Owensboro.

The reports ranged from a trace to 80 per cent barren. Three fields in one locality in which accurate counts were made had an average of 55 per cent completely barren, 19 per cent extremely small nubbins such as would be found for second and third ears of single-eared hybrids, and 26 per cent ranged from nubbins to good ears. Other fields visited ranged from a trace to about 25 per cent barren and small ears. There was some variation in individual fields as to the amount of barrenness. In some cases there would be 25 to 30 consecutive barren stalks in a row and then several plants with good ears. Often normal ear development occurred adjacent to skips in a row but this did not always hold

true. There appeared to be no consistent differences in height or size of stalks between barren and normal plants, except an occasional tall, large dark plant. The darker color was probably due to accumulation of sugars in the leaves and stalks and being overrun with saprophytic fungi. Root systems in all cases were normal. The corn made good growth early and appeared to grow normally throughout the year (although it turned dry following tasseling) and perhaps this is why the trouble was not detected until late in the season.

Many of the stalks showed no indication of having developed a shoot. Others showed evidence of shoot primordia such as for second or third ears. On some plants the shoots were well developed and the silks appeared to have emerged normally but fertilization apparently failed. In these cases the cobs were 4 to 6 inches long but appeared to have disintegrated early and were no larger than a pencil, unlike ears that fail to become pollinated due to lack of pollen. On other plants the cob was only an inch or two long. Some plants were found where the tip of the cob had a few grains but the rest of the cob was shrivelled with no grains.

No other white hybrids were reported to show this trouble but one field of yellow corn, either Indiana 844D or US13, was reported to have a high percentage of barren stalks. This same hybrid planted 2 to 3 weeks later in the same field performed satisfactorily.

Source of seed, use of 2,4-D, plant population, soil type or fertility could not be established as factors causing the trouble.

Early season conditions were quite favorable for corn but the first half of July was extremely humid in this area and temperatures remained above 90 for an extended period. It is suspected that the high humidity and temperature were factors contributing to the expression of this trouble. The corn may have been at the right stage of growth to develop female sterility under these conditions since corn planted later in the season and under different growth conditions did not exhibit barrenness.

Ky 203 has previously shown pollen sterility as reported by Josephson and Jenkins (Jour. Amer. Soc. Agron. 40: 267-274. 1948) but the present trouble seems to be an entirely different type of reaction. Neither does there seem to be an incompatibility between silks and pollen since ear shoots failed completely to develop. Ky 203 has been grown commercially for eight years and while a small percentage of barren stalks has been observed previously, to my knowledge it has never occurred on this large a scale.

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1. A pericentric inversion in chromosome 9.

In the F₁ progeny from X-rayed pollen there was detected in a single plant a long inversion in chromosome 9, which involved approximately .7 in cytological length of the short arm and .1 of the long arm. The heterozygote gave about 25% aborted pollen. Cytological determination of the inversion could be made out only from pachytene configurations which, in heterozygous form, assumed a typical large loop. One of the inversion breaks was found closely associated with the wx gene. As in the manner of translocations in maize, the inversion was followed in linkage test by the partial sterility of pollen which behaved in outcrosses like a dominant gene located at the break point.

Linkage relations between the inversion break in the short arm (hereafter designated In) and wx, and between the gene sh for shrunken endosperm and In, were tested first in separate crosses. The data presented in the following table were obtained in each case from the type of testcross in which the female parent was the double recessive.

Table 1.

Year	Family	Constitution of male parent	Non- crossovers				Crossovers				Crossing over %	Total plant
			++	+-	-+	--	++	+-	-+	--		
1947	102- 103	<u>n*</u> <u>Wx</u> <u>In</u> <u>wx</u>	78	87			3		2	2.94	170	
1948	303- 304	<u>n</u> <u>wx</u> <u>In</u> <u>Wx</u>	75		64		7	2		6.08	148	
1948	307- 308	<u>n</u> <u>wx</u> <u>In</u> <u>Wx</u>	96		87		5	4		4.68	192	
			171	78	87	151	3	12	6	2	4.57	510
1949	442- 451	<u>Sh</u> <u>In</u> <u>sh</u> <u>n</u>	170		86		4	7		4.12	267	
1949	452- 457	<u>Sh</u> <u>In</u> <u>sh</u> <u>n</u>	92		95		8	4		6.03	199	
			262		181		12	11		5.08	466	

* The symbol, n, represents the normal arrangement of chromosome 9 and it is treated as a recessive gene.

Obviously, averages of 4.57 and 5.08 can be taken only as relative measures of the respective linkage value for In-wx and sh-In since reduction of crossing over in these regions, as caused by the heterozygous inversion, will be evident in a three-point test.

The inversion break in the short arm of the chromosome was placed between the genes sh and wx. Evidence for this sequence was given by the fact that, as a result of any single crossing over within the inversion loop in the inversion heterozygote, gametes formed with the chromosome deficient for the distal .3 of the short arm (and at the same time duplicate for a small piece of the long arm, hereafter designated as df 9S, dp 9L) were tested to be void of the allele of the sh gene. Part of the counts made in 1947 from twelve crosses of the type, ♀ $\frac{Sh \quad In}{Sh \quad n}$ x ♂ sh n, gave 77 shrunken kernels out of a total of 3180 seeds on the ears of the immediate cross, the frequency being 2.42%. Twice this figure, that is, 4.84%, was taken as the total frequency of the deficient-duplicate gametes survived since the complementary class, designated as df 9L, dp 9S, should occur and transmit with equal frequency through the female side. Accompanying with cytological demonstration of a df 9S, dp 9L chromosome paired with a normal 9, the plant grown from such shrunken seeds gave 50% pollen sterility and produced all shrunken seeds when it was further tested by the recessive stock. It seemed evident, therefore, that the sh gene was located in the non-inverted region of the short arm.

The data of a three-point test, in which the heterozygote $\frac{Sh \quad In \quad Wx}{sh \quad n \quad wx}$ was used as the male parent, were available in 1949 from seven cultures; lumped numbers being given as follows:

Family	Non-crossovers		Crossovers at region				Total plants		
	0		1	2	1.2				
	ShInWx	shnwx	Shnwx	shInWx	ShInwx	shnWx	ShnWx	shInwx	
411- 437	266	225	8	12	8	3	4	5	531

Crossing over percentage: sh-In = 5.46 In-wx = 3.76

Linkage values for In-wx and sh-In obtained in this test were fairly close to that observed in the separate tests. Taking 30 as the map distance of normal chromosome between the loci sh and wx, the heterozygous inversion obviously caused a marked reduction of crossing over in the region concerned; the difference (30-5.46+3.76=20.78) being twice as much as the observed value of the heterozygote. It remained to be seen whether the reduction was greater or less in the sh-In section than in the In-wx region.

Another test was made to try to place the inversion break more precisely on the chromosome map with respect to the locus of bp, the gene for brown pericarp, which was known to be located midway between sh and wx. The bp material obtained from the Maize Coop. bearing the pedigree number 43-163 (2)(x) was supposed to be a c sh bp wx tester. It was, however, segregating for brown and colorless pericarp plants, apparently due to the segregation of P gene. When the inversion stock of the constitution p Sh In Bp was crossed on to the

brown tester, all the F₁ plants possessed red pericarp, and the inversion heterozygotes were selected again for crossing to the tester in both ways. In the progeny of the test, only red and brown pericarp plants were included in the counts for linkages since in the absence of P the phenotypes of Bp and bp were indistinguishably colorless. In table 2 the data represent the results of three types of testcross,

- namely, (1) ♀ $\frac{P}{p} \frac{sh}{sh} \frac{n}{n} \frac{bp}{bp}$ x ♂ $\frac{P}{p} \frac{sh}{Sh} \frac{n}{In} \frac{bp}{Bp}$,
 (2) ♀ $\frac{p}{p} \frac{sh}{sh} \frac{n}{n} \frac{bp}{bp}$ x ♂ $\frac{P}{p} \frac{sh}{Sh} \frac{n}{In} \frac{bp}{Bp}$ and
 (3) ♀ $\frac{P}{p} \frac{sh}{Sh} \frac{n}{In} \frac{bp}{Bp}$ x ♂ $\frac{P}{p} \frac{sh}{sh} \frac{n}{n} \frac{bp}{bp}$.

Contrary to expectation, segregation of the pericarp colors was found to be almost independent of the inversion break and of the sh gene. The general tendency of the segregation seemed quite unique among the families. Even with increased size of population, the result probably would not be changed to the other extreme. It seemed very likely that either we have dealt with another gene such as A^b or aP, in addition to the presence of bp, which determined the production of pericarp pigmentation to the similar effect, or the bp gene might be located quite a distance away from the break in the short arm. Nevertheless, neither of the possibilities has been confirmed at present.

In this connection, it is necessary to mention a test of the deficiency-bp relation. Plants grown from the shrunken seeds obtained from the cross, ♀ $\frac{Sh}{p} \frac{n}{Sh} \frac{Bp}{In} \frac{Bp}{Bp}$ x ♂ $\frac{P}{p} \frac{sh}{sh} \frac{n}{n} \frac{bp}{bp}$, were classified for their pericarp colors. The data are summarized in table 3.

Table 3.

Year	Family	Plant	Parental constitution		F ₁ plants and pericarp colors			Total
			Female	Male	Red (P Bp)	Brown (P bp)	White (p-)	
1948	344	102	$\frac{Sh}{p} \frac{n}{Sh} \frac{Bp}{In} \frac{Bp}{Bp}$	$\frac{p}{p} \frac{sh}{sh} \frac{n}{n} \frac{bp}{bp}$	0	0	2	2
	347	102	"	"	0	0	6	6
1949	572	307	"	$\frac{P}{p} \frac{sh}{sh} \frac{n}{n} \frac{bp}{bp}$	1	0	1	2
	574	307	"	"	1	0	3	4
	577	320	"	"	1	4	0	5
	578	320	"	"	5	0	3	8
	579	320	"	"	2	0	3	5
	580	320	"	"	0	1	0	1
	581	321	"	"	1	2	0	3
	582	321	"	"	0	0	3	3
Total							39	

Table 2.
Frequencies of plants in families

Cross- ing over region	Phenotypes	Type cross 1						Type cross 2					Type cross 3			
		442- 443	444- 445	446- 447	448- 449	Total	%	452- 453	454- 455	456- 457	Total	%	460- 461	462- 463	Total	%
0	<u>P Sh In Bp</u>	20	16	11	22	69		5	7	7	19		39	46	85	
	<u>P sh n bp</u>	10	8	2	11	31		8	5	4	17		19	16	35	
1	<u>P Sh n bp</u>	1	0	0	0	1	4.76	1	1	0	2	6.97	0	1	1	2.81
	<u>P sh In Bp</u>	0	2	0	2	4	(4.12)*	1	0	0	1	(6.03)*	3	0	3	(2.74)*
2	<u>P Sh In bp</u>	16	14	10	13	53		5	7	12	24		36	37	73	
	<u>P sh n Bp</u>	5	12	1	9	27	44.45	9	9	2	20	54.65	54	15	49	50.20
1.2	<u>P Sh n Bp</u>	1	0	0	0	1		0	1	1	2		2	0	2	
	<u>P sh In bp</u>	0	1	0	2	3		0	1	0	1		0	1	1	
		<u>53</u>	<u>53</u>	<u>24</u>	<u>59</u>	<u>189</u>		<u>29</u>	<u>31</u>	<u>26</u>	<u>86</u>		<u>133</u>	<u>116</u>	<u>249</u>	
<u>sh-bp</u>							45.00					54.65				50.60
0 or 2	<u>p Sh In -</u>	11	14	9	14	48		18	15	16	49		31	27	58	
	<u>p sh n -</u>	5	16	1	6	28		33	22	3	58		10	9	19	
1 or 1.2	<u>p Sh n -</u>	1	0	1	0	2		3	0	1	4		2	0	2	
	<u>p sh In -</u>	0	0	0	0	0		2	0	0	2		0	0	0	
		<u>17</u>	<u>30</u>	<u>11</u>	<u>20</u>	<u>78</u>		<u>56</u>	<u>37</u>	<u>20</u>	<u>113</u>		<u>43</u>	<u>36</u>	<u>79</u>	
	Total	70	83	35	79	267		85	68	46	199		176	152	328	
	Unclassified	4	11	10	8	33		26	12	13	51		20	23	43	

* Percentage of crossing over between sh-In when p- plants were not included.

While most families gave expected results on the basis of the supposition that the gene Bp was located in the inverted region, the appearance of brown plants in the families 577, 580 and 581 seemed difficult to explain on the same assumption. However, it was noted that families 577 to 582, inclusive, came from plants of different origin. The inconsistent result in 1949 might again be due to the involvement of another gene or genes in the latter cultures.

The transmission of a deficient chromosome through both sexes was tested against normal ones. It was found that in progenies of six cultures, consisting of 266 plants, no single gamete with the df 9S, dp 9L or the df 9L, dp 9S chromosome could go through pollen. On the other hand, deficient-duplicate gametes were successful in competition with the normal gametes in a ratio of 1:6 (actually 17:127) to transmit through the female side.

In a certain setup, i.e., $\frac{Wx}{sh\ n\ wx}$, crossing over between sh and wx under the condition of heterozygous deficiency could be used as a check on the value of the heterozygous inversion. The value was determined to be 9.3% which was fairly close to what was observed in a previous section.

Different combinations of the chromosome with normal arrangement: typical inversion; df 9S, dp 9L and df 9L, dp 9S were investigated by cytological study for the purpose of determining the role of the centromere in synapsis. General observation revealed that the most frequent type of configurations was those resulting from the initial pairing of centromeres. Occasionally, a type was also found in the same material in which the terminal regions started to pair first, resulting consequently in homologous synapsis of the co-regions. Non-homologous synapsis without centromere pairing was also observed but was least frequent.

Material with a homozygous inversion has been made available for linkage study in this condition. (Acknowledgments are due to Dr. L. F. Randolph and Dr. E. G. Anderson for their generous help when the work was undertaken at Cornell University and California Institute of Technology.)

C. H. Li

2. Distribution and recombination of X-ray induced chromosome breaks.

A general survey was made on the apparent breaks of maize chromosomes induced by X-rays of about 2000 r units with special reference to their distribution and recombination among individual chromosomes. The cytological location of the breaks was determined with the precision possible at the pachytene stage in the progeny obtained from treated pollen, and was expressed as a decimal fraction of the distance from the centromere to the end of each arm. Statistical analysis of 647 breaks on the basis of the breakage hypothesis showed that, excluding the breaks resulting in deficiencies, the breaks that produced viable rearrangements such as translocations and inversions were distributed at random among the arms of the chromosomes. There was close

agreement between the frequencies of observed breaks and that of expected according to the relative proportion of the arm length of all chromosomes measured at meiotic prophase. However, significant deviation from chance occurrence of breaks was noted in the heterochromatic regions where the knobs and the nucleolar organizer are located.

It was attempted to determine whether the frequency distribution of number of breaks in each plant agreed with the expected frequencies calculated on the basis of Poisson formula of randomness. The test gave a very poor fit. Greater departure from random distribution was noted in classes with odd number of breaks, and the observed values for classes with 2 and 4 breaks far exceeded the theoretical. Such discrepancies are due to the fact that the samples included only types that survived to the observable stage, thus representing only part of the potential breaks.

About 65% of the treated pollen that functioned transmitted structural changes of chromosomes detectable at mid-prophase or later stages of the first meiotic division. Of the total aberrations observed there were 68.5% translocations, 4.8% inversions, 24.2% deficiencies and 2.5% others.

The data on the relative frequencies of different types of translocations agreed fairly well with those reported by previous workers (cf. Catcheside, 1938. Jour. Genetics 36:321-328). Nevertheless, the results of the present investigation were not in agreement with expectations based on the breakage hypothesis, without certain modifications of the hypothesis including a proximity effect. The distribution of translocation breaks among arms and chromosomes and of the number of translocations per plant were found to be at random. This suggested that a great majority of the induced translocations must have been viable. However, some degree of differential recombination concerning translocations was noted between the chromosomes and also between the arms, since the possible recombinations were not evenly spread among the chromosomes.

Comparison of the relative frequencies of intra-arm, inter-arm and inter-chromosome rearrangements on the basis of the same number of breaks per cell or plant showed that there was no agreement between the observed and the expected values. The experimental frequencies of intra-arm changes, the majority of which were deletion types, were consistently higher than the calculated values while those of translocations were lower than the theoretical. This preferential exchange within an arm seemed to be induced by failure of re-fusion of the broken ends, which would result in the production of more deletions than inversions. More deletions occurred in the chromosomes of small size. A plausible explanation supposed that shorter chromosomes were perhaps free to move in the nucleus during or after irradiation so that rejoining may occur between two breaks in an arm at greater distance than in longer chromosomes that are less free to move. In this respect, the observations in Zea mays seem to be in line with those in Drosophila, Tradescantia and Tulip.

(This note is abstracted from part of a thesis presented to the Graduate School of Cornell University in 1948.)

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1. Brittle endosperm starch (bt bt bt).

In the course of analyses of endosperm starch of non-waxy back-mutants from mutable-waxy (wx^m) stocks, the opportunity presented itself to determine the starch composition of homozygous brittle endosperm, as an approach to the question of brittle gene action. The fact that supersugary (su du Wx) kernels contain endosperm starch assaying 65% amylose (Cameron, Genetics 32: 459), suggested that the brittle phenotype might also be due to an abnormality in starch synthesis expressed in an off-ratio of amylose to amylopectin.

The brittle phenotype is characterized by the shriveled and translucent appearance of dried kernels. Some starch is present; the starch grains are smaller than normal, and round rather than angular, indicating that they probably are not closely packed in the endosperm cells.

The granular starch of brittle endosperm was found to contain 27.8% amylose by the potentiometric titration method of Bates, French and Rundle (Jour. Amer. Chem. Soc. 65: 142). This value is well within the range reported for a large number of corn varieties and inbreds by many investigators, and may be considered "normal", i.e., unaffected by the recessive brittle allele. That the small amount of starch present does not result from some mechanical block to nutrients entering the endosperm from the plant, is shown by the occurrence of brittle endosperm mosaics.

Ruth Sager

2. Pollen mutations.

During studies of mutable-waxy, an unstable allele at the waxy locus, some data were obtained which are as yet unexplained. They are summarized here in the hope that an analogous phenomenon might have been encountered by someone with other material, in which case I would appreciate learning about it.

Wx wx plants produced red-staining (with I_2 -KI) and blue-staining pollen grains in equal numbers, as was first shown by Demerec and by Brink and MacGillivray. The red-staining grains are assumed to carry the wx allele, and the blue-staining grains the Wx allele, judging from the staining properties of pollen from Wx Wx and from wx wx plants. Similarly, wx wx wx endosperm starch stains red, while endosperm with one or more Wx alleles stains blue. In the case of the unstable allele, wx^m , it has been determined that endosperm starch is red-staining before mutation. The direction of mutation, which results in the occurrence of mosaic kernels, is from red-staining to blue-staining. From these data it was assumed that the pollen carrying wx^m

would be red-staining, while pollen carrying a Wx allele, due to mutation from wx^m to Wx, would be blue-staining. If this were so, it would provide a basis for studying time and frequency of mutation in the tassel of wx^m wx^m plants.

In general, wx^m wx^m plants, whether selfed or crossed with wx wx, give rise in the F₁ mostly to mosaic kernels plus a varying per cent of non-waxy kernels, the latter breeding true in most cases for a stable non-waxy (Wx) allele. A large per cent of blue-staining pollen was frequently found in the tassels of wx^m wx^m plants, but these plants gave rise predominantly to mosaic, rather than to non-waxy kernels. This observation led to the following study.

Plants which were wx^m wx^m were grown to maturity. A pollen sample taken from each was used in part to pollinate wx wx plants and the rest was stained with iodine and a sample of 300 grains counted to determine the per cent blue-staining. The results, summarized below, show clearly a discrepancy between the per cent blue-staining pollen and the per cent non-waxy kernels on the resulting ears. Differential pollen viability was ruled out by many previous crosses with these stocks in which the linked marker c was recovered in the ratio of 1:1 from crosses of the type: c wx/c wx x C wx^m/c wx.

Plant	% Blue-staining pollen	% non-waxy kernels
591-2	30	0
591-3	59	7
591-4	50	20
591-6	35	0
591-7	36	8 - one ear 3 - second ear
590-1	33	14
590-2	44	1
590-4	3	0
590-5	40	20 - one ear 1 - second ear
590-10	11	0
590-12	49	2
590-16	25	0

From these data it appears that blue-staining (i.e., mutated) pollen gives rise to some mosaic kernels as well as, in some cases, to non-waxy kernels. One might suspect that forward-mutation is the explanation, i.e., that the blue-staining pollen carried an unstable dominant allele which subsequently mutates to the original unstable recessive. However, the fact that no unstable dominant alleles have been found, and that the extent of blue-staining tissue increases during endosperm development, have indicated that mutation from blue-staining to red-staining (from dominant to recessive) occurs rarely if at all in this material.

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1. New data on the location of "zn" in chromosome 10.

Four families from a three-point test, including Og, zn and g, were classified, throwing the following result:

$$\text{Three-point test: } \left(\begin{array}{ccc} \text{Og} & \text{zn} & + \\ + & + & g \end{array} \right) \times \left(+ \text{ zn } g \right)$$

Family	Og zn +	+ + g	Og + g	+ zn +	Og zn g	+ + +	Og + +	+ zn g	Totals
1505	36	34	5	8	4	7	1	0	95
1506- 7	36	34	7	4	3	2	1	0	87
1508- 9	43	60	7	15	6	10	6	0	147
1510-11	44	34	18	9	3	8	3	1	120
Totals	159	162	37	36	16	27	11	1	449
	Paternal combination 321		Single crossovers (I) 73		Single crossovers (II) 43		Double crossovers 12		

According to these data the order and relative position of the genes involved would be:

$$\text{Og} - 18.93\% - \text{zn} - 12.25\% - \text{g}.$$

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1. The symbol \underline{Y}^R is now established to designate the gene for pale-yellow seeds (Maize Genetics Coop. News Letter No. 22. 1948), since it controls the shade in a yellow base. Crosses with \underline{ms}_1 and \underline{po} gave the following results:

Total of ears selfed	Color of the seeds	Plants		Medium % of germination
		+	\underline{ms}_1	
3	Yellow-orange	199	28	60
	Pale-yellow	56	15	64
	White	21	107	68
		+	\underline{po}	
3	Yellow-orange	161	19	70
	Pale-yellow	27	12	70
	White	25	59	86

The interrelations between \underline{Y}^D and \underline{Y}^R are as follows:

\underline{Y}^D -- \underline{Y}^R --	Yellow-orange
\underline{Y}^D -- $\underline{Y}^r \underline{Y}^r$	
$\underline{Y}^d \underline{Y}^d$ \underline{Y}^R --	Yellow
$\underline{Y}^d \underline{Y}^d$ $\underline{Y}^r \underline{Y}^r$	Pale-yellow .

The separation of yellow-orange and yellow seeds is sometimes difficult, the segregation obtained being 15:1 pale-yellow. The pale-yellow seeds in some crosses are very pale and could be confused with the white ones.

2. The crosses received from Dr. C. R. Burnham, containing a gene for pale-yellow, referred to as \underline{Y}_x , gave the following results in plants selfed in Brazil:

Ear selfed	Seeds	Plants		
		+	\underline{ms}_1	
4-47	Yellow-orange	161	81	6
	White	94	4	25
2-49	Yellow-orange	173	99	3
	White	66	5	27
3-49	Yellow-orange	156	97	1
	White	54	1	20
	White (viviparous)	46	--	--
8-49	Yellow-orange	86	52	0
	Pale-yellow	41	25	1
	White	52	3	13
	White (viviparous)	47	--	--

Only one ear segregated for pale-yellow and it suggests a condition similar to that reported above for a gene controlling the yellow shade. Two ears segregated for viviparous and the ears of the 49 family segregated also for B and Pl. The plants obtained from selfed seed were poor, very early maturing, and not proper for our conditions.

3. The su_x found in a Brazilian commercial variety (Maize Genetics Coop. News Letter No. 22. 1948), when crossed with a white flint strain and selfed, gave a ratio of approximately 6 normal : 1 su_x. Seeds, su_x, of this cross were sown and the plants crossed with su₁ tests. The ears secured contained: (a) All seeds apparently normal, (b) all seeds sugary or "pseudo" sugary or, (c) segregation of approximately 1 normal : 1 sugary or "pseudo". Other combinations will be investigated this summer (1950).

E. A. Graner

INDIGENOUS MAIZE

1. The Pilcomayo-Paraguay-Parana Region.

New material has been received which adds further proof that in the Guarani area the predominant type is the Yellow Soft Guarani corn and in the Caingang area the White Soft-Dent Corn. Guarani Yellow is also now being cultivated by the Caingang, but no white dent is found outside the Caingang reservations. The large-kernelled white flint is limited to the Guarani area, though much less frequent than the Soft Yellow. Pointed popcorn is scattered over the whole region.

2. Southern margin of the Amazon Basin.

New material from the Upper Amazon reaches, still in Peru, and from the Bolivian lowlands near Reyes (coll. Culer) extend considerably further the area of the Long Ear Soft corn with interlocked rows. Except the occurrence of a popcorn with spherical ears and a brachytic plant with short internodes in the Territory of Acre (Brazil), the Long Ear Soft corn is the only race present in this region.

3. Amazon Basin.

Material has been studied during the last years from two Indian tribes: Emerilhon north of the Amazon in the Territory of Amapa, and Caraja south of the Amazon in the State of Para. Since the latter is growing very well in Piracicaba, a detailed study will be possible. The material received was highly heterogeneous and heterozygous, with flint and soft kernels, in general of small size, straight or interlocked rows and many other interesting characters, which shall be reported later in more detail.

4. Caribbean Flint and Southeastern Orange Flint.

Samples, which were collected near the border of Brazil and

French-Guinea from the Paricur (Aruac) and Galibi (Caribs) Indians, contained exclusively dark-orange hard-flint corn; the former quite pure, and the latter somewhat contaminated with other races. This is, so far, the most southern point where pure orange flint has been collected. I stated in the 1948 Maize News Letter that I had my doubts whether the regions of the southeastern orange flint and the Caribbean orange flint should be united into one big coastal region; and this doubt still remains.

5. Chimu-Inca region in the Andes.

To the list of races given by Cutler (1946), I have to add one more: "Aizuma", a white flint corn with small regular cylindrical ears which is, evidently, quite related to the yellow flint "Uchuquilla".

After studying a larger number of strains of the popcorn "Pisankalla", the relation of this type to the Pointed Pop from the lowlands became much more evident.

The southern limits of this area seem to coincide both with the frontiers of the Inca empire and perhaps also with the pre-Colombian range of corn. However, a decision will be possible only after a careful study of what still has been left of maize of the Araucanians in Chile. It is, however, quite possible that these Indians, which are supposed to have cultivated a Bromus species, may not have known maize at all.

The northern limit seems to have been originally the Southern States of Colombia (Narinhos), thus including also Ecuador. To decide this question, new material from Northern Peru and from Ecuador are needed. The few samples, which I have received so far, give indications of the existence of other races than those of the Andean highlands, with slender cylindrical ears, and a more pronounced degree of denting.

6. Colombia.

Thanks to an invitation by the Colombian Minister of Agriculture, I spent a few weeks in that country, and with the efficient help of friends and colleagues there, I have been able to assemble a large collection both from the mountain area of Central Colombia (Cundinamarca, Boyaca, Antiochia, Santander, etc.), from the northern lowlands near Baranquilla and from the "Serra de St. Martha". The material has been planted, together with material from Guatemala, and is promising a very good harvest.

The following preliminary conclusions can be drawn: The Colombian area seems to have very little relation to the Inca-region in the south. It seems quite probable that, from the region of origin in the south, maize has migrated on several occasions northward along the eastern slopes of the Andes. Arriving at the upper reaches of the Amazon, it has divided its migration: one route turned west, passing through Ecuador and Peru and then turning again south, spreading through both the coastal plains and the mountain regions of the Chimu empires or its predecessors. The other route continued in a

northwestern direction, reaching the Colombian highlands.

The most primitive race in Colombia is a popcorn, "Pira", different from though related to both the Southern Pointed Pop and the Andean Pisankalla. The most characteristic and common varieties are: (1) A white, rarely yellow, soft-endosperm race with big round kernels, very large and long ears with pronounced butts at the base. This is called "Capiro" and seems identical in all details to the "Salpor" of Guatemala and the "Cacuahuacintle" of Mexico. (2) A large-kernelled, hard-yellow flint of somewhat similar ear type, which again has its counterpart in Guatemalan "Big Mountain Yellow". Denting appears sporadically in Colombia, but there is no established and accepted dent variety.

Thus it is clear that no pronounced relations exist between Colombian and Chimu-Inca maize races, while there are clear relations with the Central-American area. There is also evidence of secondary migrations of the large Colombian races to the East into the Amazon Valley, the most eastern point of Capiro being "Iauarete" in the Rio Amazonas-Rio Negro region. If there had been, as it seems possible, some connection between Chimu-Inca and Mexican races, the only connection must have been by the sea route. Archeologic relics which I have seen in the museum in Bogota, from the island of Tucumaco, favor this possibility.

7. Asiatic maize - Naga Hills in Assam.

Stonor and Anderson (1949) have already given a detailed description of the races from this region, and thus I shall give a fuller report only after having studied two generations of material obtained from the same source. As to the origin of these Asiatic races, which probably are quite old, the following hypothesis should be taken into consideration seriously. The nearest replicas of the type of ear of the Assam races can be found in the Amazon Basin (Caraja). Thus it seems quite possible that the distribution of these tropical races had been made by Portuguese sailors and merchants who travelled from Brazil to other Portuguese possessions, scattered over Southern Asia. Changing sea routes and the decline of Portuguese sea power may have been the reason that no further importation of other and better races was made, and owing to the mechanism acting in limited and isolated populations, specialized races must have been established in these backhill regions.

Himalaya Foot Hills north of the Brahmaputra. The material, collected by Stonor, is quite different from the above and would probably be classified by Anderson as belonging to the "Caribbean" races. As a working hypothesis, I am studying the relation of these races to types from the Pacific coast of Colombia and Central-American countries.

Solomon Islands. Very interesting material has been received through the help of Dr. Bridgenan, from New Guinea, and the material is promising a very good harvest. According to the information received from the collector, these races are locally considered as very old "Portuguese" introductions.

MORPHOLOGICAL AND ONTOGENETICAL STUDIES

Using Bonnet's method, the ontogeny of tassel and ear was studied in some indigenous South American races of maize, in *Euchlaena* and in *Tripsacum*. A complete homology was found in all details, and there is an especially close relation between *Tripsacum* (australe and dactyloides) and *Euchlaena* (Novogame and other types). A full report is nearly ready for publication.

It should be noted that a very great amount of parallel variation occurs.

F. G. Brieger

GENETICAL STUDIES

1. Linkage testers for subtropical regions.

Small amounts of seeds could be distributed upon request of the following constitutions:

<u>bm</u> ₂ <u>sr</u> <u>P</u> ^{WR}	<u>bt</u> <u>pr</u> <u>A</u> ₂ <u>ACR</u>
<u>bm</u> ₂ <u>f</u> <u>br</u> <u>P</u> ^R	<u>su</u> ₂ <u>y</u>
<u>Ch</u> <u>v</u> ₄ <u>g</u> ₁₋₂	<u>Y</u> <u>py</u> <u>Pl</u>
<u>sr</u> <u>bm</u> ₂ <u>P</u> ^{WR}	<u>Y</u> <u>ABPl</u>
<u>pr</u> ^R <u>br</u> <u>f</u> <u>bm</u> ₂	<u>Y</u> <u>ABplr</u> ^G
<u>lg</u> ₁ <u>g</u> ₁₋₂ <u>v</u> ₄	<u>v</u> ₅ <u>g</u> ₁ <u>Bn</u>
<u>lg</u> ₁ <u>g</u> ₁₋₂ <u>v</u> ₄ <u>Ch</u>	<u>O</u> ₂ <u>v</u> ₅ <u>ra</u> <u>g</u> ₁ <u>ij</u> <u>Bn</u>
<u>Ab</u> <u>Pl</u> (<u>Ph</u>)	
<u>Y</u> ₃ <u>Y</u> ₋₁ <u>Y</u> ₅	<u>y</u> <u>ms</u> ₈ <u>v</u> ₁₆
<u>cR</u> <u>ts</u> ₄	<u>yg</u> ₂ <u>Cish</u> <u>AR</u>
<u>cR</u> <u>ts</u> ₄ <u>Rg</u>	<u>yg</u> ₂ <u>C</u> <u>sh</u> <u>AR</u>
<u>cR</u> <u>ts</u> ₄ <u>lg</u> ₂ <u>et</u>	<u>c</u> <u>sh</u> <u>wx</u> <u>AR</u>
<u>lg</u> ₂ <u>et</u> <u>a</u> ₁ <u>CR</u>	<u>bp</u>
<u>A</u> ^b	<u>g</u> <u>ACR</u>
<u>AB</u> <u>Pl</u> <u>Cr</u>	<u>g</u> <u>ACr</u>
	<u>g</u> <u>li</u> <u>v</u> ₋₁₈

su la a₂ bt pr v₂ ACR
su Ts₅ a₂ ACR BPl
 bm gl₆ pr v₂

2. Husk color.

(a) Rose-wood (self or variegated) husks is a character due to a new effect of the P series as reported already in 1948. This color type is very common in South American maize, from the Rio Grande to Central Brazil (Bororo Indians). A North American hybrid obtained through Dr. M. M. Rhoades with white pericarp and red cob, proved, under our conditions to have light rose-wood colored husks. The character appears only when the ear is picked after the plant has dried completely in the field.

As in other pericarp colors, the deeper colors are dominant over the lighter ones, and color is dominant over colorless. The types so far studied and their proposed symbols are as follows:

<u>Pericarp</u>	<u>Cob</u>	<u>Husks</u>
<u>prrr</u> red	red	rose-wood
<u>pVVV</u> variegated	variegated	variegated rose-wood
<u>pwrr</u> colorless	red	light rose-wood
<u>pwrf</u> colorless	red	rose-wood or margins.

Crosses of prrr with a testers showed that dominant A is necessary for the formation of the rose-wood color in the husks. The aP type has brown pericarp with colorless husks. Crosses with P tester and sr prove that we are dealing with a P gene in chromosome 1.

(b) Purple husk color is due to two genes. Crosses with Abpl gave in F₂ the following: Total = 1214, purple husks = 669, white husks = 545. χ^2 for 9:7 = 0.64.

Crosses with ABPl showed in the five strains with colored husks studied that four were of the constitution AbPl, in spite of the fact that plants in the field appeared typically sun red with gray anthers. The fifth strain with lighter colored husks had the constitution Abpl.

Crosses with a North American tester AbPl gave 3 colored husks to 1 colorless in F₂. A sr tester proved that the P gene is not responsible for the purple husks. Crosses with a tester gave in F₂ a segregation and green plants with brown husks appeared. These plants are homozygous aa. The color is just the same as in the Colombian races which previously we called "Havana".

This has led to the conclusion that dominant A is necessary for producing purple husks. Thus we believe that purple husk is due

to a new dominant gene, reinforced by the Pl factor and requiring the presence of the A factor, and propose the symbol Ph ph for this new gene.

3. Yellow endosperm.

(a) More than one gene is involved in the difference of orange against yellow. In crosses between deep-orange flint from the La Plata Region and Brazilian orange hard flint a bifactorial segregation of 15 orange to 1 yellow was found.

<u>Ears</u>	<u>Total</u>	<u>Yellow</u>	<u>χ^2</u>
5	1592	107	0.60
6	2136	120	1.45

Descendants of the first lot gave in 20 ears normal F_2 and backcross ratios. In the next generation all ears again gave the expected ratios, but the proportion of non-segregating, monofactorial and bifactorial ears was not in accord with expectation, as can be seen by the following data:

<u>Descendants of (15:1)</u>		
	<u>Ears</u>	
<u>Segregation</u>	<u>Obtained</u>	<u>Expected</u>
Non-segregating	47	29
Bifactorial	48	76
Monofactorial	48	38

<u>Descendants of Backcross 3:1</u>		
	<u>Ears</u>	
<u>Segregation</u>	<u>Obtained</u>	<u>Expected</u>
Bifactorial	30	27
Monofactorial	51	54

<u>Descendants (3:1) Monofactorial</u>		
	<u>Ears</u>	
<u>Segregation</u>	<u>Obtained</u>	<u>Expected</u>
Non-segregating	38	34
Monofactorial	63	68

<u>Descendants of Backcross 1:1</u>		
	<u>Ears</u>	
<u>Segregation</u>	<u>Obtained</u>	<u>Expected</u>
Monofactorial segregating	44	44

The frequency of ears with a monofactorial segregation is as expected, while that of ears with bifactorial segregation is abnormal. No explanation has been found at the present time and new plantings were made to check the results. The classification is very sharp; The recessive class being of a golden yellow, and the dominant one of orange shades, from deep to light. For the two pairs of factors, causing the difference between orange and yellow, we propose the symbols Or or and Or₂ or₂.

(b) In order to know how many genes are involved in the difference between orange and yellow, a special test was made last summer. Seeds of backcrossed ears segregating 1 orange : 1 yellow, of 11 different origins were planted, including different shades of yellow and orange. Crosses were then made in the following manner:

every yellow was crossed with other yellows and with all heterozygous oranges. The orange types were crossed inter se. In order to check the dosage effect, every cross was made reciprocally, plant to plant, and at least two ears were secured of each cross. All were crossed with a y y tester. The data have not yet been fully analyzed but the results can be summarized as follows:

Cross	Segregations observed		
Yellow x Yellow	All yellow	Orange and yellow	All orange
Orange x Yellow	--	Orange and yellow	All orange
Orange x Orange	--	Orange and yellow	All orange

This added one more proof to our hypothesis that more than one factor is responsible for the difference between orange and yellow. Some ears were planted this summer to test the segregation in F₂.

(c) We do not agree with some authors about the role of modifiers in differentiating orange and yellow. Our point of view is that the modifiers act only, as in other genetical segregations, in occasionally disturbing the ratios. We do not yet have enough data to confirm the hypothesis that a selected modifier complex is responsible for all the different shades of orange and yellow. Our working hypothesis is that several complementary factors are involved and proper tests must be synthesized for proving this assumption. As in the color of the aleurone layer, several basic genes are involved, besides dosage and modifier effects which cause a variation in shades.

N. Kobal

4. Soft and hard endosperm.

Most indigenous races of maize contain soft endosperm, with the following exceptions: southeastern orange flint, Caribbean yellow flint, flint races of Colombia and of Central America, northeastern little flint, and some isolated races such as Guarani white flint, Andean, Uchuquillo and Aizuma. To this one should add the various popcorn races. A study was started to determine the genetic nature of the difference of translucent from opaque kernels. So far all crosses of translucent x translucent and of opaque x opaque have bred true. The segregation between the two types, however, does not always give a monofactorial segregation, and even when the segregation was clearly monofactorial, factor interaction and dosage effects were very pronounced. Ratios vary from 3 translucent : 1 opaque to 1 translucent : 1 opaque, though segregates occur where the opaque condition appears to be dominant. Serious complications are caused by the action of genes for deep orange endosperm color, or by other closely linked genes. It may be remembered that these same color factors or genes closely linked also cause an increase in the amount of pseudostarchy kernels in orange sugary strains.

Mario P. Mezzacappa

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1. A mutants from aged a^P seed.

Several mutants from the a^P allele to new alleles have been found when plants arising from eight-year-old seed of $a^P a^P$ constitution were pollinated by recessive a pollen. Two of the mutants were to alleles similar to recessive a in their aleurone and plant color effects but produced the dominant brown pericarp of a^P . They do not respond to the action of Dt. A third mutation was to an unstable condition which gives mosaic (pale and colorless sectors) aleurone as well as variegated culms (red-brown and brown or green stripes) presumably due to mutations of a^P to a . That this unstable a^P allele also mutates to A is indicated by the presence of numerous deep colored dots of color in the aleurone similar to those produced on a Dt kernels. The mutability of this allele is not affected by Dt.

2. Gene for unreduced eggs.

A recessive gene has been found which produces haploid male gametophytes but on the female side gives chiefly egg cells with an unreduced number of chromosomes. Ears from the homozygous plants appear highly sterile with a few plump (diploid) seed scattered among the numerous shriveled (triploid) kernels. Classification of segregating families is easy and good ratios are obtained in backcross and F_2 progenies. The limited studies of megasporogenesis which have been made indicate that the first meiotic division is normal; production of diploid eggs presumably arises at either the second meiotic division or during the development of the female gametophyte. Genetic studies, using linked genes in a heterozygous condition, of the constitution of the diploid eggs are underway. This gene promises to be valuable for the study of crossing over.

3. Genic induction of plastid mutation.

A second case, resembling that of the *iojap* gene, has been found where a specific gene induces irreversible plastid mutations. This new gene is closely linked with R and is probably an allele of *luteus-1* judging by its interaction with albino genes. Plants homozygous for this gene, and not carrying other chlorophyll mutants, have broad sectors of green and yellow tissue. Ear buds arising from the luteus sectors give all luteus populations when outcrossed by green males; ears from green sectors give all green seedlings irrespective of the constitution of the male parent. In many ways this new gene seems to be better suited for the study of gene-cytoplasm interaction than is the *iojap* gene.

4. Inversion in long arm of chromosome 3.

The breakage points in paracentric inversion 3a are just to the left of the knob in the long arm and very near the end so that in the inverted chromosome the knob lies close to the distal end of the

long arm. The locus of Ragged is to the left of the proximal break while lg_2 , A and et all lie within the inverted region and show almost complete linkage with one another and with the inversion. Owing to the greater length of the inversion some two-strand doubles occur. This inversion promises to be of some value in connection with the study of mutation at the A locus since crossing over is effectively suppressed in regions lying on either side.

5. New mutable loci.

Two new unstable genes were found in material provided by E. G. Anderson which had been exposed to irradiation from the Bikini atom bomb. Good F_2 ratios are found. One of these mutable genes is a luteus which mutates to normal giving plants with green stripes on a luteus background. The mutability of this allele is autonomous in that its rate is not influenced by the action of other loci. The second unstable type is a mutable pale green but the mutability of this gene is apparently controlled by the action of another gene which segregates independently. This latter case is similar to the a-Dt situation and to the Activator type of induced mutability which is being so extensively studied by McClintock.

M. M. Rhoades and
Ellen Dempsey

6. Male sterility.

A case of cytoplasmic male sterility has been analyzed which exhibits a unique type of inheritance. Sterility is complete so that no viable pollen is produced. The behavior of the male sterile plants in crosses is peculiar in that sterile plants are recovered in the progeny only when the inbred line Kys is used as the pollen parent. The F_1 population shows a good 1:1 segregation of male sterile and normal plants. When male steriles are outcrossed to a large number of unrelated lines, the F_1 progeny are all normal. Furthermore, continual backcrossing to male sterile plants for four generations failed to produce any male steriles. When these normal backcrossed plants were used as the female parent in crosses with Kys, sterility was recovered.

The F_1 hybrids of outcrossed Kys yield only normal plants when crossed with male steriles. These hybrids were backcrossed to Kys for six generations, using Kys as the female, and still they gave only normal plants in crosses with male steriles even though they are 98.5% pure Kys. However, when Kys was used as the male in the backcrosses, sterile plants were produced.

The inheritance of this male sterile condition was found to involve an interaction between the cytoplasm and a dominant gene for sterility (Ms), in addition to a male gametophyte factor (S^{Ga}) which is associated with a suppression of male sterility. Kys is homozygous recessive for ms and s^{Ga}. Thus, crosses of male sterile plants

(Msms s^{Ga}s^{Ga}) with Kys (msms s^{Ga}s^{Ga}) segregate for the sterility gene (Ms) and yield normal and male sterile offspring in a typical backcross ratio. All of the unrelated lines studied were found to be MsMs s^{Ga}s^{Ga}. The F₁ progeny of an outcrossed male sterile plant is composed of only normal individuals since the suppressor (s^{Ga}) is present. s^{Ga} pollen can not compete successfully against the pollen carrying the dominant gamete factor (s^{Ga}) so that in the subsequent backcrosses to male steriles only s^{Ga} pollen effects fertilization and the offspring are all normal due to the action of the suppressor gene. On the other hand, when these male fertile backcrossed plants, which are heterozygous s^{Ga}s^{Ga}, are used as the female parent in crosses with Kys, there is no gametophyte competition. Half of the progeny lack the suppressor gene and the male sterile condition can be expressed.

This same scheme explains the action of the Kys outcross hybrids in backcrosses to Kys. When the heterozygous s^{Ga}s^{Ga} hybrids are used as the male parent only s^{Ga} pollen functions and all of the individuals in the progeny carry the suppressor gene. However, when the hybrid is used as the female parent, the suppressor segregates and some of the offspring yield sterile plants when used in crosses with male steriles.

Drew Schwartz

7. Chemical studies on the action of A.

The original isolation by Sando and coworkers of the flavonol isoquercitrin from brown (aa B Pl) plants and of the anthocyanin chrysanthemine from purple (A B Pl) plants has been interpreted to mean that the A gene acts by substituting an atom of hydrogen for one of oxygen in isoquercitrin. However, as reported elsewhere, a number of brown pigments distinct from isoquercitrin are present in the brown plant and are affected by A action; moreover, anthocyan pigments in addition to chrysanthemine are present in the purple plant, absent in the brown. Since isoquercitrin is not responsible for the brown phenotype of the aa B Pl plant it might be questioned whether this pigment is affected by the A gene. This point has been investigated by separation of the pigments by the paper chromatographic technique followed by spectrophotometric analysis. Spectral absorption curves characteristic for isoquercitrin have been obtained by this method from extracts of both purple (A B Pl) and brown (aa B Pl) plants. However, there is strong indication that more of this pigment is present in husks of brown than in those of purple plants. Hence it appears that the A gene does affect the amount of isoquercitrin present, but in view of the other pigments involved, it is by no means valid to conclude that the action of this gene is concerned with the step which makes the difference between isoquercitrin and chrysanthemine.

8. New dottable A^d allele.

Of the seven original A^d derivatives from A^bEt/a et plants, six carried et (News Letter No. 23:54-56. 1949). More extensive data

now available support the conclusion that these dilutes arise by crossing over between genes comprising \underline{A}^b . In testing these \underline{A}^d cases for dottability, six were found to be negative in this regard. One, tentatively designated \underline{A}^d-2 , has a high rate of dotting. Plentiful dotting occurs on endosperms of the constitution $\underline{A}^d-2/\underline{a}^{dl}/\underline{a}^{dl} \underline{Dt} \underline{Dt} -$. Moreover, $\underline{A}^d-2/\underline{A}^d-2/\underline{A}^d-2$ endosperms on selfed ears of homozygous plants are dotted. Tests indicate that the dotting is determined by the \underline{Dt} gene as is the case with recessive \underline{a} . There is no reported case of a dottable \underline{A}^d gene among the large number of mutants obtained from $\underline{A}^b/\underline{A}^b$ plants. Since the \underline{A}^d-2 mutant originated from an $\underline{A}^b \underline{Et}/\underline{a} \underline{et}$ plant and carried \underline{et} , it is tempting to consider that synapsis and crossing over in the mother cell were such that the \underline{A}^d-2 derivative now carries the proximal (\underline{A}^d) component of \underline{A}^b adjacent to and on the same strand with recessive \underline{a} . By reason of the presence of the latter, \underline{A}^d-2 would now appear to dot. If this were the case it would be reasonable to expect that \underline{A}^d-2 should occasionally give rise to a dottable recessive \underline{a} and that the occurrence of the latter would be associated with crossing over. From a number of $\underline{A}^d-2/\underline{A}^d-2$ plants crossed using $\underline{a}^{dl} \underline{a}^{dl} \underline{Dt} \underline{Dt}$ pollen this past season two kernels with colorless, dotted endosperms were obtained. Although it is not known whether their occurrence is associated with crossing over, these mutants carry a dottable \underline{a} derived from \underline{A}^d-2 .

John R. Laughan

9. Illinois chemical strains.

The 1949 crop completed the 50th generation of continuous selection in the Illinois oil and protein strains. The results of this long-term experiment in mass selection are now being summarized, and publication in some detail will follow in due course.

Results of the "regular" phase of this experiment through 1947 were briefly summarized in last year's Maize News Letter. The analyses of the 1949 crop have not been completed at the time of this writing. Mean oil and protein contents of the 1947, 1948, and 1949 crops are shown in the following table:

Year	% oil		% protein	
	High Oil	Low Oil	High Protein	Low Protein
1947	13.45	.76	19.24	5.11
1948	14.25	1.04	19.14	5.50
1949	15.36	1.01	not complete	

The mean oil content of the 1949 crop of Illinois High Oil was more than one per cent above that of any preceding crop. Conditions were exceptionally favorable for the development of high oil content in this strain during 1949, yet there appears to be evidence

that progress is still being made by selection in this strain.

The first crop of the "reverse selection" experiment described in the last News Letter was grown in 1948. As shown by the table following, one generation of this selection markedly affected the composition of both "high" strains. Ear-rowing of seed ears of "reverse" high protein in 1949 indicated that the plant and ear types of the "reverse" strains are very similar to those of the "regular" strains. There seemed to be no indications that recent outcrossing was involved in the marked changes in chemical composition.

Selection	% oil		% protein	
	High oil	Low oil	High protein	Low protein
Regular	14.25	1.04	19.14	5.50
Reverse	13.45	1.10	18.20	5.53

Earl R. Leng and
C. M. Woodworth

10. Short-plant types.

Fourteen unrelated short-plant stocks were grown in the nursery in 1949, along with a number of additional derived and recovered lines. A number of these stocks were crossed with multiple linkage-testers. An active search for new short-plant mutations is continuing.

The "Oakes dwarf" gene, provisionally located in the short arm of chromosome 3 (Maize News Letters No. 15 and No. 16) was found not to be the same as Singleton's rd. Crosses between rd and the "Hy dwarf" gave normal F₁'s, also indicating that the "Hy dwarf" gene is not rd. In this connection, it should be noted that this "Hy dwarf", which was obtained by a number of corn workers from a common source, is not a mutation arising directly from inbred Hy, but was found in one of the more extreme Hy variants.

Earl R. Leng

11. Row number studies.

Variability within inbred lines of maize has long been observed. Hy2 and R4 are highly variable, while many other lines are relatively uniform. Dr. R. A. Emerson's work has pointed out that environmental conditions may affect row number.

In 1946 approximately 40 ears were selected from standard selfed material of each of the inbred lines, Iowa L289, Indiana WF9, Illinois 90, Illinois R4, and U.S.D.A. C.I. 187-2. No effort was

made to select for row number when these ears were picked out. These were "ear hilled" in 1947, "ear rowed" in 1948, and "ear hilled" again in 1949.

Replicated, randomized block plantings were made of some of these sub-lines in 1948 and 1949. In all five inbreds studied, highly significant differences existed between the means of certain sub-lines. Extreme differences as observed during the past growing season are indicated in table 1.

Table 1. Primordia* number of inbred lines

Inbred	High row number sub-line (\bar{X})	Low row number sub-line (\bar{X})	Primordia number required for significance (1% level)
L289	5.98	5.77	.08
WF9	8.94	7.67	.22
90	10.87	10.41	.40
R4	9.16	8.50	.39
187-2	8.44	6.97	.23

* Row number equals primordia number x 2.

Statistically significant differences exist between means of the F_1 crosses of sub-lines when crossed on to the same tester, i.e., a sub-line of another inbred. F_2 and backcross data are inconclusive, although in some cases, these means too are significantly different.

Studies were made over a two-year period on intra-plant variation in row number within three inbred lines, and their three possible single crosses. Mean values of these are indicated below. Node 1 is the uppermost ear-bearing node, node 5, the lowermost node studied.

Table 2. Primordia number at successive nodes

Pedigree	Node				
	1	2	3	4	5
L289-22B	6.12	6.08	5.43	4.91	5.10
WF9-24	8.56	9.00	8.75	8.44	8.78
90-4	11.89	11.19	10.42	9.64	9.08
L289 x 90	8.07	7.27	7.03	6.64	6.45
L289 x WF9	7.43	7.00	6.91	6.59	6.48
WF9 x 90	10.41	10.14	9.52	9.03	8.89

It appears that the later differentiated ears possess higher row number than those differentiated when the plant is smaller, except in the case of WF9, where no such gradation exists.

An attempt was made to evaluate to what degree non-genetic factors would affect row number. Inbred and hybrid plants were cut off slightly above ground level shortly after their emergence. This treatment was continued for varying lengths of time, usually until some of the plantings died. Counts of row numbers at each of the five nodes indicate that this treatment was highly effective in reducing row number. The magnitude of this reduction was highly variable, but in all observed cases, positive.

It appears likely that at least some of the commonly used inbred lines are genetically heterogeneous for row number, and that selection can shift the mean row number normally associated with that line.

D. E. Alexander

12. Reciprocal crosses.

Among a large number of reciprocal single crosses tested during the past season, some exhibited differences in early plant vigor, days to silking, plant and ear height, row number, ear length and yield. It is known that the maternal effect on seed quality and early plant vigor may be great, thereby causing the large differences observed in early plant growth. It appears, in some cases, that large differences in early plant vigor are of a great enough magnitude to affect mature plant characteristics as plant and ear height, silking date, ear length and yield. Generally, these differences showed little tendency to carry thru into the F_2 generation. If cytoplasmic factors are involved, the variability added by segregation of multiple factors in the F_2 may have prevented their detection.

Reciprocal backcrosses showed inequality in ear length and yield as shown in the following table.

<u>Pedigree</u>	<u>Ear length inches</u>	<u>Pedigree</u>	<u>Yield bushels</u>
WF9*(WF9 x R30)	6.87	187-2(187-2 x R30)	69.0
WF9 (R30 x WF9)	6.77	187-2(R30 x 187-2)	64.1
(WF9 x R30) WF9	7.26	(187-2 x R30)187-2	67.6
(R30 x WF9) WF9	7.04	(R30 x 187-2)187-2	62.2
Diff. for Sign.	.16		5.4

* Seed parent appears first in pedigree.

It is interesting to note that backcrosses were shorter eared where inbred WF9 was used as seed parent in making the backcross and when R30 was the seed parent in the original single cross. These differences cannot logically be explained on the basis of seed quality. They may possibly arise if the lines were composed of a mixture of sub-strains and different plants were used as male and female. However,

this would be unlikely because the seed was from a bulk of approximately 12 plants. It should be stated that these differences were exceptional and not of general occurrence in the material studied.

L. F. Bauman

13. Floral histogenesis in maize.

For a number of years work has been done on the developmental morphology of the maize ear and tassel. Early studies were published in the Journal of Agricultural Research 60:25-38. 1940, and in the Annals of the Missouri Botanical Garden 35:269-287. 1948. These papers dealt with the visible external changes in the shoots of the maize plant during the differentiation and development of the tassel and ear.

During the past two years work has been done on the histology of different stages in the development of the tassel and ear. Five ear types, four-row, flint (eight-row), 16-rowed types, fasciated (20 or more rows), and ramosa are being used. These histological studies trace the changes from the vegetative to the floral shoot and the beginning of the various plant and flower parts in the different zones of the shoots that give rise to them. The purpose of these studies is to see when, where, and in what order the flowers and flower parts originate. These studies will probably be published in a Station bulletin in the near future.

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1. Crossing over differences in reciprocal backcrosses in corn.

In translocation 5-9a (break in the long arm of 5 beyond the subterminal knob and in the short arm of 9), $\underline{c\ sh\ wx}$ and $\underline{v_2}$ are in the translocated pieces of chromosomes 9 and 5 respectively. The percentages of recombination observed in reciprocal backcrosses for plants heterozygous translocation, homozygous translocation ($\underline{c\ sh\ wx}$ now beyond the knob in the long arm of 5) and in standard normal plants were for $\frac{C\ +\ +}{c\ sh\ wx}$:

		<u>Region 1</u>	<u>Region 2</u>	<u>Totals</u>	<u>No. of ex- reciprocals</u>
$\frac{T}{+}$	♀	3.0	6.6	3779	19
		3.7	12.5	5949	19
$\frac{T}{T}$	♀	4.6	19.4	6394	23
		4.3	23.0	6433	23
$\frac{+}{+}$	♀	5.0	22.9	2336	--
		4.2	27.5	3554	--

3. Linkage studies with brown midrib₄.

No evidence of linkage of bm₄ was obtained with the factors lg₁ - gl₂ - B - v₄ - fl in group 2, ts₄ - na in group 3, pr in group 5, ms₁ - su₂ in group 6, ra₁ - gl₁ in group 7, v₁₆ - j₁ in group 8, Wc - sh - wx in group 9, and g₁ in group 10. Factors in groups 1 and 4 have not been tested.

Robert E. Bothun

4. Corn pollen size in varieties, hybrids and inbred lines.

The inbred lines are in two groups, those with 4 or less generations of selfing and one with more than 4 generations of selfing. Each group compared is based on 4 different stocks; one plant of each stock. One hundred normal pollen grains taken at random were measured for each plant; the 400 measurements for each group being averaged. The results are as follows:

Pollen sizes \pm S.E.

	Hybrids	Varieties	Inbred lines with	
			more than 4 selfing	less than 4 selfing
Means	30.17 \pm 0.12 <u>1</u>	30.42 \pm 0.13	31.78 \pm 0.10	32.10 \pm 0.11
S. Dev.	2.34 \pm 0.08	2.53 \pm 0.09	2.01 \pm 0.07	2.24 \pm 0.08

Comparison and significance between

	Hybrids and:			Varieties and:		Inbred lines
	Varieties	more than 4 selfing	less than 4 selfing	more than 4 selfing	less than 4 selfing	
Means	0.25 \pm 0.17	1.61 \pm 0.15	1.93 \pm 0.16	1.36 \pm 0.15	1.68 \pm 0.17	0.32 \pm 0.15
<u>Dev.</u> S.E.	1.4	10.7**	12.0**	8.6**	9.8**	2.1*
S.Dev.	0.18 \pm 0.12	0.33 \pm 0.11	0.10 \pm 0.11	0.52 \pm 0.11	0.29 \pm 0.12	0.23 \pm 0.10
<u>Diff.</u> S.E.	1.4	3.0**	0.9	4.7**	2.4*	2.3*

** Highly significant, 1% level of probability

* Significant, 5% level of probability

1 Each unit = 3u

There are significant differences between pollen size of inbred lines and of varieties and hybrids; the pollen of the inbreds being the largest, the standard deviation of the variability of the inbreds being the smallest.

The following explanation is suggested: Since some qualitative genetic factors are expressed in the pollen, factors for quantitative characters may also be expressed. We expect that there would be a correlation between the expression of the genes in the sporophyte and in the gametophyte.

The variability of pollen grain size would be related to the degree of heterozygosis of the plant that produces the pollen.

The greater pollen size of inbred lines is considered to be the expression in the haploid gametophyte of the selection of the better balanced homozygous diploids. On this basis inbreds with longer inbreeding might be expected to be larger contrary to that observed. Further tests are being made of these relations and their importance in plant breeding and genetics.

Jose L. Blanco*

* This work was conducted with the collaboration of Doctor E. Vieitez, who made the taxonomic analysis.

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1. Transmissibility of chromosomal aberrations induced in a nuclear reactor.

A study is in progress concerning the effects of irradiating maize pollen in a nuclear reactor on chromosomal aberrations transmissible through the male gametophyte. The work is being done at the California Institute of Technology in cooperation with Dr. A. E. Longley and Dr. E. G. Anderson and is made possible by a Johnson faculty fellowship from the University of Nebraska.

Crosses were made in 1947 between untreated female plants and male plants of which the tassels had been irradiated in a nuclear reactor. In 1948 the F_1 plants with abnormal pollen were outcrossed as males to one of the following good pachytene spreaders: L289 x I205, L289, or I205. In 1949 at least ten seeds of each outcross were planted at Pasadena and the resulting plants sampled for sporocytes and pollen.

The observations on pollen sterility are recorded in table 1. Only progenies in which at least two plants showed pollen sterility of ten per cent or more were included in the counts of the segregating progenies.

A cytological study of the chromosomal aberrations is in progress.

Table 1. Number and percentage of progenies of F₁ plants with abnormal pollen outcrossed to normal plants, segregating for pollen sterility of 10% or more.

Time of tassel exposure in nuclear reactor	Total number progenies	Number segregating progenies	Per cent segregating progenies
None	9	2	22.2
1 minute	55	24	43.6
2 minutes	98	44	44.9
4 minutes	84	44	52.4
8 minutes	27	10	37.0

Rosalind Morris

2. Preparing slides for determining percentage of pollen abortion.

Determinations of percentages of aborted pollen in maize are commonly made on slides prepared by emptying the pollen from an anther into an aqueous solution of I₂KI. When the coverslip is placed on the drop, the aborted pollen grains tend to move to the outer edges, resulting in an uneven distribution of the normal and aborted pollen grains. Getting representative areas for the purpose of making accurate determinations on a sampling basis is therefore impossible with such preparations.

A method of preparing slides has been devised whereby it is possible to obtain a fairly satisfactory distribution of the two types of pollen grains on a microscope slide. The essential steps of the method, all of which must be done with the aid of a dissecting microscope using a direct source of light, are as follows: An anther, the pollen of which has been prestained in a concentrated solution of I₂KI, is placed in a small drop of hot 1% aqueous agar on a warmed slide. The anther is dissected and all of the pollen grains are forced out of the anther into the agar. The entire drop of agar and the pollen in it can be viewed at one time under a dissecting microscope. It takes only a moment of vigorous stirring to secure a fairly even distribution of the two types of pollen. The initial stirring is done when the agar is hot and in a liquid phase. One must make certain that there is a reasonably uniform distribution of pollen grains with respect to both numbers and types within the area occupied by the agar. Special attention must be given to the edges of the drop. The stirring is completed as the viscosity of the agar increases and preceding its change into the gel phase. The coverslip is applied

when the viscosity of the agar has increased sufficiently to hold the pollen grains firmly in position, but before the agar has changed to the gel phase. If a small enough drop is used initially, a slight pressure on the coverslip with the needles will result in a thin film of agar under the slip. The film of agar must be thin in order to have all of the pollen grains in one plane of focus when the counts are being made.

It takes some practice to become proficient in the use of this method.

T. H. Pittenger and
E. F. Frolik

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1. Inbreeding depression and non-allelic gene interaction.

Material representing five levels of inbreeding was developed from two populations of prolific corn. The genetic representation of the material and their relative percentage of heterozygosity are as follows:

<u>Per cent Heterozygosity</u>	<u>Material</u>
0	Longtime inbred lines, P_1 and P_2
100	F_1
50	F_2
	Backcrosses
	(1) $B_1 = F_1 \times P_1$
	(2) $B_2 = F_1 \times P_2$
75	Double backcrosses
	(1) $B_{12} = (F_1 \times P_1) \times P_2$
	(2) $B_{21} = (F_1 \times P_2) \times P_1$
25	Double backcrosses
	(1) $B_{11} = (F_1 \times P_1) \times P_1$
	(2) $B_{22} = (F_1 \times P_2) \times P_2$

The various levels of heterozygosity were developed from the two populations, CI21-NC7 and NC16-NC18. Twenty-five replications containing one plot of each of the following: P_1 , P_2 , B_1 , B_2 , B_{12} , B_{21} , B_{11} , B_{22} and two plots of F_1 and F_2 were grown in 1948. The data for eight characters were analyzed to determine the presence of

non-allelic gene interactions, the presence of which would result in non-linear relationships between performance and per cent heterozygosity.

Many non-linear relationships were found to exist but could have been partially explained by the particular environmental conditions under which the test was grown. Material is available for testing again in 1950, at which time several locations and different planting dates will be used to provide more information concerning genotype-environmental interactions.

H. F. Robinson

2. Type of gene action for grain yield.

Yield records were obtained on 45 single crosses in 1949 which represented five S_1 lines selected on the basis of high yield performance of test crosses and five S_1 lines selected for their low performance on test crosses. These two groups of lines had been selected in the same manner through two cycles of recurrent selection (after Hull, Amer. Soc. Agron. 37. 1945). The ten single crosses of high x high (having two high performing parental lines) averaged 67.4 bushels per acre; the ten single crosses of low x low averaged 46.0 bushels per acre; and the 25 single crosses of low x high averaged 55.5 bushels per acre. Four of the five highest performing single crosses were of the high x high parental combination. These data seem to be more nearly in agreement with the expectations on the basis of additive effects of dominant or partially dominant gene action, and do not support the hypothesis of over-dominance of factors governing grain yield in corn (if the gene frequencies were at or near the equilibrium point in the starting population).

Paul H. Harvey

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1. Growth of inbred and hybrid maize.

The growth of inbred and single and double cross maize hybrids in terms of both fresh weight increase and dry weight increase was studied during the germination period and the early post-germination period in one instance and during the grand period of growth and the maturation period in another instance. No relation was found between size of the embryo and/or size of the seed and the ultimate size of the shoot of the plants developing from the embryos.

A method was developed for the determination of the relative efficiency of the protoplasm synthesizing processes of the inbreds and the hybrids. The hybrids were found to have relatively more efficient

protoplasm than the inbreds. In every case where the hybrids exhibited distinct vigor, this vigor was associated with an early attainment of rates of fresh weight and dry weight increase higher than those of the inbred parents. Double cross hybrids showed no distinct growth advantages over single cross hybrids but did exhibit greater uniformity.

W. Gordon Whaley

2. Developmental morphology of "Corn Grass".

The mutant corn grass upon which W. R. Singleton has reported in earlier News Letters has been subjected to a developmental morphological analysis. The evidence indicates that the mutation acts to change the pattern of meristematic activity.

The meristematic activity of corn grass differs from that of normal controls with respect to the formation of leaf primordia, the formation of stem tissues, and also with respect to the general rate of development and maturation. The corn grass plants show a greater degree of sensitivity to the light period than do normal plants.

W. Gordon Whaley

3. Gene action in the Rg mutant.

An investigation of the action of the Rg gene upon the growth and development of maize plants has yielded an abundance of data from which a few observations are cited below.

There are indications that the action of the Rg gene produces different end effects in plants grown under varied environmental conditions. Much of the material for this developmental study was grown in the greenhouse (from germination to maturity, both by liquid culture techniques and in large clay pots of soil). We find that progeny from Rg/rg stock (both cross- and self-pollinated) show no lethality under greenhouse conditions. Neither was zygotic mortality observed in these Rg/rg x Rg/rg crosses. These results are in contrast to those reported by Brink and Senn (Jour. Heredity 22:155-161. 1931) obtained from plants grown under field conditions.

By examining each leaf of the progeny 1-2 times daily until maturity was reached, the first visible manifestations of the character were determined and the extent and progression of "ragged" was recorded. Certain progeny (of the inbred Rg/rg stock) always showed the character earlier and exhibited it to a greater degree. It is believed that these plants are homozygous for the Rg gene. Segregation ratios also support this idea, although owing to the number of plants grown (a total of 256 plants) we cannot, at present, be certain of this. Viability and pollen production of these "early ragged"

seedlings (early as compared to the time of ragged manifestation by Rg/rg stocks) appears to be good, although in many cases these plants are female sterile. If, in the course of further investigations, these "early ragged" seedlings prove to be homozygous for the Rg gene then here is another example of a dosage effect, in that a "double dose" of the Rg gene results in an earlier initiation of gene action and/or a more widespread action.

It has been pointed out elsewhere (Mericle, Amer. Jour. Bot., February, 1950) that the initial anatomical manifestation of the Rg character is expressed early in the developing leaves by a "plasmolytic-like shrinkage" of the protoplasts of certain cells followed by a cytolysis of these cells. It was further suggested (from the appearance of cells surrounding a cytolysed area) that a diffusible substance may be the product of the Rg gene action. Additional physiological investigations are now in progress to inquire further into the nature and mode of action of this diffusible substance which may be produced by the Rg gene action.

Leo W. Mericle

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1. Mutation at the P locus.

A. Variegated pericarp in heterozygous combination with red cob, colorless pericarp (V/WR) shows a higher frequency of mutation in grade of variegation than homozygous variegated (VV). R. A. Emerson reported this fact in 1921. It is confirmed in the present studies with stocks which are believed to be unrelated to those which Emerson tested. The procedure used was similar to Emerson's. V/WR plants were selfed and the offspring classified for (1) grade of variegation and (2) cob color. Variegation grade was scored against a set of standard ears varying from class 1 in which each kernel, on the average, showed a single stripe of red pericarp, to class 7 which is heavily striped. The mean grade of variegation on the V/WR ears was about 1.5 classes, on the average, above the corresponding value for the VV ears.

B. Different colorless (or near-colorless) pericarp alleles, or genes closely linked with them, differentially stimulate the mutation of variegated to self color in VW plants, as R. A. Emerson reported in 1929. Our procedure was to cross a series of red-cobbed, colorless (WR) pericarp inbred field corn strains with inbreds Oh40B and Wisc67C which are white cobbed with colorless or near-colorless pericarp (WW). These WR/WW heterozygotes were then pollinated by variegated. The test cross offspring carrying the red cob allele are red-cobbed and those receiving the white cob allele have variegated cobs, and, thus, may be distinguished from each other readily. The red-cobbed alleles (or genes linked with them) carried by inbreds WF9,

WiscM13, Wisc22 and Wisc38 tested in this way against the white-cobbed Oh40B allele gave definitely lower average grades of variegation and fewer mutations from variegated to self color than did the white-cobbed Oh40B allele. Similar tests involving Wisc67C and a series of red-cobbed, colorless pericarp alleles from inbreds A334, 111B, 79A, 153, 355, and 143A showed smaller differences. In certain combinations the W_W heterozygotes had the higher variegation grade and in other combinations the reverse was true.

C. The self colors (red and orange) at the P locus, in contrast to variegated and mosaic, represent relatively stable alleles. Certain of them, however, mutate in somatic tissue to alleles lower in the dominance series with measurable frequencies. Preliminary studies show that the mutation rates vary widely. The extent to which the differences are due to the P alleles themselves and to modifiers is not known. The procedure followed was to cross the self-colored stock to a colorless (or near-colorless) pericarp strain and then to score the kernels on the F_1 ears for number of colorless sectors one millimeter wide or larger. Mutation rate is expressed as number of such mutant sectors per 1000 kernels. A self-red of uncertain origin but believed to have come originally from a farmer's open-pollinated variety showed a mutation rate of .02, based on about 800,000 kernels. Two plants sampled from the Strawberry Pop (red pericarp) variety differed greatly. One gave no mutations; the other showed a rate of .41. An orange stock of Cornell origin gave no mutations in about 50,000 kernels classified. Another orange derived from a mutant sector on an ear from a red-cobbed, white pericarp field corn hybrid showed a rate of .05. No mutations were found among 7000 kernels of a self-red derived by mutation from a "Crow Creek" variegated pericarp. On the other hand, two separate mutations from variegated to self-red in an unrelated variegated stock gave rates of 1.9 and 3.4 based on about 512,000 and 313,000 kernels, respectively. These latter values contrast with mutation rates of about 10-70 per 1000 kernels observed in the limited number of variegated pericarp stocks which we have scored for mutations from variegated to self-red. Most of the mutant sectors on the red and orange ears involved less than a single kernel. Because of this circumstance, data concerning the qualitative character and heritability of the changes are difficult to obtain.

D. Methyl-bis (β -chloroethyl) amine applied to pollen in the vapor phase is not effective in altering the mutability of variegated pericarp. Two levels of treatment were tested, the higher of which approached the lethal dosage. The pollen treatments were severe enough to result in numerous partially shrivelled kernels on the ears to which the pollen was applied. Pollen from a waxy VV stock was used, waxy serving as a means of detecting contaminants. The control and treated lots of pollen were applied to a red-cobbed, colorless pericarp single cross between two dent inbreds. The ears borne by the F_1 plants were scored for (1) grade of variegation and (2) number of mutations to self-red. The respective scores for the controls and the treated series did not differ significantly from each other. The sub-class in the treated series comprising the plants reared from the partially shrivelled F_1 seeds likewise did not differ significantly

from the controls in variegation grade and frequency of mutations to self-red.

E. In order to facilitate their studies on mutation at the P locus the Wisconsin group is interested in securing seed of the mutant types which appear occasionally in commercial corns. Seed from ears bearing patches of mutant kernels are especially desired.

Ronald Anderson
Douglas Knott
Robert Nilan
Walter Plaut
R. A. Brink

2. Mutagenic action of nitrogen mustard.

An apparatus was described in last year's News Letter by which mustard gas vapor may be applied to pollen in accurately graded doses. Continuation of the work with mustard gas has given the following results. Chromosomal derangements leading to partial fertility in the F_1 plants are induced with high frequency in mustard treated pollen. Most of them appear to be deficiencies. Few, if any, reciprocal translocations occur. The relation between dosage of mustard applied to the pollen and frequency of partially sterile F_1 plants is linear. The incidence of readily detectable point mutations relative to sterility-inducing chromosomal derangements is low. Following pollen treatment the number of entire losses of dominant endosperm characters is much higher in F_1 endosperms than in the associated embryos. Fractional losses of different dominant characters in F_1 endosperms were 1.4 to 4.0 times as high as entire losses.

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1. Inheritance of chocolate colored pollen.

In a plant of the genetic constitution, $li\ r^{rr}\ A_1A_2\ B\ Pl$, the pollen was of a chocolate color. Through self-pollination a strain with that pollen color was obtained.

Some crosses of these plants with plants of a constitution, $Li\ r^{rr}\ A_1A_2\ b\ Pl$, and a yellow pollen plant were made. In the F_1 the pollen was of a light chocolate color. The chocolate color is due to a dominant factor for which we use the symbol Co.

The following relations of the phenotypes of the mentioned backcross were obtained:

(a)	$B\ Pl\ Co$	-	178	chocolate pollen
	$B\ Pl\ co$	-	169	yellow "
	$B\ pl\ Co$	-	180	" "
	$B\ pl\ co$	-	173	" "
	$b\ Pl\ Co$	-	167	chocolate "
	$b\ Pl\ co$	-	171	yellow "
	$b\ pl\ Co$	-	166	" "
	$b\ pl\ co$	-	181	" "
	Total		1,385	

(b)	$Li\ Co$	-	698	chocolate pollen, green leaves
	$Li\ co$	-	127	yellow " , " "
	$li\ Co$	-	139	chocolate " , lineate "
	$li\ co$	-	719	yellow " "
	Total		1,683	

The chocolate pollen has developed only in plants with purple anthers. This means that there is an interaction of the factor for purple anthers and chocolate color.

A linkage of 15.8% exists between the factor for chocolate pollen and the factor for lineate leaves, located in chromosome 10.

2. p^{bor} , an allele at the P locus.

In a strain of orange pericarp and red cob, a plant with grains of orange pericarp and a red base and red cob was observed. Through self-pollination a homozygous strain for this character was obtained.

Some plants of this character were crossed with plants of orange pericarp and white cob, the others were crossed with plants of colorless pericarp and white cob. In the F_1 generation the grains had an orange pericarp, a red base and red cob in both crosses.

The F_1 generation of the first group of crosses was outcrossed with plants of orange pericarp and white cob and the second group was outcrossed with plants of a colorless pericarp and white cob.

For the factor for the grain with red base the symbol \underline{p}^b was used. The following relations of the phenotypes of the above mentioned outcrossed F_1 generation were obtained:

(a) P_1 :	$\underline{p}^{bor}/\underline{p}^{bor}$	x	$\underline{p}^{-ow}/\underline{p}^{-ow}$	
F_1 :	$\underline{p}^{bor}/\underline{p}^{-ow}$ (orange pericarp, red base, red cob)			
F_1 Outcross :	$\underline{p}^{bor}/\underline{p}^{-ow}$	x	$\underline{p}^{-ow}/\underline{p}^{-ow}$	
Progeny :	$\underline{p}^{bor}/\underline{p}^{-ow}$	-	569	orange pericarp, red base, red cob
	$\underline{p}^{-ow}/\underline{p}^{-ow}$	-	548	orange pericarp, white cob
	Total		1,117	
(b) P_1 :	$\underline{p}^{bor}/\underline{p}^{bor}$	x	$\underline{p}^{-ww}/\underline{p}^{-ww}$	
F_1 :	$\underline{p}^{bor}/\underline{p}^{-ww}$ (orange pericarp, red base, red cob)			
F_1 Outcross :	$\underline{p}^{bor}/\underline{p}^{-ww}$	x	$\underline{p}^{-ww}/\underline{p}^{-ww}$	
Progeny :	$\underline{p}^{bor}/\underline{p}^{-ww}$	-	731	orange pericarp, red base, red cob
	$\underline{p}^{-ww}/\underline{p}^{-ww}$	-	750	colorless pericarp, white cob
	Total		1,481	

The character, orange pericarp, red base, red cob - \underline{p}^{bor} is allelomorphous to \underline{p}^{-rr} .

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West Virginia University
Morgantown, West Virginia

An "indeterminate growth" character, apparently the third recorded, segregated in two rows of first generation selfs from a single ear of a farmer's open-pollinated, yellow dent variety from Pocahontas County, West Virginia. Plants were pale green in early stages, grew tall with 26 or more short nodes, developed eight or more series of brace roots, and tasseled (in transplants from field to greenhouse) in November. No ear shoots appeared under these conditions. A limited amount of seed from first and second selfed generations of the parent line is available.

J. Lincoln Cartledge

II. MAIZE PUBLICATIONS -- 1949

(Including certain 1948 publications not previously listed
and some early 1950 publications.)

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III. SEED STOCKS PROPAGATED AND RECEIVED

This past summer Mr. James E. Wright, Jr. propagated approximately 115 cultures. Most of these were planted to renew viability of old stocks, to incorporate genes into new linkage testers and to purify stocks that had been outcrossed in order to increase vigor. The work of crossing weak stocks to vigorous in-bred lines was continued.

A complete inventory of material on hand was presented in News Letter No. 22. Inasmuch as relatively few stocks have been added to the Coop. since that letter, no additional extensive list is given in this issue. Many of the genes have been incorporated into new combinations with other genes, thus increasing the variety of gene combinations available.

The Coop. has received the following new gene cultures during the past year:

<u>bl</u> ₃ -- blotched leaf	H. W. Simmonds Imperial College of Tropical Agriculture Trinidad, B.W.I.
<u>de</u> ₁₇ -- defective seed	R. A. Brink Department of Genetics University of Wisconsin Madison 6, Wisconsin
<u>bm</u> ₄ -- brown midrib	C. R. Burnham Div. of Agronomy & Plant Genetics University of Minnesota University Farm St. Paul 1, Minnesota
<u>BB</u> -- pseudonormal	H. C. Eyster
<u>Bb</u> -- light green	Charles F. Kettering Foundation
<u>bb</u> ₁ -- light green; grows into albino	Yellow Springs, Ohio
<u>bb</u> ₂ -- similar to <u>bb</u> ₁ , except white color is chalky white	
Yellow seedling - Chromosome 6.	
Pigmy and Albino - Chromosome 6.	

The following communication was received from Dr. William L. Brown, Pioneer Hi-Bred Corn Company, Johnston, Iowa that is of special interest:

"Last summer I grew for study approximately 150 strains of North American Indian corn representing 35 tribes distributed largely in the northern Great Plains and the Southwest. I was able to increase seed of most of these, small quantities of which have been placed in cold storage. It is now almost impossible to obtain some of these from tribal remnants and, since there may be other people interested in working with them, it may be worthwhile to mention that they are available in small quantities. These are, for the most part, those varieties that Longley worked with and reported on in 1938."

Julian P. Craigmiles