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FOREWORD

As in the past eight years the task of editing and assembling the News Letter has been most capably performed by Miss Ellen Dempsey and all of us who profit by the publication of these research items are indebted to her. Our thanks are also due to Mr. Karl Rinehart for his assistance in reading proof.

A portion of the expenses incurred in connection with cutting the master sheets, running, assembling and binding was met by a grant from the National Science Foundation.

M. M. Rhoades

II. REPORTS FROM COOPERATORS

AGRICULTURAL ALUMNI SEED IMPROVEMENT ASSOCIATION
West Lafayette, Indiana

1. Further observations on the Rf inhibitor carried by CI.44.

Preliminary observations on the suppression of the fertility restoring gene Rf by CI.44 were reported in the last News Letter and the problem was studied further during the past season. CI.44 was crossed on K6T, K55T, Ky21T and Kyl22T, 4 naturally restoring lines which had been backcrossed into T cytoplasm, and these crosses were compared with the cross Oh45T Rf(I153) x CI.44 reported previously. One of the 2 plants of K55T and also one of the 2 plants of Ky21T used as seed parents proved to be heterozygous for Rf rf and their progenies gave 1:1 segregations for fertile and sterile plants. Otherwise all of the plants from these crosses were fully fertile with no indication of fertility suppression by CI.44. The cross Oh45T Rf(I153) x CI.44 yielded 46 late partials (similar to those reported previously) and 3 fertiles, 2 of which were very late plants. The reciprocal crosses of CI.44T by K6, K55, Ky21 and Kyl22 also were grown and all plants in these progenies were fully fertile.

The male sterile single cross WF9T x CI.44 was crossed by the following 14 restoring inbred lines:

B14T Rf(I153)	K6
B14 Rf(Tx127)	K55
C103T Rf(I153)	Ky21
C103T Rf(Ky21)	Kyl22
CI.42AT Rf(Tx127)	Oh43T Rf(K6)
HyT Rf(Tx127)	Oh45T Rf(I153)
I153	W153R

One plant of C103T Rf(Ky21) proved to be heterozygous for Rf rf and gave a 1:1 segregation of fertile and sterile plants in its cross. In addition a single plant among the 28 plants in the progeny from the cross involving B14 Rf(Tx127) was sterile. Otherwise all plants from all of these crosses were fully fertile except those from the cross involving Oh45T Rf(I153). Of the 59 plants from this cross 5 were partials and the remaining 54 were a little late in shedding pollen and were somewhat sparse shedders.

The general conclusion to be drawn from these observations, and a few other miscellaneous observations not reported, is that the fertility suppression of CI.44 is very specific and in the tests so far conducted is restricted to crosses involving Oh45T Rf(I153).

M. T. Jenkins

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Gödöllő, Hungary
Department of Plant Breeding

The main objective of the research work conducted in this Department is to produce maize hybrids with a high protein and oil content. The experiments performed in order to reach this target are theoretically twofold, part of them being connected with the increase of protein content and others being related to the analysis of the theoretical foundations of heterosis. The present study is an account of experiments connected with the increase of protein content.

For the production of hybrids, attempts were made to establish lines of genetically very different origin. One procedure is to produce lines from the existing open pollinated improved varieties with 10 to 11 per cent protein content. This method, however, provides for slow progress only.

Zea x Euchlaena hybrids.

Another source of this breeding work is the hybridization of Zea mays with Euchlaena mexicana. Variability in the F_1 generation is presented in Table 1. In order to transform the F_1 generation with a higher protein content into a maize variety of the cultivated type, repeated top crosses were performed (Table 2). This brought about a reduction of the average protein content to 14.1 per cent but the segregating generations allowed the selection of individuals with 15 to 16 per cent protein content.

Table 1
Protein Percentage in Intergeneric Crosses of Maize
(Gödöllő, 1957)

No.	Crude Protein %	No.	Crude Protein %	No.	Crude Protein %
1	17.1	9	14.8	17	17.5
2	12.9	10	14.4	18	15.4
3	13.2	11	15.5	19	15.6
4	13.2	12	16.6	20	15.5
5	12.1	13	15.0	21	14.5
6	14.0	14	13.1	22	14.1
7	14.5	15	12.8	23	12.2
8	13.4	16	14.4	24	15.8

Table 2
Protein Percentage Changes in Repeated Top Crosses
(Gödöllő, 1954-57)
(As Related to Absolute Dry Matter)

No.	Combinations	Variety at First and Second Top Cross	Protein Percentage		
			Before Top Crossing	After	
				First Top Crossing	Second Top Crossing
				1956	1957
1.	Zea x Euchlaena	Bankuti	16.6	12.2	10.7
2.	Zea x Euchlaena	F-korai (early)	15.6	14.2	14.1
3.	Zea x Euchlaena	F-korai (early)	17.5	14.0	11.1
4.	Zea x Euchlaena	F-korai (early)	17.5	14.0	10.3
5.	Zea x Euchlaena	Land variety	15.4	13.7	13.2

Breeding of biochemical mutants.

In 1958 an open pollinated variety (Fk) and different lines have been irradiated at dosage rates of 10 and 15 kr according to the usual methods. In order to accelerate breeding work, the X_1 has been investigated too, and only the grains of 300 selfed individuals with a protein content above 8 per cent were planted as the X_2 generation. Table 3 shows the variability of a part of the material.

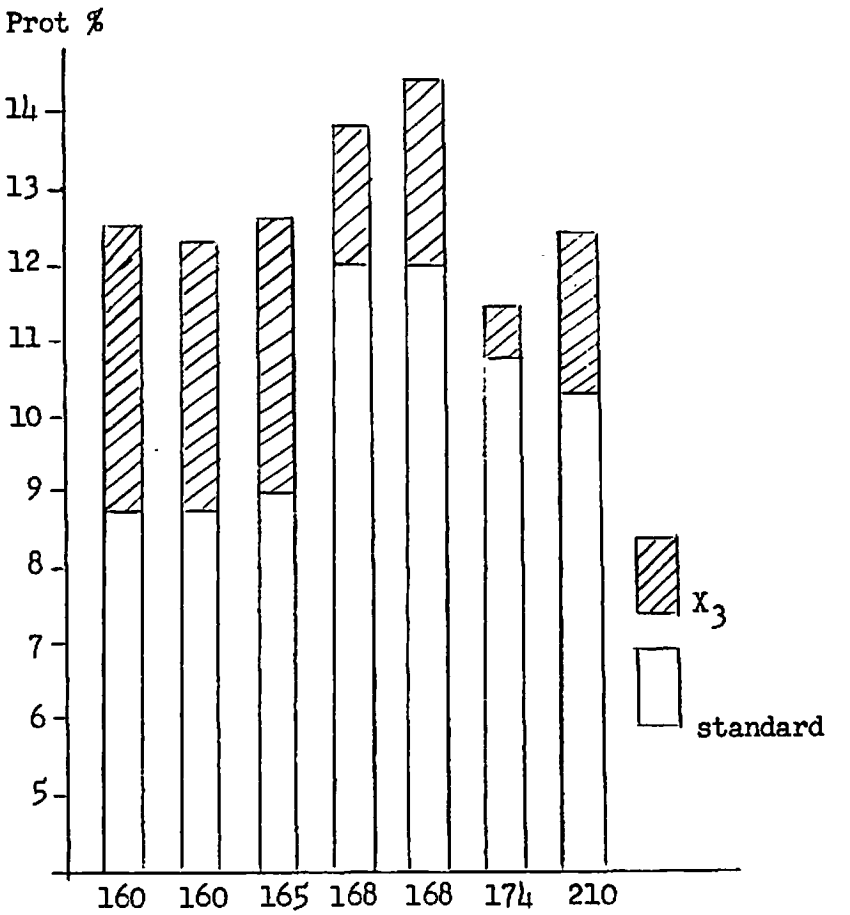
Table 3
Variability of the X_2 Generation Selected From the Irradiated X_1
(Gödöllő, 1958, 1959)

Dosage Applied (kr)	No. Of the Starting Material	Mean Protein Content	Variation Range in X_2		Absolute Deviation Per Cent
			From	To	
15	Fk	11.3	8.3	15.2	6.9
10	S 160	8.8	8.6	13.1	4.5
15	S 160	8.8	8.4	14.4	6.0
10	S 165	9.0	8.4	15.4	7.0
10	S 168	12.0	11.4	12.3	0.9
15	S 168	12.0	10.2	15.5	5.3
10	S 174	10.8	10.6	13.3	2.7
10	S 200	13.3	10.1	16.1	6.1
10	S 210	10.2	14.1	15.4	1.3

Each entry represents the mean value of individual analyses of 18 selfed ears from the 24 individuals of a plot. The mean values of the X_2 plots of the other lines showed a variation range from 0.9 to 7.0 per cent. Our expectations concerning a hereditary increase of the variation range of the material due to irradiation have been verified in the X_2 generation.

In the X_3 generation too, only the X_2 sub-lines with a protein content above 8 per cent were maintained. The protein percentage was, probably under the influence of meteorological factors, lower than in X_2. Figure 1 presents the mean protein percentage in the best X_3 lines obtained from the inbreds, as related to the standard variety "F" korai (early) and to the starting material.

Figure 1. Average Protein Content of X_3 Maize Lines (Gödöllö, 1960).



According to the data, we succeeded in obtaining forms with hereditarily higher protein contents from 6 lines.

This increase of the average values surpasses the 2.86 per cent increase observed in the first five years of the Illinois cycle and, consequently, it is more considerable than the progress obtainable by simple selection in 5 years. The principal value of this breeding method consists in the fact that differences can be hereditarily fixed, using a reduced material to a more favorable extent and in a shorter period, than using untreated material.

For a genetically more exact analysis of the effects of mutagens in 1962 further lines were irradiated. When the X_1 and the starting material were treated, the line C5 gave in 1962 the results shown in Table 4.

Table 4

Treatment	Value		Number of Ears Examined
	Minimum	Maximum	
Control	8.2	13.2	20
15,000 r	7.2	14.2	29
7,000 r	8.0	15.4	38
Neutron (500 REP)	8.5	14.5	22

The variation range increased once more here. A definite evaluation of the experiment will be possible on the ground of the data from the X_3 and X_4 generations.

Summary

With various methods, lines with a high protein content were obtained from which in the meantime some promising single and double crosses have been produced.

ANDHRA UNIVERSITY
Waltair, India
Department of Botany

1. Pachytene pairing in auto-tetraploid maize.

A study of pachytene associations in the pollen mother cells of a colchicine induced auto-tetraploid maize showed that (1) pairing is between homologous parts only of pairs of chromosomes at any point and (2) the four homologues may form two separate pairs or one or two exchanges of partner may take place in an association.

The data on the frequency of different numbers of exchanges revealed that the occurrence of one exchange does not interfere with the occurrence of a second one in an association since different numbers of exchanges fit a Poisson distribution.

Of a total of 195 associations, 80 showed association of all four homologous centromeres, 35 of these being with no exchange elsewhere in the association. The centromere associations probably represent points of exchange of partners located at the centromere. This is supported by the following consideration. Analysis of metaphase I configurations in 60 nuclei revealed that 74% of the possible multivalents are formed. An essential requirement for multivalent formation is one or more exchanges of partners in the pachytene association followed by formation of appropriate number of chiasmata distributed at appropriate places along the paired chromosomes in the association. However, only 48.7% of the observed pachytene associations showed one or two exchanges of partner. In addition to these, 19% of the observed pachytene associations were those with association of all four homologous centromeres

with no exchange of partners elsewhere in the association. Only when these are also taken as representing an exchange of partners located at the centromere, the percentage of multivalents expected will be close to the observed.

It is also observed that the number of times an exchange occurs in an arm is proportional to its length. In the case of chromosome 6, however, the absence of exchanges from the short arm is significant ($P = 0.0024$). This is probably due to the special features of this chromosome; the short arm is the shortest in the complement and it is anchored to the nucleolus by the subterminal nucleolus organizer, both of which features hamper the formation of an exchange.

Although the exchange points show a random distribution along the length of the chromosome, they show a tendency to cluster in certain regions of the chromosome, which indicates that the initial points of pairing are probably mostly associated with the centromere, knobs and the ends of the chromosomes or with regions adjacent to them.

On the basis of data with respect to chromosomes 3, 6, 9 and 10 the mean length of the "pairing block" (the region paired between two consecutive exchange points or point of exchange and the end of the chromosome in single exchange cases) increases with increase in the length of the chromosome while the mean number of pairing blocks increases with increase in length of the chromosome up to a certain limit beyond which it decreases. In the case of chromosome 6, however, the rise in the length of the "pairing block" is not so sharp as in other chromosomes probably due to the special features of this chromosome.

J. Venkateswarlu

2. Chiasma frequency in colchicine-induced auto-tetraploid maize.

Chiasma frequency was determined from analysis of metaphase I configurations in the pollen mother cells of tetraploid sectors in the tassels of two colchicine-treated maize plants. The average number of chiasmata per nucleus was 35.7 and of half-chiasmata per chromosome was 1.785. The chiasma frequency in pollen mother cells of diploid flowers in the same inflorescence was 16.06 per nucleus and 1.61 per bivalent (half-chiasmata per chromosome).

The mean chiasma frequency per tetraploid nucleus is more than twice as great as that per diploid nucleus. A comparison between them was made by calculating

$$t = \frac{X_t - 2X_d}{\sqrt{V_{X_t} + 4\,V_{X_d}}}$$

where X_t and X_d are the means, V_{X_t} and V_{X_d}, the variances of the means respectively of the tetraploid and the diploid. The t value obtained was 7.6 and this gives a probability of less than one in a thousand that they could be equal and that the difference is a chance one. The mean chiasma frequency per tetraploid nucleus is thus significantly greater than twice that in the comparable diploid.

The number of rod bivalents was smaller than ring bivalents in the pollen mother cells of the diplod flowers while in those of the tetraploid flowers, the ring bivalents were fewer than the rod bivalents. Further, the half-chiasma frequency per chromosome in the bivalents of the tetraploid (1.49) was less than that in the bivalents of the diploid (1.61) and the half-chiasma frequency increases with increase in the number of quadrivalents. Thus the substantial increase in the chiasma frequency in the tetraploid is accountable solely by those chromosomes which form the multivalents.

J. Venkateswarlu

ATOMIC ENERGY ESTABLISHMENT TROMBAY
Byculla, Bombay-8, India
Biology Group

1. A new inhibitor of aleurone and plant colour.

Under the title "Pigmented silkscar" (MNL 36:104), we reported that this stock, which was collected locally, inhibited the aleurone pigmentation completely when crossed as the female parent with homozygous colored aleurone stock. In the reciprocal cross, there is only a partial inhibition of pigmentation. Since it is very unlikely that we obtain the pigmented silkscar phenotype again, we wish to disassociate it from the aleurone inhibiting effect. We propose the symbol I_2 (Inhibitor$_2$) to denote the factor(s) responsible for this effect. In addition, the I_2 I_2 stock also seems to possess the capacity to inhibit plant color. The tests made so far are summarized below:

Cross	Average pigmentation grade of kernels or plants	Remarks
A A C C R R X I_2 I_2	2.81/5	Partial inhibition of aleurone color.
I_2 I_2 X A A C C R R	1.00/5	Complete inhibition of aleurone color.
A A B B Pl Pl X I_2 I_2	3.66/5	Partial inhibition of plant color.
A A B B Pl Pl ⊗	4.94/5	
(I_2 I_2 Wx Wx X $\frac{\text{T7-9 wx}}{\text{T7-9 wx}}$) X A A C C R R wx wx	-	I_2 shows linkage with wx. Recombination 18.36%. (Data based on a single cob bearing 98 kernels.)

Table 1

Comparison of Types and Numbers of Kernels Obtained from Crosses Involving Inhibitors, Inhibitor Sources, Different R Alleles and R^rK Combination.

Cross	Number of F_1 kernels scored	Average aleurone pigmentation grade	Remarks
1) AA CC RR x I_2I_2	797	2.97	Local inhibitor
2) AA CC RR x I_1I_1(Coe)	49	2.14	Of common origin
3) AA CC RR x I_1I_1(Coop)	151	1.95	Of common origin
4a) AA CC $R^{d:Pony}$ x I_2I_2	298	1.00	$R^{d:Pony}$
4b) AA CC $R^{d:Catspaw}$ x I_2I_2	111	1.13	$R^{dilute:Catspaw}$ replacing R
5) AA CC R^{sc} x I_2I_2	173	2.03	R^{sc}($R^{self-colored}$) is a mutation of R^{st}
		1.40	($R^{stippled}$) to R^{sc} and is non-paramutagenic
6) AA CC RKRK x I_2I_2	71	1.30	RrK abnormal chromosome 10 knob present.

AA CC RR = Colored aleurone.

The present data suggest that I_2 may be either on chromosome 9 or chromosome 7. However, the recombination value of 18.36% between I_2 and wx is about the same as that between I_1 (C^I) and wx and it would not be surprising if I_1 and I_2 turn out to be allelic.

Comparisons between I_1 and I_2 have been made regarding their expression against a common colored aleurone tester. Various R stocks in a common background have also been tested against I_2. These were included at the suggestion of Prof. R. A. Brink and the seed was kindly made available by him. The data are summarized in Table 1.

The following observations are made:

(1) I_2 seems to have somewhat less capacity to inhibit aleurone pigmentation than either I_1 (Coe) or I_1 (Coop). The differences could be due to differences in the genetic background. If the differences are real, two classes of colored kernels would be expected on test crossing the I_1 I_2 heterozygote on A C R. This test is under preparation.

(2) All the colored aleurone stocks carrying different R alleles are inhibited much more than the standard A C R. The significance of this observation is not clear. One would have anticipated that at least there should have been less inhibition by I_2 against A C R^{sc} than with A C R.

N. K. Notani
Chandra Mouli

2. UV--Irradiation of $\widehat{A_1 Ds}$ pollen.

Pollen grains with the genotype $\widehat{A_1 Ds}$ (without Ac) were irradiated with ultraviolet light obtained from a germicidal lamp. The idea was to see if Ds can be "mutated", inactivated or deleted without affecting the A_1 locus. The change so brought about should be detectable as full or partially colored kernels in the cross to an appropriate tester (a_1^s sh_2 or $\widehat{A_1 Ds}$--both with ac). We have now tested 2279 UV--irradiated gametes. Not a single colored kernel has been obtained.

(Seeds for this study were kindly made available by Dr. Barbara McClintock.)

Chandra Mouli
N. K. Notani

3. Role of chemical composition in radiosensitivity of seeds.

(a) Protein content: Maize strains differing in their protein content were tested for their radiosensitivity. The low protein (L.P.) strain having only about 5% protein in seeds, was compared with the high protein (H.P.) strain which had about 23% protein. The two strains differ in their rates of growth, L.P. being the slower of the two. Because one of the criteria of radiobiological damage is seedling height in a finite period direct comparison would not be possible. Since differences in protein content between L.P. and H.P. appear to be primarily due to

differences in zein content (which is localized in the endosperm), it seemed feasible to produce hybrids, by reciprocally crossing H.P. with L.P., differing in their endosperm protein contents but having identical germs. When such crosses were made, kernels from (L.P. X H.P.) and (H.P. X L.P.) phenotypically resembled those of L.P. and H.P. respectively. Analysis of the amino acid content, more or less confirmed the above observation--(L.P. X H.P.) amino acid content was slightly higher than L.P., and (H.P. X L.P.) amino acid content was somewhat lower than H.P. (Table 2).

Table 2
Amino Acid Content of L.P., H.P., (L.P. X H.P.) and (H.P. X L.P.) Lines (mgm/gm of Seed)

Amino Acid	L.P.	L.P. X H.P.	H.P. X L.P.	H.P.
Aspartic acid	2.904	3.20	8.80	14.46
Glutamic acid	8.08	9.04	27.56	33.16
Threonine glycine	4.00	3.72	11.60	15.76
Histidine	4.0	2.60	7.56	12.00
Alanine	1.00	1.32	2.84	4.92
Tyrosine	8.56	9.12	26.64	39.96
Lysine	2.32	3.04	10.40	16.00
Valine	1.60	2.40	2.40	2.20
Methionine	2.36	2.08	2.68	1.84
Phenylalanine	3.28	2.44	4.40	3.28
Arginine	1.88	1.72	2.60	2.04
Proline	4.28	4.60	4.16	4.60

Seedling height data were recorded 11 days after sowing and survival data after 24 days. Growth rates of the two hybrids were the same. Data are summarized in Table 3.

Table 3
Height and Survival of L.P., H.P., (L.P. X H.P.), and (H.P. X L.P.) Plants Following Irradiation with Co^{60} Gamma Rays

Treatment of Seeds*	H.P.		L.P.		(H.P. X L.P.)		(L.P. X H.P.)	
	Ht. (Cm.) Mean	Survival (%)	Ht. (Cm.) Mean	Survival (%)	Ht. (Cm.) Mean	Survival (%)	Ht. (Cm.) Mean	Survival (%)
Control	11.34	87.80	7.41	92.98	12.36	94.29	12.45	100.00
5,000 r	9.45	75.68	6.40	83.33	12.00	89.65	10.80	83.33
10,000 r	5.08	64.82	4.12	67.44	9.08	91.22	9.63	82.14
15,000 r	3.16	44.07	2.94	45.65	-	-	-	-
20,000 r	-	-	-	-	5.65	87.50	5.70	92.00
25,000 r	2.31	3.64	2.25	25.00	-	-	-	-

*Seeds were stabilized for their H_2O content over $CaCl_2$.

It is apparent from these data that the protein content of the endosperm has very little influence on the radiosensitivity of the germ. It is also noteworthy that both the hybrids exhibit conspicuous radioresistance when compared to either L.P. or H.P.

(b) Oil content: Low oil (L.O.) and high oil (H.O.) strains were likewise compared. L.O. has about 0.75% oil and H.O. about 15%. Oil is for the most part localized in the germ. Surprisingly the H.O. strain appeared to be more radiosensitive than the L.O. This result might be due to the fact that the germ size of the L.O. strain is very much smaller than that of the H.O. strain (see Table 4).

Table 4
Germ and Endosperm Weights of Low and High Oil Strains of Maize

Maize strain	Average wt. of whole kernel (mgms.)*	Ratio of whole kernel wt. L.O./ whole kernel wt. H.O.	Average germ wt. (mgms.)*	Average endo-sperm wt. (mgms.)*	Ratio of germ wt./ endo. wt.
High Oil	170.30	1.76	25.10	145.20	0.173
Low Oil	300.00		13.20	287.00	0.046

*Measurements made on 50 kernels. Consequently, the actual total deposited dose may be less in L.O. than H.O.

(Foundation stocks of Ill. L.P. and Ill. H.P. and considerable background information were kindly supplied by Drs. D. E. Alexander, R. W. Jugenheimer, E. R. Leng, and Mr. R. J. Lambert, University of Illinois, U.S.A.)

N. K. Notani
P. S. Chourey
Chandra Mouli

4. P^{32} treatment of cytoplasmic male sterile seeds of maize.

Cytoplasmic male sterile seeds of an inbred line WF9-21MS were treated with P^{32} in an attempt to inactivate the (presumable) plasmids and/or episomes conditioning the male sterility. The source of male sterility is the Texas or "T" type cytoplasm. Earlier attempts made by Brawn (MNL 37, 86, 1963) to "cure" maize of its plasmids with heat and certain chemicals known to affect plasmids, were unsuccessful.

We have argued that if nucleic acids are the main carriers of genetic information, then it might be easier to inactivate these particles by incorporating P^{32} in their nucleic acid. Decay of the P^{32} atom is accompanied by 3 events: (1) emission of a particle, (2) an equal and opposite recoil for the nucleus, (3) transmutation. In bacteria and viruses the transmutation and recoil components are more efficient in inactivating.

Technique: Seeds were soaked in a carrier free P^{32} solution (10 μc per seed) for 48 hours in petri dishes and are now being grown in the field.

(Foundation stock of inbred WF9-21MS and background information were kindly supplied by Dr. W. J. Mumm, Crow Hybrid Corn Co., Milford, Illinois, U.S.A.)

P. S. Chourey
N. K. Notani

AUBURN UNIVERSITY
Auburn, Alabama

1. Inheritance of blotched leaf.

Blotched leaf (bl) was first reported by R. A. Emerson (Cornell University Agr. Exp. Sta. Memoir 70:1-16, 1923). N. W. Simmonds presented data (MNL 24:26-27, 1950) showing linkage of a similar character, which he called blotched-3 (bl_3), with some undetermined "anthocyanin locus" he thought likely to be the R factor.

A blotched leaf character was observed in some breeding material at this station. In linkage tests with a series of translocations obtained from Dr. C. R. Burnham, a linkage of 21.3 $\pm$ 2.64% recombination was obtained between the character and T2-9c (2S.49 and 9S.33). Unfortunately the cross with the other interchange marking the short arm of chromosome 2 failed. However, two other interchanges involving the short arm of chromosome 9, T6-9 and T9-10b, showed no linkage with blotched leaf. The T1-8a culture was segregating for the B factor and this factor gave a recombination value of 22.97 $\pm$ 4.89% with blotched leaf.

There was considerable variation in expression of the blotched leaf character. It seems likely that Emerson's blotched leaf and Simmond's blotched leaf-3 were the same and that Simmond's "anthocyanin locus" was "B" and not "R". These data would locate "blotched leaf" on the short arm of chromosome 2.

Edward M. Clark

BEAR HYBRID CORN CO., INC.
Decatur, Illinois

1. Amylose breeding progress.

In our 1952 MNL report (Newsletter 26, page 5) the ae gene was reported and its influence on increasing amylose at the expense of amylopectin was noted.

Progress of the hybrid development program and studies of the ae influence in various endosperm combinations were reported in the Agronomy Journal, 50:595-609, 1958.

Currently, amylomaize hybrids, with amylose content between 50-70%, are being grown under the Bear brand name, Amicorn. Hybrids containing 70-80% amylose have been developed and will be available for general production by 1967.

Source stocks containing up to 85% amylose have been developed by the use of recurrent and reciprocal recurrent selection. These sources are currently being used to develop hybrids with above 80% amylose. The amylose increase in sources has averaged about 2% per year by growing multiple generations.

Marvin L. Vineyard
Robert P. Bear

BOSTON COLLEGE
Chestnut Hill 67, Massachusetts
Department of Biology

1. Further studies on the characteristics of teosinte chromosomes.

a. Florida teosinte: Microsporocytes of 16 additional F_1 hybrid plants of maize-Florida teosinte were examined. One-fourth of these plants had well-spreading pachytene chromosomes. Therefore a clear observation of the characteristics of Florida teosinte chromosomes could be made. Chromosomes 1, 2 and 3 were knobless. There were three knobs on chromosome 4; in addition to two terminal knobs there was an internal knob on the short arm. The two arms of chromosome 5 were terminated by knobs. An internal knob was present on the first knob position of the long arm of chromosome 6 and a small terminal knob on the short arm of the same. There was a large internal knob on the long arm of chromosome 7. The long arm of chromosome 9 had a large terminal knob. Chromosomes 8 and 10 had no knobs.

As previously reported, In9 in the short arm of chromosome 9 was observed in all of the F_1 plants having well-spreading pachytene chromosomes. However, the paracentric inversion in probably the long arm of chromosome 3 was found only in certain plants, indicating that this inversion existed as a heterozygote in the teosinte parent. Anaphasic evidence showed that this In3 had increased the frequency of crossovers within the inverted segment of In9.

The previous study of Longley (1937) reported that Florida teosinte like other teosinte varieties from southern Guatemala, had only terminal chromosome knobs. It has now been found in the present study that there were three conspicuous internal knobs. Possibly the teosinte employed in this study came from a different population from that of Longley's. It might also be possible that the knob substance in teosinte and its relatives represents an unstable heterochromatin. This heterochromatin may transpose from one position to the other, or from one chromosome to the other, by an unknown mechanism.

b. Jutiapa teosinte: Study of the microsporocyte divisions of 27 additional F_1 hybrid plants of maize-Jutiapa teosinte (from Guatemala) was carried out. At pachytene the chromosomes were generally identifiable even though the spreading quality was not as good as expected. There were two types of chromosome 1; one was knobless, while the other had a large terminal knob on the short arm. Chromosome 2 had a large knob terminating the short arm. Both arms of chromosomes 3, 4, 5 and 6 had probably medium-sized knobs. Chromosomes 7 and 8 were knobless. A small terminal knob was present on the short arm of chromosome 9. There were two types of chromosome 10; one was knobless, the other with a large terminal knob on the long arm. It was frequently observed that the teosinte chromosome 10 was longer than its maize homologue by this knob.

A paracentric inversion in the long arm of chromosome 1 was found in five of the plants studied. The average of five separate measurements of the length of the inverted segment was 18.1 μ (Table 1). The distance from the distal point of breakage to the centromere was equivalent to about 55.3 μ, or 67% of the length of the long arm.

Table 1
Length and Per Cent of the Long Arm Occupied by Inl in Jutiapa Teosinte

Cell No.	Length of inversion in microns	Length of long arm in microns	Per cent of long arm
1	17.7	78.3	22.6
2	16.8	78.4	21.4
3	13.9	63.9	21.7
4	24.7	103.0	24.0
5	17.6	91.1	19.3
M	18.1	82.9	21.8

In the short arm of chromosome 9 of all the plants studied, a paracentric inversion was observed. This points to the fact that the teosinte plants employed in the crosses were homozygous for this inversion. Furthermore this inversion appeared the same as that previously reported in this teosinte and the other varieties of teosinte of both Mexico and Guatemala.

c. Huixta teosinte from Guatemala: Pachytene chromosomes of 17 additional F_1 hybrids of maize-Huixta teosinte were found, last year, to differ from those previously studied (Ting, 1958) in having all of the knobs terminally located. Chromosomes 1 and 10 were knobless. There was a large knob on the short arm of chromosomes 2 and 4. The long arm of chromosome 3 had also a large knob. Both arms of chromosomes 5 and 6 were occupied by a medium-sized knob. A small knob was on the long arm of chromosome 7 and a medium-sized knob on the short arm of chromosome 8. There was also a small knob on the short arm of chromosome 9.

No inversions or any other structural changes of the chromosomes were found. All of the chromosomes from teosinte associated closely with

their maize homologues. Chromosome behavior at anaphases I and II was regular.

Y. C. Ting
Francine Torres
E. Dancewicz

2. Chromosome inversion of a maize tester plant.

During last year a cytological examination of a maize tester strain was made. The marker genes of this strain are *j* v_{16} *ms* (hetero). This tester was obtained from Professor P. C. Mangelsdorf of Harvard University. At pachytene a paracentric inversion was found in the short arm of chromosome 8 of one of the plants. This inversion was practically terminal and it appeared the same as the In8 found in certain other strains of maize (McClintock, 1933, 1959) and in some Mexican teosintes (Ting, 1958). The length of the inverted segment was equivalent to about 50 per cent of the length of the short arm. Evidence of crossing over, such as bridges and fragments at anaphase I and bridges at anaphase II, was found.

Since the chance of the occurrence of two identical inversions in nature is practically nil, the existence of these aberrations can be used as a reliable marker of germplasm interchange among distinct species as well as among different varieties of the same species. Therefore, the In8 in this maize tester is considered as one more evidence of introgression between maize and teosinte. The direction of the introgression and the effect of this inversion on the frequency of crossovers in the heterologous chromosome pairs are under investigation.

Y. C. Ting
Francine Torres

3. Further study of the selfed progenies of a variegated-leaf homozygote (vl/vl).

Last year the selfed progenies of a variegated-leaf homozygote (vl/vl) were under further investigation. A total of 129 plants was available. Four of these were practically albino and died while they were at the seedling stage. This is completely different from a previous study (Maize News Letter, 1963) in which no albino seedlings were observed. The remaining 125 plants resembled average maize plants in size. However, it was found that the degree of variegation in the leaf-chlorophyll content of this mutant varied very strikingly. Subsequently these plants were classified into four classes by the size of the chlorophyll-deficiency area in per cent of the total leaf area.

Class A had three plants with 85 per cent of the total leaf-area deficient in chlorophyll. Class B had 21 plants with 75 per cent of the leaf-area deficient in chlorophyll. Class C had 44 plants with 50 per cent of the leaf-area deficient in chlorophyll. Class D had 57 plants with 25 per cent deficient.

Through this observation it appears likely that the variations in the area of the leaf chlorophyll deficiency might be regulated by a second nuclear element in addition to the structural genes in the chromosome. This element inhibits the synthesis of chlorophyll by inactivating the enzyme catalyzing the reaction of chlorophyll synthesis. The time of operation of this element varies from plant to plant in accordance with the cellular environment. For the albino seedlings, the inhibiting element functions when the seeds just start to germinate. For the Class A plants this element starts to function later than that of the albino seedlings. Likewise, the element of Class B plants acts later than that of Class A plants, and that of Class C, later than that of Class B, and that of Class D, later than that of Class C.

Y. C. Ting

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1. Progress towards perennial Zea diploids.

Experiments were reported last year which show that one can make steady progress in increasing the perennial expression of maize-teosinte derivatives at the 4N level by the simple breeding technique of repeated mass selection for perennialism. Thus at the 50% maize level, it has been possible to increase the incidence of perennial segregates from an original level of about 0.3 to 0.75 in only 4 cycles of selection. Similarly, the production of basal branches, an attribute of perennialism, was increased from about 4.4 per plant to about 10.0. At the 75% maize level, only two generations of selection have increased the incidence of perennial segregates from 0.0 to about 0.35. Unhappily, either genetic or agronomic investigations of perennialism are difficult, if not meaningless, at the 4N level.

The situation is entirely different when working at the diploid level. As outlined, one can easily obtain large populations of maize-perennial teosinte diploids by producing the F_1 triploid generation, backcrossing this triploid to maize, and then intercrossing the resultant array of aneuploids. The chromosome number is rapidly stabilized at 20 because of gametophyte selection for euploidy. Due to the high degree of preferential pairing in the F_1 triploid, the first post triploid generation carries a high theoretical proportion of perennial chromatin, somewhere between 40 and 45%, depending upon certain assumptions.

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*Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

Since 4 separate experiments, involving now thousands of putatively perennial segregates derived by as many as 5 successive generations of intensive selection for perennialism or perennial attributes, have completely failed to yield any perennial diploid segregates at all, it is clear that diploidy imposes special difficulties upon the task of reconstructing a genotype which might confer perennialism. One does not even make progress in increasing the expression of perennial attributes, such as basal branch production. Thus in the 2nd post triploid generation, the number of basal branches per plant was 3.3. In the 3rd generation it was 2.9, and in the 4th, 3.1. At the same time production of apparently normal pollen by diploids increased from 88% to 95%, while the net viability of seeds and seedlings increased from 66% to 85%. (The pollen studies noted here represent scores taken from side-by-side outdoor-grown samples. The studies reported last year [MNL 37:8-11] were from greenhouse collections, and were extremely erratic by comparison. It is suggested that the data from outdoor collections should be regarded as valid.) These disappointing experiences with diploids can be explained if perennial teosinte, during its evolution as a tetraploid, has accumulated many mutations, such that if one attempts to rediploidize segments carrying them, an essential function may not be represented, and such "sheltered" tetraploid factors then act as haploid gametophyte or diploid sporophyte lethals. If the loci from perennial teosinte which confer perennialism are linked with such lethals, they will be eliminated inexorably in diploids. Moreover, while the phenotype of the triploid F_1, and to a lesser extent, the first post triploid generation, look decidedly intermediate between maize and teosinte, the advanced post triploid generations become, in spite of contrarywise selection pressure, extremely maize-like.

It has been possible to rule out the idea that one fails to recover perennialism in diploids because of the absence of tetrasomy by directly doubling with colchicine several of these advanced generation diploids. Thus of nine confirmed and 12 probably newly redoubled tetraploids, none showed the slightest degree of perennialism or increase in the expression of perennial attributes, and all died at the same time as their nondoubled sib mates.

Although an exhaustive study has not yet been made, several diploids having perennial teosinte segments have been examined at pachynema. Since no large, classical rearrangements have been found so far, it seems certain that if they exist, they are certainly not generalized, and cannot explain the general elimination of teosinte characters. The weight of evidence is therefore that one has to deal with subvisible, or "genetic" factors.

As reported last year, only one breakthrough in this otherwise bleak picture has been found. One plant, a trisomic, found in a first post triploid generation, "Clone 85-12," was obtained which although very weak and extremely difficult to maintain, did appear to be perennial. Although it is so far completely self sterile, it does produce pollen about 5% of which stains with IKI. Some of this pollen was used to effect fertilization of perennial teosinte ovules. Of the triploids thus obtained, one was fertilized again by 85-12 pollen. Of the resulting aneuploids, two were saved, with 22 and 24 chromosomes respectively.

These were grown in greenhouse culture, and upon the demise of the first culms, basal and axillary began to produce offsets which could be separated and thence develop into fully normal plants which again produced many fully totipotent basal branches. It is sufficient to say that the 22 chromosome derivative, which has been retained as "Clone A," has a vigorous, fully perennial phenotype and it can be cloned with ease to any desired number of propagules. Out-of-doors it is extremely vigorous, and its basal branches become at least pseudo-rhizomatous. Although it produces about 70% staining pollen, and a multitude of vigorous, 4-rowed distichous ears, it is also nearly self sterile. Only 16 seeds have been obtained from numerous pollinations. Of these, 10 germinated successfully. Two proved to be albinos, while the remaining eight grew to maturity. Three were trisomics, and five were euploids. Even though five of the eight were grown out-of-doors, none showed any perennial tendency or attribute. No basal branches at all were produced under the same conditions in which the sexual parent, Clone A, formed as many as 16 basal branches during one growing season and was still growing strongly when freezing temperatures brought the season to a close. The one ear produced by the sexual derivatives of Clone A was polystichous and the plants were otherwise maize-like. This appears to be the best evidence that loci from perennial teosinte are selectively eliminated in diploids.

While Clone A and 85-12 are nearly self sterile, they may be crossed together, and viable progeny obtained. Of 18 seeds so obtained, 10 grew successfully; one of these appears to be a perennial whose root tips yield perfectly repeatable counts of both 20 and 21 chromosomes.

Clone A may also be crossed with perennial teosinte, and the resulting triploids crossed back to Clone A. From the ensuing aneuploids, one can select those with the lower chromosome numbers for further work. Unlike previous experiences, all such populations at the near-diploid level which were derived ultimately through 85-12 (by way of Clone A) are essentially perennial populations with a few exceptional annuals. Even though as a group these also tend to be strongly self sterile, a few seeds can be obtained by sib pollination.

In summary, the study of pollen abortion, seedling and seed lethality, and inability to transmit teosinte characteristics, including perennialism, at the diploid level or near diploid level, support the idea that perennialism-conferring loci in teosinte are linked with diploid-lethal factors which make perennialism difficult to recover in diploids. Clone 85-12 may have arisen by means of a fortuitous crossover such that a key perennialism locus may be brought at least to a diploid or near-diploid background. The evidence that 85-12 may represent such a specific event is that near diploids derived through it are not only unique in being essentially all perennial, but all possess, in addition, a photoperiodic response such that on long days only terminal inflorescences are produced, while in a short day regime, both terminal and lateral inflorescences appear. In either regime, however, at least Clone A continues to produce totipotent basal branches, and therefore is still perennial even in a short day regime.

Because of the perennial nature of the material, Clone A now represents an easy means to produce any number of additional perennial diploids

and near diploids that could be desired. If the remaining task is essentially one of obtaining fortuitous crossovers in order to place perennialism-conferring loci into maize chromosomes, one then appears to be in the position of having the means by which any degree of pressure may be applied to the problem that it may require.

D. L. Shaver

2. A simple mechanical method of inducing tetraploidy.

Heat shock and colchicine treatments, historically speaking, have yielded a very low percentage of success in producing maize tetraploids. Recently, genetic methods of introducing new chromosomes from diploids into existing tetraploids have been proposed and come into vogue. These methods, involving the genes elongate, asynaptic, and ameiotic, however, require several generations to bring the inducing gene into the background one wishes to tetraploidize before one succeeds in deriving the desired diploid gametes. In an agronomic situation, one eventually faces the problem of linkage of the inducing genes to unfavorable factors. Moreover, the inducing gene itself is introduced into the new tetraploid, where it is then undesirable.

Two experiments have shown that one can easily introduce desired chromosomes from diploids into existing tetraploids by the straightforward procedure of crossing a 4N female by the desired diploid. If the resulting shriveled seeds are dissected and the embryo proper is removed and shallowly planted in moist soil, or other situation where the developing embryo can soon persist on its own photosynthate, no difficulty is encountered in growing these triploid embryos. (The dissection procedure was suggested by Ellen Dempsey.)

Triploids produce much pollen in the greenhouse, or out-of-doors on Long Island, but they may not if grown outside in less favorable environments. One can collect this pollen in conventional pollen bags, or by shaking it directly off of the tassels, and then straining it through a stack of U.S. Standard Sieves (W. S. Tyler Co., Cleveland 14, Ohio). A top screen with a mesh size of 149 microns removes clumped pollen and loose anthers. The next screen with a mesh size of 125 microns catches a small proportion of the very largest pollen grains from triploids (and tetraploids). No diploid has yet been found to produce pollen grains which "stay" in this screen. The next screen with 105 micron mesh catches most of the large grains. These were discarded in this experiment. The next screen with 74 micron mesh catches most of the viable small grains, while the last, 53 micron mesh, seems to catch only aborted or dried grains.

The following results were obtained by sibbing a population of triploids with the 74 and 125 micron pollen fractions:

74 Micron Pollen		125 Micron Pollen	
Chrom. No.	No. of Individuals	Chrom. No.	No. of Individuals
20	1	23	1
21	1	30	2
22	1	33	3
23	4	34	2
24	2	36	5
25	5	37	4
26	2	38	4
27	1	39	1
28	1		
29	4		
30	1		
32	1		

It is evident that the screening procedure results in two nearly discrete populations. If one sibs among the 36-39 chromosome individuals, one obtains a new population having many individuals with 40 or more chromosomes. The chromatin of such newly constituted tetraploids should derive approximately 45% from the original diploid parent.

D. L. Shaver

3. A successful colchicine method for Zea.

In producing tetraploids of Zea, the "target" for colchicine action is the undifferentiated meristem of the shoot apex. Probably because of complications involved in the various techniques of getting colchicine through the several multicell-layered embryonic leaves which ensheath the apex, production of tetraploids by means of this mitosis inhibitor has with few exceptions been disappointingly difficult.

Faced with the necessity of deriving tetraploids from a 2N population directly, a method of applying colchicine proposed by Dr. L. F. Randolph in one of the early Maize Genetics Cooperation News Letters was used in conjunction with colchicine techniques suggested by Dr. J. Van't Hof at Brookhaven. Seeds of maize were germinated until the primary root reached about $1\frac{1}{2}$" in length. About $\frac{1}{4}$ to $\frac{1}{2}$ inch of the root tip was cut off under distilled water to expose the large, empty xylem vessels of the differentiated portion of the root. The seedling was then suspended on hardware cloth above an aqueous colchicine solution in such a way that only the decapitated root tip was immersed, and the young seedling therefore was forced to supply its entire transpiration stream through the decapitated root.

Effective colchicine concentrations were determined by dissecting out the shoot apex, smearing, and determining the proportion of colchicine metaphases present. In this manner, proper concentrations were determined for the specific material used. Possibly another strain or variety could have a different optimal level. In this work, one chose the lowest level at which all observed metaphases were "arrested." It was found that there is an accumulation of colchicine effects from one treatment

period to the next. Thus, 0.025% colchicine did not produce 100% arrested metaphases during the first 24-hour treatment, but during the second 24-hour treatment, all were arrested. The practice was eventually adopted of using a higher concentration for the first cycle of treatment than during succeeding ones.

In the first experiment, 500 maize seedlings were given three 24-hour treatments in 0.05% colchicine, interrupted by 24-hour recovery periods in distilled water. Of the 16 survivors of this treatment, 4 were confirmed tetraploids (their pollen produced full seed sets when tested upon an established tetraploid), and 2 were probably tetraploid (produced pollen, some of which "stayed" on a 125 micron screen). In the second experiment, 500 maize seedlings were treated for the first 24 hours in 0.05% colchicine, allowed to recover for 24 hours, and then treated for an additional 24 hours with 0.025% colchicine. Of 152 survivors of this treatment, 5 were confirmed tetraploids and 10 were probables. There appeared to be only one case of 2N-4N sectoring. The rest appeared to be cases where the entire shoot apex had become tetraploidized.

Dr. J. Van't Hof's "colchicine bible": Use colchicine from a source where the quality of the chemical is consistent, e.g., L. Light & Co., Ltd., Colnbrook, England, and store in the dark in a desiccator over $CaCl_2$. Always make up fresh solutions immediately before use. Always establish optimal levels for each new variety by experimental dissections and fixations.

D. L. Shaver

4. A mechanical method of classifying large numbers of seeds for waxy.

The work of O. E. Nelson of establishing the map characteristics of the wx cistron of maize has established an exceedingly useful system for the study of the nature of mutation in a higher organism. However, in the initial steps of producing and screening for mutations produced by radiations or chemical mutagens, one is faced with a Herculean task of physically classifying maize kernels for wx in sufficient number that one can realistically expect to recover a meaningful number of induced mutants.

In order to speed this task, a Red Devil paint shaker model 30 was obtained. The sides of two one-gallon paint cans were lined with floor sanding paper with a no. 2 grit (Behr-Manning Co., Troy, N.Y.). The machine was connected to a standard greenhouse timing switch so that runs could be made while the machine was unattended. It was found that this machine could easily scarify 2,000 grams or ca. 11,000 kernels at one time. Doubtless it could be adapted to larger containers and this could be greatly increased. It was necessary to perforate the ends and sides of the cans with numerous small holes to allow the dust to sift out, and thus avoid "filling" of the sandpaper. Frictional heating of the can and its contents could be avoided by setting the timing switch to 15 minutes on, 15 minutes off, etc. Two and one half to three hours running time was sufficient to scarify the seeds for wx classification.

As a result of this scarification treatment, the pericarp and aleurone layers were uniformly worn away, especially on the edges and tips of the kernels, while a recessed "germ" or embryo usually escaped damage. Such seeds could then be dipped in weak IKI solution, and rapidly spread upon an absorbent material to dry while being classified for wx. Searching for mutant kernels was as simple as looking for a red-brown ball among many black ones. Small batches were stained at a time, after rinsing away the loose dust, and immediately searched for mutants since the contrast between wx and Wx kernels is best just after staining.

It should be emphasized that the usefulness of the method depends upon having kernels in which the germ is recessed. This was the case in well-pollinated ears of M14, but is not true of many other inbred lines. From a practical point of view, even with M14 the method will be found unsatisfactory unless full sets of seed are obtained so that resulting kernels will be flat instead of round. Perhaps isolation-detasseling production of subject kernels is the most practical way of obtaining the needed numbers and quality of seed.

D. L. Shaver

5. id maize.

Several attempts have been made to mate id/id plants carrying a newly found id gene (Shaver, MNL 31:94) with id/id plants having the classical C30 id allele (Galinat and Naylor, AJB 38:38–47). Various manipulations of photoperiod have succeeded in inducing flowering, but the small ears produced have always been barren. Since it seemed impossible to mate homozygous id plants, an alternative procedure was employed, that of mating normal plants in segregating families from the two id sources. Of 16 ear progenies so obtained and grown in Florida this winter, 7 segregated for the id phenotype, indicating that the id genes from the two sources are allelic. It is interesting that the id/id plants, planted November 10, 1963, were not induced to flower as of January 28, 1964, in spite of the fact that they were grown in a regime which induces teosinte (the interval between sunrise and sunset on December 21 being only $10\frac{1}{2}$ hours). This experience agrees with observations in Florida a year ago. Homozygous id/id plants, seeded October 15, 1962, were not induced as of March 10, 1963, at Princeton, Florida.

D. L. Shaver

6. Relative biological efficiency of monoenergetic fast neutrons on chromosomes in maize.

Investigations on the relative biological effectiveness (RBE) of densely ionizing radiations (with high LET, rate of linear energy transfer) are of importance in both fundamental and applied radiobiology. The difficulty in determining RBE on the basis of chromosomal exchanges or 2-break aberrations is that the dose-response curves differ for radiations of different LET and dose rate. Maize seeds of Yg_2/yg_2 genotype were used to study the RBE of fast neutrons vs. X rays.

The maize material used in these experiments has the advantage for RBE studies of yielding a basically first order dose-response curve ($Y = \alpha + \beta D$) with low (X rays) as well as with high (fast neutron) LET radiations. The frequency of yellow-green ($\underline{yg_2}$) sectors in leaves 3, 4 and 5 of young plants grown from irradiated $\underline{Yg_2}/\underline{yg_2}$ seeds served as a quantitative measure of response. The mutant sectors are believed to be due mostly to simple chromosome breakage and deletion. An exposure apparatus was used which produced essentially equal dose rates in five rings of seeds placed so as to intercept neutrons of 0.43, 0.65, 1.00, 1.50 and 1.80 MeV. Dose average LET values for these energies are 72, 67, 58, 57.5 and 42.5 kev/μ, respectively.

Two experiments were performed at dosages that gave responses which were linear, below saturation levels, and overlapping in range for X rays and neutrons. These ranges in dosages were 32.8 to 126.4 rads of neutrons and 1,500 to 15,600 rads of 250 kvp X rays.

RBE values, calculated from relative slopes (α) of linear regression lines for N and X, ranged from 42 to 135 (average 78) in Experiment 1 and from 48 to 106 (average 68) in Experiment 2. Monoenergetic fast neutrons of 0.43 MeV were the most efficient in producing $\underline{yg_2}$ sectors as shown by the yield of sectors per krad and highest RBE values.

The RBE values obtained in these experiments are higher than commonly reported and in the neighborhood of those found by Neary *et al.* (Int. J. Rad. Biol. 6:127) for plant chromosomes when the dose-squared term of low LET radiation response is minimized. With regard to maximum permissible levels of radiation for man, these results suggest the alternatives that either chromosome breaks in plants have a much higher RBE than comparable reactions in man and need not be considered, or that the problem of chromosome damage *per se* in human tissues be reexamined after exposure to high LET radiations and/or low LET radiations at low doses or dose rates.

H. H. Smith

7. Relative biological efficiency of muons and π^- mesons.

Until recently meson beams of sufficient intensity for cytogenetic studies have not been available. The Alternating Gradient Synchotron (AGS) at the Brookhaven National Laboratory now produces almost pure π^- meson and muon (μ^- particle) beams suitable for use in biological experiments. The mesons were generated in the AGS by bombarding a beryllium target with highly accelerated protons of about 28 BeV energy.

Dormant seeds of $\underline{Yg_2}/\underline{yg_2}$ maize were exposed in two experiments: (1) to 1,275 rads of nearly pure muons; and (2) to 3,360 rads, comprising about 2,060 rads of muons and about 1,300 rads of π^- mesons. To compare these effects with those of better known radiations, seeds of the same material were treated with 250 kVp X rays. The frequency of $\underline{yg_2}$ sectors was scored in leaves, 3, 4, 5 and 6 of the seedlings.

The muons were found to have about the same mutational efficiency as the X rays (average RBE = 1.01). The calculated results for π^- mesons indicated a relative mutational efficiency of about 3 (average RBE = 3.16). The greater effectiveness of π^- mesons is considered to be due to the energy deposition through strong interactions of these particles with protons and neutrons of the atomic nuclei. These interactions result in nuclear disintegrations producing stars and showers of lightly ionizing and heavily ionizing secondary particles such as protons, alpha particles and heavier nuclei. Since these higher LET tracks are much more efficient than X ray or other low LET radiations (such as muons) in breaking maize chromosomes, π^- mesons would be expected to have the higher RBE observed.

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H. H. Smith
R. G. Woodley
A. Maschke

8. Effects of X-irradiation on intracistron recombination at the Wx/wx locus.

Research work by O. E. Nelson (Science 130:794) has demonstrated intracistron recombination at the waxy locus in maize. Also, Roman (CSHSQB, 1958) has demonstrated an increased rate of intragenic recombination in yeast by using ultraviolet irradiation. This research was conducted to determine if X-irradiation would influence the rate of intracistron recombination in the waxy locus in maize.

The present authors are grateful to Dr. Oliver E. Nelson for providing the seed stocks described in this research. The procedures used for the pollen assay are essentially those published by Nelson.

Waxy alleles, wx^C, wx^{90} and wx^{H21} were available in the homozygous condition and all possible combinations among the parents. The wx^C and wx^{H21} stocks had been backcrossed six times, and wx^{90} three times, to inbred M14.

An acute dose of 200 r of X-irradiation was applied to each maize plant during meiosis. The plants were irradiated in air with a G.E. Maxitron 250 X-ray machine (30 ma, 250 kv, 1 mm Al filter at 50 cm).

At the time of irradiation sporocytes were collected. These were later scored as to the stage of meiosis. The area of collection was marked with ink in an attempt to simulate a "synchronous" system and to determine the stage(s) of meiosis at which the tassel was irradiated. The marked tassel area was used as the center of the target area and pollen used in the assay was taken from this area. Tassels to be used in the pollen assay were collected before anthesis and stored in 70% alcohol.

Nelson (1959) states that if the frequency of black staining (Wx) pollen grains in the population from a cross between two mutants is significantly higher than the frequency in either parental stock, this would indicate that the two mutations occupy different sites within the region.

The figure for each stock presumably could include back mutation, suppressor mutation and contamination from wind-blown pollen.

In our research we used a similar approach. Parents and the heteroallelic crosses were irradiated and in addition, unirradiated parents and heteroallelic crosses were used as controls. Half the Wx frequency of each control parent (spontaneous back mutation, etc.) was subtracted from the Wx frequency of the control heteroallelic material. Also half the Wx frequency of each irradiated parent (spontaneous and induced back mutation, etc.) was subtracted from the Wx frequency of the irradiated heteroallelic material. Then the corrected Wx frequency of the control heteroallelic material was compared with the corrected Wx frequency of the irradiated heteroallelic material (Z test).

The data in Table 1, for the irradiated and control parents and control heteroallelic crosses, are bulked from several plants. However, the data for each irradiated heteroallelic cross are from one plant.

Our results, Table 1, with the control parents and the heteroallelic material gave a lower rate of back mutation and of spontaneous recombination rate than reported by Nelson (1959). In his research, Nelson points out that the residual genotype may affect the recombinational process within the waxy locus, and this may apply here.

Some researchers assume that genetic recombination takes place in zygonema and pachynema, i.e. when the chromosomes are paired. Pontecorvo (1958) states that crossing over may take place well before meiotic prophase. It may be assumed that the stage in which recombination takes place is the critical stage to affect recombination, but this may not necessarily be so. In an attempt to elucidate this problem the stages of meiosis at the time of irradiation were recorded.

Irradiation apparently increased the rate of recombination in each treated heteroallelic cross, except one (Table 1); however, statistical significance was obtained for only three of them.

Table 1
Parents and Heteroallelic Crosses

	Control		Irradiated	
	Est. no. of microspores x 10^3	$\bar{X}$ no. Wx x 10^{-5} $\pm s_{\bar{x}}$	Est. no. of microspores x 10^3	$\bar{X}$ no. Wx x 10^{-5} $\pm s_{\bar{x}}$
wx^C	428	0.00 ± 0.00	125	0.80 ± 0.80
wx^{90}	732	0.27 ± 0.19	530	1.70 ± 0.57
wx^{H21}	410	0.24 ± 0.24	92	3.26 ± 1.88
wx^C x wx^{90}-1	644	28.40 ± 2.10	82	72.67 ± 9.38*
wx^C x wx^{90}-2	644	28.40 ± 2.10	168	18.48 ± 3.32
wx^C x wx^{H21}-1	335	27.77 ± 2.88	99	33.27 ± 5.79
wx^C x wx^{H21}-2	335	27.77 ± 2.88	50	41.81 ± 9.12
wx^{90} x wx^{H21}-1	235	1.28 ± 0.74	160	13.14 ± 2.87*
wx^{90} x wx^{H21}-2	235	1.28 ± 0.74	97	22.57 ± 4.81*

*Exceeds 1% level of significance.

With respect to the relationship between an enhancement of recombination frequency and stage of meiosis it was found that: (1) the heteroallelic cross wx^{C} x wx^{90}-1 gave a significant and higher increase in recombination frequency, and had a higher proportion of cells in pachynema (Table 2) than wx^{C} x wx^{90}-2. (2) In the heteroallelic cross wx^{C} x wx^{H21} the same relationship was found between recombination frequency and stage of meiosis. However, the increases in recombination were not significant and the proportion of stages in pachynema was lower than in either of the wx^{C} x wx^{90} crosses. (3) In the cross wx^{90} x wx^{H21} the relationship between recombination frequency and proportion of cells in pachynema is evidently not the same as above in that, although both plants gave a significant increase in recombination, the plant with the lower proportion of cells in pachynema gave the higher recombination frequency.

Table 2
Stages of Meiosis (Percentage) of Irradiated Heteroallelic Crosses

	Pre-pachytene	Pachytene	Diplotene	Diakinesis	Metaphase	Anaphase	Telophase
wx^{C} x wx^{90}-1	30.8	69.2	--	--	--	--	--
wx^{C} x wx^{90}-2	--	26.9	59.8	13.3	--	--	--
wx^{C} x wx^{H21}-1	--	--	--	--	72.3	16.1	11.6
wx^{C} x wx^{H21}-2	--	19.0	70.3	5.5	3.2	2.0	--
wx^{90} x wx^{H21}-1	21.3	39.9	23.9	14.9	--	--	--
wx^{90} x wx^{H21}-2	--	2.8	54.2	38.5	4.5	--	--

The wx^{90} and wx^{H21} alleles lie closest together. It is with the heteroallelic cross between these alleles that irradiation produced the greatest increase in genetic recombination.

There is some indication from these studies that irradiation in prepachynema and pachynema may be critical stages for stimulating recombination. However, it is realized that maize may not be the most desirable organism to determine this.

R. W. Briggs
H. H. Smith

9. Experiments with ethyl methane sulfonate (EMS) and radiation.

Two experiments have been conducted to study the mechanism of mutation induction by EMS.

I. The possibility of point mutation or inactivation of a small segment of chromosome was tested using four endosperm markers located on the short arm of chromosome 9. Seeds, homozygous for I Sh Bz Wx, were soaked in 0.1 M or 0.05 M aqueous solution of EMS at 25° C for 2 or 5 hours, without buffer, following 24 hours of presoaking in 25° C running tap water. The plants from the treated seeds showed markedly reduced growth, but survived well in the field. They were used as male and as female parents with the recessive tester, C sh bz wx/C sh bz wx. The results are summarized in Table 1. As the plants from the treated seeds showed

Table 1

Loss of Expression of Endosperm Dominant Markers. I Sh Bz Wx Homozygous Seeds Were Presoaked in Water, Soaked in EMS Solution and Crossed with the Recessive C sh bz wx Homozygous Tester. Culture 627 Is the Tester Stock and Those with the Letter E Are the Treated Stocks.

Female	Male	Treatment	No. tested		No. of mutations	Kind of mutation	No. of seeds mutated
			plants	cobs			
627	62E1	Control	27	40	0	-	0
62E1	627	Control	91	119	0	-	0
627	62E2,9,10	EMS soaking*	163	163	2	wx;sh	4 wx; 2 sh
62E2,9,10	627	EMS soaking*	238	242	2	wx;wx	31 wx;13 wx

* 62E2 = 0.1 M EMS for 2 hours, responsible for wx;sh mutations.

62E9 = 0.05 M EMS for 2 hours, responsible for wx;wx mutations.

62E10 = 0.05 M EMS for 5 1/4 hours, no mutations found.

chimeric mutated or altered tissue, in both tassels and ears, the following remarks may help in evaluating the mutagenic effect of EMS. When the treated plants were used as males, pollen was collected from the whole tassel of each plant, and sometimes pollinated to a few tester ears, so the number of plants tested should be emphasized rather than the number of cobs or seeds. On the other hand, when the treated plants were used as females, each cob may be regarded as an independent unit, even though more than one was obtained from a single plant. Table 1 shows that four independent mutations or losses of dominant markers were obtained, three wx and one sh. In all four cases, the possibility of the deletion including bz, which is located between sh and wx, cannot be excluded because of a color inhibitor I, but the losses of expression of only one proximal marker, Wx or Sh, may be noteworthy.

EMS was also applied to seedlings as drops in the opening of the young leaves split vertically with a razor blade, but this method was not as successful as the soaking method.

The nucleoside analogue, 5-bromodeoxyuridine, with or without 5-fluorodeoxyuridine, was also applied by soaking seeds, but at the concentration of 5×10^{-4} M BUdR, neither reduction of growth nor mutation was seen.

II. Somatic mutation in Yg_2/yg_2 heterozygotes was tested to determine the dose-response relationship of mutagenic agents. This system, in which losses of the dominant green marker (Yg_2) are expressed as small yellow-green sectors in leaves, worked well with lower dosages of the mutagen tested. The dosage which gave about 20% reduction of seedling height induced maximum numbers of sectors for reliable scoring. For scoring purposes both leaf 3 and leaf 4 of greenhouse-grown plants were used, but only the data from leaf 3, which gave more sectors than leaf 4, will be reported here.

A. X rays. Four combinations of Yg_2 and yg_2 were tested for response to X-irradiation after 24 hours presoaking in running tap water at 25° C. Curves were constructed from the data given in Table 2. Yg_2 homozygote showed a two-hit type of response (number of sectors increased quadratically with dose). This is as expected because there are two alleles of Yg_2 or of other dominant genes governing normal green chlorophyll production. The two reciprocals of heterozygotes showed a very rapid increase of sector frequencies with an increase in X-ray dose. Differences observed between the reciprocals might be due to a difference in hydration during presoaking. The response to increased radiation dose of these two heterozygotes seemed to be more linear than quadratic. The recessive yg_2 homozygote also showed yellowish sectors (lighter than the background tissue), but the yield of sectors was insufficient to interpret in terms of the type of response curve produced.

B. Fast neutrons. As a type of densely ionizing radiation, fast neutron irradiation of dry seeds of the four different stocks was tried, but most of the doses used were too high for reliable scoring of sector frequency. With the lowest dose, 500 rads, the frequency of yellow-green sectors was highest in both heterozygotes. Yg_2 homozygotes showed fewer sectors and the yg_2 homozygote showed least sectors among the four stocks.

Table 3

Frequency of Yellow-green Sectors in Leaf 3. Seeds Were Presoaked in Water for 24 Hours at 27° C and Soaked in EMS solution for 5 Hours at 27° C. Fifteen Seeds Were Used in Each Lot.

EMS Concentration (M/l)		0	0.005	0.01	0.02	0.03	0.04	0.05	0.06
$Yg_2,Y/yg_2,y$	1.	0	2.067	6.333	11.000	11.643	17.000	(13.00)	(15.50)
$Yg_2,Y/yg_2,y$	2.	0.071	1.786	5.400	10.231	11.154	(19.000)	(6.00)	
$Yg_2,Y/Yg_2,Y$	1.	0	0.533	1.539	2.667	5.083	(5.625)		
$Yg_2,Y/Yg_2,Y$	2.	0.067	1.071	1.733	2.167	6.000	(6.000)		

( ): Less than 70% of leaves scorable.

Table 2
Frequency of Yellow-Green Sectors in Leaf 3*

Genotype	X-ray dose, r						
	0	250	500	750	1,000	1,500	2,000
Yg_2/Yg_2	0.00	0.032	0.158	0.444	1.200	(1.692)	(1.214)
Yg_2/yg_2	0.050	1.258	2.211	1.722	(1.667)	(1.875)	(1.000)
yg_2/Yg_2	0.050	0.419	0.850	1.631	2.313	2.882	2.400
yg_2/yg_2	0.00	0.097	0.100	0.111	0.250	0.444	0.133
No. of seed in each lot	20	32	20	20	20	20	20

*Seeds were presoaked in running tap water at 25° C for 24 hours, then X-rayed.
( ): Less than 70% of leaves scorable.

C. EMS. In this experiment, only Yg_2, Y/Yg_2, Y and Yg_2, Y/yg_2, y were used. These two stocks of seeds were very alike in appearance, except that the color of endosperm was slightly lighter yellow in the heterozygotes (YYy) than in the homozygote (YYY). Seeds were soaked in 0.005 M to 0.06 M fresh EMS solutions for five hours at 27° C after 24 hours of presoaking in oxygenated deionized water at 27° C. Results are shown in Table 3. The yield of yellow-green sectors in the heterozygotes was about twice that in the homozygous Yg_2, and 5 to 7 times higher than in the X-ray treatment at their maximum yields. With EMS, however, the response of yield relative to the concentration was approximately linear in both stocks. Although many variables accompanied by soaking treatment must be investigated for proper interpretation, the result is interesting for the study of the mutation-inducing mechanisms of EMS.

D. Temperature and EMS. The effect of temperature during post incubation of EMS-treated seeds was tested with a yg_2 heterozygote. Seeds were soaked in 0.025 M EMS solution for 48 hours at 3° C, after presoaking as described above in the X-ray experiment. Seeds were rinsed with cold (3° C) water and incubated in the dark at several different temperatures ranging from 3° C to 33° C. Yield of the sectors increased at the higher temperatures tested, up to about twice as much as at the low temperature.

E. Recent mutations with EMS. A partial survey of the 1963 harvest from EMS-treated material has revealed 4 mutations in chromosome 9 marker genes. The treatments consisted in soaking seeds of a homozygous dominant stock for 24 hours at 27° C in water, then in 0.05 M EMS (5 hours at 27° C or 48 hours at 3° C). The silks of plants developed from EMS-treated seeds were pollinated with a multiple recessive stock. Four independent mutations were found on 4 different ears in a total of 181 cobs scored to date. These mutations were 1C, 1sh, and 2wx.

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1. A genetic factor for blotched leaves showing variable inheritance.

A factor causing leaf blotching, which may be the same as that described by Emerson (Cornell Memoir 70, 1923) has been under study for several years. It behaves as a recessive gene, but with peculiarities. It appeared in an M23 inbred line of sweetcorn from Wisconsin; it causes internal breakdown of scattered areas of leaf tissue during late preflowering. The blotching does not appear on the first 3 to 4 leaves, and has not been detected on leaf sheathes or on culms. It appears not to be due to fungi or bacteria, nor to be spread by insect vectors. Symptoms are clear, but show many grades of severity within selfed or sibbed families. Blotching is completely absent in F_1 in crosses with other lines, and segregates in convincing 3:1 ratios in F_2 from such crosses. Pollen of blotched plants is normal, and full-set ears are obtained. Linkage has not been established.

Within the M23 line, progenies from selfing or sibbing of severely affected plants remain severely affected, on the average; but progenies from inbreeding of phenotypically normal or slightly affected plants regularly show a buildup toward severe symptoms. Selection for normality has thus usually, although apparently not always, failed to yield permanently normal sublines. Table 1 shows the correlation between parents and progeny from sibs and a self within the inbred line in one year. Grades of blotching are: 0, none; 1, very slight; 2, slight; 3,4, moderate and severe (combined).

Table 1
Leaf Blotching in Sibbed or Selfed Progenies of Inbred M23

Plant No.	Parent plants (Blotch grade)				Number of progeny plants in grades (Blotch grade)			
	0	1	2	3,4	0	1	2	3,4
7 x 15				x			6	35
8 x 26				x				20
6 x 9		x		x		1	18	8
25 x 27	x		x		9		8	24
12 ⊗		x			32	4	6	

The fairly orthodox behavior of F_1 and F_2 after outcrossing is shown in Table 2.

Table 2
Blotching in Progenies From Outcrossing With M23 Inbred

Outcross parent	No. of plants with blotching in F_1	No. of plants in F_2, in grades (Blotch grade) 0	1	2	3,4	% blotched plants in F_2
Me 41	0/23	76			20	21
M 3722	0/50	40			11	22
P 39A	0/50	17		1	3	19
Ia 2000	0/48	32	2	2	8	27
Lu. Hill	0/46	45	1	1	17	30

Data on selection in advanced generations, within the inbred line, are shown in Table 3. In the left half of the table, selection of phenotypically normal plants did not fix a normal line, and when selection was relaxed (in F_7) the progeny showed more severe symptoms. In the right half of the table, selection within higher grades of blotching led to severe blotching. In F_6 a sib between a 0 and a 3,4 grade failed to break the trend toward severe symptoms. The asterisks indicate the grade of the plant or plants used to produce the next generation. Two asterisks mean a sib; one, a self.

Table 3
Selection in Advanced Generations of M23 Sublines

Generation	Principal Selection Directed Toward: Normal (Blotch grade) 0	1	2	3,4	Severe (Blotch grade) 0	1	2	3,4	
F_2	6	8**	4	0	0	0	3	27**	
F_3	12*	0	4	2	0	0	6	35*	
F_4	6*	4	14	26	0	0	0	48	
					2	0	9*	32	Families not derived from previous starred plant
F_5	9*	6	5	0	0	3	4	30*	
F_6	2*	6	18	5	0	0	3	21	
					2*	6	18	5*	
F_7	2	9*	7	12	0	1	4*	18	
F_8	1	2	3	18	0	0	0	15	

In two instances (not shown in Table 3) normal sublines appear to have been obtained from selfs in affected families. These have remained normal for 3 to 4 years. The behavior of this blotch factor may be explainable by a combination of interactions involving modifiers, incomplete dominance, and/or variable penetrance, but it may be a function of some form of paramutation or gene conversion. Perhaps a less complete or more variable type of conversion is taking place here than in some other systems.

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1. Partial desynaptic maize.

In one backcross progeny, (0_7 x In 3a) x In 3a, segregation for normal and partially sterile (59% ovule abortion, on average) plants was observed. Examination of the microsporocytes of the partially sterile plants revealed that the sterility is due to the premature separation (desynapsis) of several homologous chromosomes.

The partial desynaptic plants had been crossed with the normal testers. Meiosis and fertility of all the F_1 plants were normal. Results of segregation for normal and partial desynaptic plants of eleven F_2 progenies (Table 1) indicate that partial desynapsis is due to a recessive gene.

Table 1
Segregation in the F_2 Progenies Resulting From Crosses Between the Normal and Partial Desynaptic Plants

Progeny No.	No. of normal plants	No. of partial desynaptic plants	Total
8008	46	11	57
8010	141	41	182
9021	143	50	193
9022#	142	5	147
9023	106	25	131
9024	63	15	78
9025	143	38	181
9026	191	56	247
9027	250	80	330
9056	92	29	121
9057#	156	25	181

deviates from 3:1 ratio.

In order to test whether or not the new gene was allelic to the asynaptic mutant, as, crosses were made between the partial desynaptic and asynaptic plants. All the F_1 plants resulting from such crosses were normal. The F_2 plants were classified into normal and sterile ones according to the seed set on the mature ears. If the new and asynaptic genes are not allelic, a ratio of 9 normal to 7 sterile plants should be expected. This is true in 11 out of 14 F_2 progenies tested (Table 2).

Besides desynapsis, other abnormalities such as the formation of a plasmodial mass, uncoiling of chromosomes, chromosome breakage, etc. which have been described in asynaptic maize can also be observed in the microsporocytes of the partial desynaptic plants. However, the partial desynaptic maize differs from the asynaptic one in several aspects which are briefly described as follows:

For the asynaptic maize both Beadle (1930, 1933) and Miller (1963) have reported that the intensity of asynapsis varies from complete to very

Table 2
Segregation for Normal and Sterile Plants (Asynaptic and Desynaptic) in the F_2 Progenies Resulting From Crosses Between Partial Desynaptic and Asynaptic Plants

Progeny No.	No. of normal plants	No. of sterile plants	Total
9028	161	98	259
9058	37	25	62
9059	40	29	69
9061	30	25	55
9062#	49	11	60
9063	62	50	112
9065	39	22	61
9066	30	19	49
9067	40	31	71
9068	20	21	41
9069	17	13	30
9070#	14	24	38
9071#	50	23	73
9072	24	29	53

deviates from 9:7 ratio.

low. The degree of desynapsis has been checked in many desynaptic plants. On the average, only 35% of PMC's have 2 or more univalents at MI, but the number of univalents never exceeds 10. Thus, the action of the partial desynaptic gene is weak as compared to that of as.

In the asynaptic sporocytes, Miller (1963) has shown that the intensity of asynapsis at zygotene or pachytene is similar to that at diakinesis or MI, indicating that the homologous chromosomes which failed to pair at diakinesis or MI did not pair at early prophase I. Several hundred pachytene configurations of 8 desynaptic plants have been checked carefully. In no case was a completely asynaptic bivalent observed. Only occasionally, a small region of a bivalent was not synapsed. The failure of pairing of chromosomes in the partial desynaptic plants, thus, occurs after the pachytene stage.

The abnormal spindle described by Beadle and Miller in asynaptic sporocytes has not been observed in the desynaptic maize.

Double foldback of a portion of a bivalent has been found at the pachytene stage in desynaptic maize. Its occurrence varied from anther to anther. More foldbacks were found in chromosome 4 than in other chromosomes. Since such foldback bivalents were rare, the direct relationship between foldback and desynapsis is doubtful.

Work is in progress to locate this gene in a specific linkage group.

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Part of this work was carried out in the Department of Botany, Indiana University while the writer was on the Visiting Research Scientist Program, sponsored by the National Academy of Sciences of the United States, 1961-1963.

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1. An analytic study on the chloroplast lesion of w_3 mutant seedlings.

The recessive allele of the W_3 gene was incorporated into five Mexican maize races by appropriate breeding methods. From segregation progenies, albino and normal seedlings were selected for studies. Investigation of genic and cytoplasmic effects of the five maize races on the physiological function of homozygous mutant w_3 w_3 and normal W_3- were conducted on the following relationships:

(1) Photochemical conversion of protochlorophyll to chlorophyll-a in etiolated seedlings.

(2) Aerobic and anaerobic (nitrogen atmosphere) photosensitivities of chlorophyll-a in etiolated seedlings.

Selected seed stocks were germinated in the dark. The etiolated normal and mutant seedlings were allowed to complete photoconversion of protochlorophyll to chlorophyll-a under either aerobic or anaerobic conditions. It was found that chlorophyll-a formation was similar for both normal and mutant seedlings under anaerobic illuminated environment. The quantities of chlorophyll-a increased within minutes after the etiolated seedlings were anaerobically exposed to light. It soon reached a plateau; no more chlorophyll increment was observed thereafter. However, if the photoconversion process of protochlorophyll to chlorophyll-a was conducted in air, normal and mutant behaved quite differently after the conversion of chlorophyll. After a brief lag period, the chlorophyll-a content of normal seedlings increased linearly with time of exposure in light. The mutant seedlings, however, lost pigment and became fully bleached after approximately 10 minutes of illumination. These same phenomena have been observed by Koski (1), Koski and Smith (2), and by Anderson and Robertson (3). Phenotypically the w_3 mutant seedling can be characterized by its inability to retain chlorophyll-a once formed. Tests were carried out on the w_3 mutant in varied background genotypes. In all, w_3 mutant and normal seedlings of five maize races were tested. Slight influence of varied genetic backgrounds was found for w_3/w_3 genic action in the seedlings. It appears that a fundamental change in chloroplasts, such as that triggered by the homozygous recessive w_3 gene, is rather autonomous from other cellular activities.

References:

1. Koski, V. M., Arch. Biochem. 29:339, 1950.
2. _____, and Smith, J. H. C., Arch. Biochem. Biophys. 34:189, 1951.
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2. $C^{14}O_2$ assimilation by light-grown w_3 albino and etiolated normal and mutant seedlings.

Both normal and mutant w_3 seedlings have been found to be able to form chlorophyll-a under a nitrogen atmosphere.

It is questionable if the newly transformed chlorophyll-a is photosynthetically active. In order to clarify this problem, normal and mutant seedlings were grown under light and in darkness for two weeks. The seedlings were placed in a desiccator-type reaction chamber and flushed three times with nitrogen. In this nitrogen atmosphere, the seedlings were illuminated for 25 minutes with 800-900 Ft. C. light. The treatment allowed the etiolated normal and mutant seedlings to transform all of their protochlorophyll to chlorophyll-a. After the photoconversion of chlorophylls was completed in about 25 minutes, a normal aerobic photosynthetic environment with $C^{14}O_2$ was restored. Seedlings were allowed to photosynthesize for 10 minutes. The experiment was terminated. The seedlings were killed in liquid nitrogen, and the total radioactivity determined by the method previously described in the Newsletter (MNL 37:18, 1963).

For normal green seedlings, no correlation was found between chlorophyll concentration and rate of carbon dioxide fixation. This suggests that the rate of photosynthesis is controlled by the enzymes or enzymic reactions involved in photosynthesis rather than by pigment content under light conditions. Generally, green plants produced chlorophyll in excess amount of that required in coordinated activity with the other factors or cofactors in photosynthesis.

It is, however, somewhat surprising that the light-grown albino seedlings accumulated a higher amount of C^{14} than either etiolated normal or mutant seedlings. The latter contained some chlorophyll-a while the light-grown albino seedlings were completely depleted of any kind of photosynthetic pigments. Apparently the newly transformed chlorophyll-a is not photosynthetically active. A Wood-Werkman type of carboxylation of pyruvate to form a four-carbon dicarboxylic acid would account for the entry of $C^{14}O_2$ into these three types of seedlings. As to the higher rate of CO_2 assimilation by the light-grown albino seedlings, we postulate that the variable growth conditions under light and darkness may impart a differential stimulus for internal physiological activity in maize seedlings. This in turn is responsible for the observed differences.

Table 1
Relative Rates of Carbon Dioxide Assimilation by Normal and Mutant Seedlings Grown in Light and Darkness#
Race: Nal-Tel

Entry*	Total radioactivity	Sample dry wt. mg.	Cmp / sample mg.	%
1.	680,330	104.97	6,481	100.00
2.	1,570	122.55	13	0.20
3.	1,610	48.00	34	0.52
4.	2,880	125.10	23	0.35

Seedlings preincubated under anaerobic illumination for 25 minutes before reaction.

Table 2
Relative Rates of Carbon Dioxide Assimilation by Normal and Mutant Seedlings Grown in Light and Darkness
Race: Celaya

Entry*	Total radioactivity	Sample dry wt. mg.	Cmp / sample mg.	%
1.	183,760	134.65	1,365	100.00
2.	1,540	98.73	16	1.17
3.	4,540	127.25	36	2.63
4.	7,140	235.60	30	2.19

Table 3
Relative Rates of Carbon Dioxide Assimilation by Normal and Mutant Seedlings Grown in Light and Darkness
Race: Maiz Dulce

Entry*	Total radioactivity	Sample dry wt. mg.	Cmp / sample mg.	%
1.	1,280,050	213.76	5,988	100.00
2.	3,350	80.76	41	0.68
3.	8,650	135.30	64	1.07
4.	9,700	392.20	25	0.42

Table 4
Relative Rates of Carbon Dioxide Assimilation by Normal and Mutant Seedlings Grown in Light and Darkness
Race: Arrocillo Amarillo

Entry*	Total radioactivity	Sample dry wt. mg.	Cmp / sample mg.	%
1.	498,675	67.79	7,356	100.00
2.	1,030	46.70	22	0.30
3.	4,300	62.22	69	0.94
4.	3,120	58.91	53	0.72

*Entry Number: 1 = Light-grown normal seedlings
2 = Etiolated normal seedlings
3 = Light-grown mutant seedlings
4 = Etiolated mutant seedlings.

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3. Excised corn roots.

Is heterosis determined, at least in part, by the roots of plants? Our early efforts to use excised corn roots in a study of this question quickly demonstrated that we did not know how to grow such roots. Neither

did any one else, so far as we know, except J. E. McClary (Proc. Nat. Acad. Sci. 1940), and our attempts to repeat his work failed. However, a statement of his, "root tips often become abnormal and cease to grow, ...if they are allowed to penetrate the agar,...", set us on a course that promises success. There seem to be two important phases of the problem, viz., the composition of the medium and the physical nature of the substrate.

Composition--Our best medium, to date, has been Shive's "best" solution (Curtis & Clark--Introduction to Plant Phys. 1950, p. 384), plus 1% agar, 5% dextrose, 0.05% yeast extract, 30 ppm glycine and 5 ppm nicotinic acid.

Substrate--Our own early work corroborated McClary's quoted statement. Consequently, we sought a means of keeping the roots on the surface. Thus far, the best substrate has been a thin layer of the agar medium (15 ml in a 10 cm Petri dish) with an S-shaped piece of #20 wire imbedded in it and a $3\frac{1}{2}$" Blue Streak coffee filter on top. Aluminum wire has been used because of its cheapness, although growth seems to be a little better when platinum wire is used. The wire is simply a support for the coffee filter during sterilization, and can be dispensed with if three filter disks are used instead of one.

Using the best substrate and the best medium (but without nicotinic acid), with a single cross hybrid, Io B8 x NY H1, at a temperature of ca. 20°C, we have managed, with difficulty, to keep one set of roots growing for 17 subcultures covering a period of a little over 6 months. Only time and more experiments will tell whether the addition of nicotinic acid improves the solution sufficiently to extend the time of culture indefinitely.

Curiosity tempted us to a premature comparison of inbred and hybrid roots, with these results: Io B8--ca. 3 mm per day, slender; NY H1--ca. $4\frac{1}{4}$ mm per day, plump; Io B8 x NY H1--6 3/4 mm per day, medium thickness (6 subcultures).

A satisfying study of heterosis in excised corn roots must wait until it is possible to culture such roots for an indefinite period of time.

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1. Transmissibility of light endosperm phenotypes during progressive conversion of R-locus expression.

Many weakly pigmented phenotypes are observed on ears of $\underline{R}\underline{R}^{st}$ heterozygotes where the $\underline{R}$ allele has been kept heterozygous with $\underline{R}^{st}$ for several generations. The number of such weakly pigmented kernels increases with the number of generations that $\underline{R}$ has been heterozygous with $\underline{R}^{st}$. Since

the frequency of light phenotypes increases with each generation, that is, the penetrance of R decreases, it is of interest to know if selection may account for the altered incidence of R expression in the aleurone tissue.

Homozygous RR plants (represented as R^6R^6) from inbred W22 background were isolated after six generations with R^{st}. The lightest and darkest kernels were selected from the R^6R^6 ears to be planted out for testing. Some plants of each class were self pollinated to give $R^6R^6R^6$ endosperm phenotypes; an equal number of plants were pollinated with r^gr^g to give the $R^6R^6r^g$ phenotype. This mating plan made it possible to score aleurone pigment where two and three R^6 alleles were present. The scores reported are ear means based on sets of 50 kernels from each ear where each kernel was scored against a set of standard kernels ranging from colorless (zero) to a fully pigmented class (score of 22).

Table 1 summarizes the ear means of the two selected classes of kernels. Less than half a class interval now separates the two groups whose scores in the previous generation were separated by scores of ten or more intervals on our scoring standard. Another result which can be seen in the table is that no difference is evident between $R^6R^6r^g$ and the $R^6R^6R^6$ expressions. It is concluded that the initial difference upon which selection was based cannot be selected for. It would appear that it is the penetrance conditions which are being progressively altered from one generation to the next as R is maintained with R^{st}. The aleurone scores can be thought of as measures of penetrance for the R locus.

Table 1
Comparison of Selected Light and Dark Endosperm Phenotypes After R Has Been Heterozygous with R^{st} for Six Generations

	Self-color selection	Light selection	Self-color selection	Light selection
	$R^6R^6R^6$	$R^6R^6R^6$	$R^6R^6r^g$	$R^6R^6r^g$
	19.73	18.86	18.46	19.52
	18.64	20.40	19.88	19.00
	19.66	18.55	18.84	18.82
	19.18	20.64	19.08	20.18
	18.32	20.02	18.00	19.68
	19.70	18.46	20.30	19.06
	19.76	17.18	21.16	16.70
	18.96		19.72	17.49
	20.02		20.66	21.28
	20.02		18.22	18.74
	19.10		20.60	19.44
				20.16
pooled $\bar{X}$	19.37	19.16	19.54	19.17

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Clifford Gibian

2. Progressive conversion of R-locus expression through eight generations.

The demonstration of progressive reduction of endosperm pigment conditioned by the R locus raises the question of whether the end-point of such treatment can be detected short of the recessive, completely colorless phenotype or whether extended treatments over many generations--more than ten--will give R phenotypes indistinguishable from those conditioned by r. Typical testcrosses where the R alleles from the RR^{st} heterozygotes are scored in one dose give colorless or very nearly colorless phenotypes after three or four generations with R^{st}. Yet when these same R^3 or R^4 alleles (three or four generations with R^{st}) are brought into aleurone tissue through the female for two dose expressions ($R^4R^4r^g$, for example) or if R^4R^4 homozygotes are selfed ($R^4R^4R^4$ endosperm), self color phenotypes are common and considerable pigment can still be found on most kernels of the ear.

To continue observations on the ability of these progressively treated R alleles to produce pigment, aleurone color is scored where the treated R genes are brought through the female only or both through the male and female. RR homozygotes extracted after treatment for a varying number of generations with R^{st}, were selfed for three dose aleurone pigment. RR^{st} plants were pollinated with r^gr^g to give the two dose aleurone (RRr^g). The alleles in question were carried in inbred W22 background. Ear mean scores are based on samples of 50 kernels from each ear. Each kernel was scored against a set of standard kernels represented by 0 (zero), the colorless class, through 22, the fully pigmented class.

Table 1 shows the scores of RR homozygotes which have been selfed. The data show a steady progression toward the colorless phenotype with an average reduction of half a class interval per generation. The same results can be observed in Table 2 where the R alleles are given as two dose expressions since R is removed directly from the RR^{st} heterozygote. In Table 2 the treatment is carried to the eighth generation. Sampling errors account for some of the year to year unevenness in reduction of pigment, nevertheless, the trend over the seven and eight generations is clear.

Table 1
Pigment Scores for Aleurone with Three R Alleles After R Has Been Heterozygous with R^{st} from One to Seven Generations

	R^{1*}	R^2	R^3	R^4	R^5	R^6	R^7
Pooled $\bar{X}$ (6 ears/ treatment)	21.96	21.65	20.55	19.58	19.48	18.97	18.65

*Superscripts represent number of generations R has been heterozygous with R^{st}.

Table 2
Pigment Scores for Aleurone with Two R Alleles Present Where R Has Been Heterozygous with R^{st} for One to Eight Generations

	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
Pooled $\bar{X}$ (6 ears/ treatment)	21.92	21.71	21.21	20.28	19.67	20.00	19.48	18.76

The mechanism of R pigment control still remains to be discovered. Since the extreme phenotypes cannot be selected for, it appears that it is the penetrance conditions which are being progressively altered from one generation to the next as R is maintained with R^{st}. This progressive penetrance control of R expression provides an interesting genetic phenomenon with "memory" capabilities. The endosperm pigment system cannot only be manipulated in a directed way—i.e. taught to respond--but its "I.Q." can be tested by means of the "read-out" supplied by the endosperm pigmentation. Thus the pooled ear means constitute gene-treatment histories reaching back as far as eight generations, recalled now and summarized as a single figure.

B. C. Mikula
Scott Warren

3. Light controlled diurnal rhythm in corn seedlings.

Germinating seeds were found to show diurnal responses in water uptake beginning 24 hours after initial contact with water. Seeds of inbreds W22 x W23 were placed in shallow, glass-covered germinating pans and maintained eight days under the controlled light and temperature conditions of two growth chambers. Seeds were germinated during this period on pads of germinating paper soaked with distilled water. One chamber environment was maintained on alternating 12 hour light and 12 hour dark cycles; the other chamber had constant light conditions. A chamber light intensity of approximately 1700 foot candles was diffused through a white cloth placed over the glass-covered pans; ambient temperature within the chamber was 22.5° C. From the start of germination, 20 seeds were weighed each 12 hour period (at the beginning and end of each dark cycle).

Tables 1 and 2 show the typical rhythmic patterns. Those seeds grown on the 12:12 cycle (LD) show a clear rhythm beginning after the first 24 hours of germination. This rhythm continues for the next three days. The seeds were then transferred to constant light conditions (LL). After transfer to LL conditions, the rhythm is damped and the rate of weight gain is reduced with the loss of the rhythm. It may also be noted that under the LD conditions it is during the dark period that most activity is taking place.

Where seeds were started under LL conditions there is no apparent rhythm in water uptake. After four days under LL the seeds were transferred to LD conditions where a pronounced rhythm becomes observable and a marked

Table 1
Hybrid W22 x W23 Seeds Started Under Alternating Conditions of 12 Hrs. Light and 12 Hrs. Dark, Then Shifted to Constant Light 108 Hrs. After Start of Germination

	Time in Hours														
	12	24	36	48	60	72	84	96	108	120	132	144	156	168	180
	L*	D	L	D	L	D	L	D	L	L	L	L	L	L	L
Wt. gain in gms.	.70	.37	.11	.17	.01	.27	.07	.44	.11	.13	.13	.04	.08	.01	.06

Table 2
Hybrid W22 x W23 Seeds Started Under Constant Light Conditions Then Transferred to Alternating 12 Hour Light and Dark Periods 108 Hours After Start of Germination

	Time in Hours														
	12	24	36	48	60	72	84	96	108	120	132	144	156	168	180
	L	L	L	L	L	L	L	L	L	D	L	D	L	D	L
Wt. gain in gms.	.94	.28	.04	.10	.05	.12	.21	.27	.24	.62	.16	.56	.05	.55	.16

*L = light period 12 hours; D = dark period 12 hours.

change in rate of water uptake takes place. After these first eight days seeds become too difficult to manage by the above weighing procedures.

Rhythmic activity such as that outlined above has important experimental implications. During certain periods of great activity in the plant, the biologist is quiescent; during the greatest activity periods of the biologist, the plant is quiescent!

B. C. Mikula

4. Antigenic substances connected with the R locus.

A fundamental assumption in biology is that genetic information must be translated into molecular information in the form of protein. Because immunological mechanisms of animals are able to detect foreign protein, laboratory animals provide a means for the detection of gene-related antigenic materials of plants. Since a great many important basic questions hinge on the ability to detect gene-related molecules, an attempt was made to see if any of the alleles of R might produce distinct, antigenically active substances. To overcome difficulties experienced by others who have used plant materials as antigens, a minimum preparation was given the plant extracts which were injected into young rabbits. Fresh roots of a W22 x W23 hybrid containing the genes $R^r r^g$ were harvested after five to six days of germination on pads soaked with distilled water. Roots were ground in normal saline with a mortar and pestle in an ice

bath. Coarse cellular material was spun down at low centrifugal speeds until the supernatant fluid could be assured of passing through a syringe. Within an hour after harvest the root extract was injected into young rabbits by two routes: one group of animals was inoculated intravenously and a second group was inoculated intramuscularly using antigen with an adjuvant. The injection program was continued eight weeks at the end of which time the animals were exsanguinated and the blood allowed to clot. The serum was collected and stored at -72° C.

Gel diffusion plates were prepared with wells from which antiserum was allowed to diffuse out against the antigens prepared from fresh roots by the method mentioned above. Two separate precipitin bands could be detected where $\underline{r}^g$ or $\underline{R}^r$ was used as the diffusing antigen. It is concluded that the alleles $\underline{R}^r$ and $\underline{r}^g$ produce antigenically detectable substances which can now be identified by means of our antiserum.

J. R. Frey
B. C. Mikula
Scott Warren

DEKALB AGRICULTURAL ASSOCIATION, INC.
DeKalb, Illinois

1. 7x7 latin square experiments for special purpose studies.

In 1963 four sets of eight 7x7 latin square experiments were planted, two sets on the Dayton, Iowa, and two on the DeKalb, Illinois, research farms. Each set of two experiments had duplicate entries. One purpose for planting this series of experiments (which will not be discussed in detail here) was to accurately measure agronomic differences between hand harvest and machine harvest methods.

A. TR vs. T Cytoplasm Comparison

In addition to the comparison between harvest methods, these experiments were designed to show differences in yield, moisture, and plant quality between sterile T-cytoplasm, and restored sterile T-cytoplasm.

In each 7x7 latin square, six of the entries were composed of three pairs of single crosses, one member of each pair being the sterile version, the other member a restorer version of the same single cross. In this latter version, the TR line was used as the female parent. For the purpose of this test, one to one segregating backcross progeny of T sterile and TR restored-sterile plants were pollinated by the same male inbred so that each pair of single crosses differs only by Rf genes and their linkage complex. It also follows that each female inbred of a pair had an equivalent amount of backcrossing. Most pairs were either in the 5th or 6th backcross generation. The 7th entry in each experiment is a standard commercial single cross hybrid with normal cytoplasm.

Table 1

	Entry No.							Entries Thinned to 16,000 Pl/A.	
	1	2	3	4	5	6	7		
Cytoplasm	TR	T	TR	T	TR	T	N	LSD*	C.V.%
				Expt. 224 (H)					
Yield bu/A	142.0	143.5	131.6	131.7	122.6	118.2	129.1	9.83	6.86
Moisture %	17.3	17.4	16.1	16.3	18.6	19.3	17.6	1.04	5.44
T.D.Pl.	0.7	1.9	2.0	1.7	1.1	1.7	1.4	N.S.	--
				Expt. 225 (M)					
Yield bu/A	144.9	142.9	125.7	123.1	107.8	113.6	127.9	9.83	7.12
Moisture %	17.3	16.6*	16.4	16.4	18.9*	19.6	17.3	.60	3.12
T.D.Pl.	1.7	2.9	2.6	2.7	1.9	0.4	1.3	N.S.	--
				Expt. 226 (H)					
Yield bu/A	123.7	125.1	175.5	176.2	156.6*	147.2	136.1	7.44	4.59
Moisture %	20.0*	21.0	19.1	19.3	19.4	19.4	17.9	.64	3.02
T.D.Pl.	1.0	0.1*	0.4	0.4	0.0	0.0	1.1	.77	--
				Expt. 227 (M)					
Yield bu/A	117.4	121.6	162.7	160.0	141.4	141.3	135.2	8.07	5.29
Moisture %	19.7*	20.9	19.6	19.6	19.6	19.3	18.1	.83	3.89
T.D.Pl.	.4	.9	.6	.6	.4	.4	.6	N.S.	--
				Expt. 228 (H)					
Yield bu/A	126.4	128.6	146.6	148.1	155.7	155.6	128.1	6.25	4.05
Moisture %	16.3	16.1	15.6	15.6	16.3	16.0	15.4	0.47	2.72
T.D.Pl.	1.4	2.1	3.3	2.6	5.3	5.7	1.4	2.31	--
				Expt. 229 (M)					
Yield bu/A	122.8	116.1	144.9	140.6	144.8	156.8*	124.3	10.22	6.90
Moisture %	16.7	16.0*	15.9	15.7	16.4	16.4	15.7	.48	2.72
T.D.Pl.	2.6	2.9	2.9	2.6	3.7	2.6	2.0	N.S.	--
				Expt. 230 (H)					
Yield bu/A	136.2	139.9	150.8	148.1	133.3	136.2	123.1	7.24	4.80
Moisture %	15.0	15.0	15.9	15.7	15.7	15.7	15.0	0.58	3.46
T.D.Pl.	2.6	1.6	2.4	3.4	3.1	2.1	0.9	1.39	--
				Expt. 231 (M)					
Yield bu/A	126.1	133.9	123.7	131.7	132.6	131.4	114.1	10.47	7.51
Moisture %	15.1	15.1	16.0	16.0	16.0	15.6	15.3	.36	2.09
T.D.Pl.	3.7	1.6*	4.3	4.7	2.9	2.0	1.1	1.83	--
Expts.				Average Yield Bu/A.					
224/225	143.45	143.20	128.65	127.40	115.20	115.90			
226/227	120.55	123.35	169.10	168.10	149.00	144.25			
228/229	124.60	122.35	145.75	144.35	150.25	156.20			
230/231	131.15	136.90	137.25	139.90	132.95	133.80			

(H) = Hand harvested; (M) = Machine harvested.
*Significant at the five per cent level.

Data from each experiment are summarized in Table 1 for yield in bushels per acre, per cent moisture, and total damaged plants (T.D.Pl.). The pairs of single crosses are entries 1 and 2, 3 and 4, 5 and 6, with No. 7 being the check entry. With the exception of entry 5 in experiment 226 and entry 6 in experiment 229, no pair member significantly outyielded the other. In the case of moisture, five pairs differed significantly and two pairs differed in total damaged plants. Of the 24 pairs of single crosses compared, eleven yielded more with a TR female and thirteen yielded more with a T female. When the yields of the entries from hand harvested and picker-sheller harvested experiments were averaged, the number of TR and T female single crosses outyielding the other was the same, namely six.

B. Restorer Gene Expression

By using the TR line in the female or "A" position, it was also possible to detect any inhibiting or enhancing action on the part of the pollinator inbred with respect to fertility restoration in the crosses containing Rf germ plasm. From a cursory examination of the plants at pollen shedding time, it was found that entries 1 and 5 in experiment 224 had a higher proportion of fertile plants than the expected 1:1 ratio of fertile to sterile. Entry 5 of experiment 226 exhibited only partial fertility. However, its sterile counterpart, entry 6 in the same experiment, exhibited the same degree of partial fertility. These results, even though from incomplete data, suggest that further investigation of this mode of fertility expression is warranted.

Loring M. Jones

2. Parthenogenesis.

In parthenogenesis in maize, the fate of the male nucleus that normally fertilizes the egg is unknown. The possibility has been raised that in parthenogenesis both male nuclei fuse with the polar nuclei to form a tetraploid endosperm. To check this possibility, the white inbred line 4Co82 was crossed reciprocally with the yellow inbred line W22. It was hoped that dosage effects would indicate tetraploid endosperm, as follows:

a) 4Co82 as female x W22 as male -
normal endosperm yyY (pale yellow)
tetraploid endosperm yyYY (medium yellow)

b) W22 as female x 4Co82 as male -
normal endosperm YYy (strong yellow)
tetraploid endosperm YYyy (medium yellow)

The expected (tetraploid) class of endosperms was not detected. Another test set based on stippled gave the same negative result.

Sherret S. Chase

3. Flint studies.

For the separation, in segregating progenies, of kernels of greater degrees of flintiness from those with lesser degrees of flintiness, sugar solutions have proven convenient and effective. With a graded series of solutions, kernels with small differences in specific gravity can be separated readily.

Sherret S. Chase

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1. A new kind of brachyism.

A new kind of brachyism has been observed in certain stocks of the white inbred, Tx61M. This brachyism is different from any that the authors have seen before. It affects only the internodes above the ear. The striking phenomenon results in two or more leaves being abnormally close together, the number depending upon the degree of brachyism as well as a reduction in plant height.

Some plants have as many as seven leaves which give the general appearance of coming from the same node. This degree of the character gives a circular, bushy, arrangement of the leaves with the tassel sitting in the middle of the circle.

The authors prefer coining the word "brachyism" instead of using "brachysm" as given in the dictionary.

A. A. Fleming
G. M. Kozelnicky

2. Inheritance of variations between stocks of the same inbred line of maize.

Using the P_1, P_2, F_1, F_2, Bc_1, and Bc_2, significant differences between generations were obtained on four and eight variables considered on an individual plant basis for two different experiments involving three stocks of the same inbred line. Five of the variables which were considered on a plot basis also were affected significantly by generations.

Based on the Sewall Wright Method, the minimum number of genes differentiating the parents ranged from -1.76 for total number of leaves to +6.97 for plant height. Powers Partitioning Method was applied to the mode of inheritance for three characteristics in Experiment 1 and two characteristics in Experiment 2. The range in major genes or their equivalents differentiating the parents for any one characteristic under study was two to four pairs.

W. S. Jordan
A. A. Fleming

3. Pollination techniques with Zea mays.

Four experiments were conducted to determine the effects of certain pollination techniques on seed set of corn. Data were obtained on 300 single-cross plants in each experiment.

(a) A comparison of polyethylene (a new plastic type) and glassine shoot bags. Ten characteristics were used for comparison. A better seed set was obtained by the use of glassine bags.

(b) The effect of time of pollination on seed set. Five time-periods were used to divide the day into five pollination periods. No significant differences in the number of kernels per ear for the five periods were obtained. However, on the basis of average daily temperatures, the number of kernels per ear decreased as the temperature increased.

(c) The relationship of length and age of silks at time of pollination to seed set. No significant differences in seed set occurred between "cut" silks and "uncut" silks. Two- to three-day-old "non-mass" silks produced an average of 318 more kernels per ear than five- to ten-day-old "mass" silks.

(d) Pollen viability in cold storage. Comparisons were made between pollen stored 48 and 72 hours under four temperatures ranging from 2° to 25° C. Fresh pollen, the control, set 140 times more seed than stored pollen. The 48-hour storage gave better results than the 72-hour storage.

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1. The tunicate locus dissected and reconstituted.

In previous News Letters we reported that the two components of the Tu locus could be separated by crossing over. When compared in isogenic stocks produced by repeated backcrossing on the inbred A158, the two loci proved to be different in their phenotypic effects; lines heterozygous for the locus, tu^d, having longer glumes, both staminate and pistillate, than lines heterozygous for tu^1. We have now reconstituted the Tu locus by restoring its separate components to their original positions on the same chromosome. Plants heterozygous for both tu^1 and tu^d in a trans configuration were crossed on two inbred strains of the genotype tutu. It was assumed that the progeny of these test crosses would consist of the heterozygous genotypes, tu^1tu and $tutu^d$, in approximately equal numbers and that the great majority of the plants would fall into these two categories. It was assumed further, however, that there would be rare crossovers between the two components and that these would be of two complementary types, tu^1tu^d and tutu.

In a winter crop in Florida and a summer crop in Massachusetts a total population of 10,090 plants were classified. Of these, eight were identified as Tutu and seven as tutu.

The rate of "mutation" (crossing over) involved in reconstituting the locus, 1 in 1261, is of the same order as that, 1 in 1319, which occurred in the experiment involving the dissection of the locus.

The experiment on reconstituting the tunicate locus shows why pod corn, which Weatherwax and others have assumed to be a mutant form, has never been reported in pedigreed cultures although millions of ears of inbred strains and their first-generation hybrids have been studied by corn breeders. Pod corn, as the type represented by the Tu locus, can appear as a mutant only in stocks of half-tunicate maize. If our genetic analysis is valid, it cannot occur as a mutant in modern commercial nontunicate maize.

It now appears that there may have been two kinds of wild corn: one of the genotype, $tu^{l}tu^{l}$, the other of the genotype, $tu^{d}tu^{d}$. When these were brought together under domestication by the American Indians, hybridization would have produced—as it did in our experimental cultures—two new types: (1) an extreme form of pod corn which the Indians in parts of both South and Middle America preserved (and still do) for its supposed magical properties; (2) a nonpodded corn similar to modern corn in lacking conspicuous glumes, which is more productive and in other ways more useful than pod corn as a cultivated food plant.

We now have some evidence, still quite preliminary in nature, that one of the components of the Tu locus, tu^{d}, is itself compound. Crosses to test this possibility have been made.

P. C. Mangelsdorf
W. C. Galinat

2. Prehistoric maize, teosinte, and Tripsacum from Tamaulipas, Mexico.

We have finally analyzed the archaeological maize and other specimens which MacNeish uncovered in Romero's Cave in southwestern Tamaulipas, Mexico, in 1954. The collection includes 3015 intact or nearly intact cobs, 457 cob fragments, 47 pieces of stalk, 9 leaves, 219 husks, 8099 tassels or tassel fragments, 151 quids of chewed stalks, young ears, or tassels, 5 specimens of Tripsacum, 9 of teosinte, and 4 of maize-teosinte hybrids.

The great majority of the cobs, about two thirds of the total, were classified as belonging to the race Chapalote or its precursors or derivatives. This race is found today only in western Mexico but it was once much more widespread. The prehistoric wild corn uncovered in caves in the Valley of Tehuacan in southern Mexico is related to Chapalote (Mangelsdorf et al., SCIENCE 143:538-545) as is also the earliest prehistoric corn from Swallow Cave in Chihauhau and from a number of sites in the southwestern United States (MNL 32).

The earliest prehistoric cobs from this cave, like those from caves in the Tehuacan Valley, were non-Tripsacoid, having soft glumes and rachis tissues. These were soon replaced by Tripsacoid cobs which appeared first at about 1500-1400 B.C. and became the predominating type in the two succeeding culture phases from 1400 B.C. to A.D. 800.

Of even greater interest than the tripsacoid maize is the prehistoric teosinte. The earliest specimen occurred in feces at 1800-1400 B.C., the remaining specimens in two succeeding phases. Four specimens, classified as maize-teosinte hybrids, occurred in the remains dated at 1400-400 B.C. Except for pollen grains which may be those of teosinte described by Barghoorn et al., these are the first prehistoric specimens of teosinte to be reported. Prehistoric Tripsacum had previously been reported from a cave in the Ozarks.

P. C. Mangelsdorf
R. S. MacNeish
W. C. Galinat

3. Tripsacum a possible amphidiploid of Manisuris and wild maize.

Several writers have suggested that Tripsacum is a polyploid hybrid having Manisuris as at least one of its parents. It now seems possible that the other parent may have been wild maize. Two lines of evidence have suggested this. (1) As reported in last year's News Letter, the transmission frequency of six dominantly-marked Tripsacum chromosomes in a maize-Tripsacum hybrid was 32.2 per cent, indicating the presence in Tripsacum of only one homeolog for each of the recessively-marked maize chromosomes. Subsequent cytological studies by Chaganti tend to verify this. They show that most of these segregates carried additional unmarked Tripsacum chromosomes. One plant, for example, with 12 Tripsacum chromosomes carried alleles of only 3 of the recessive marker genes of maize, leaving the remaining 9 addition chromosomes unmarked. As many as 3 of these might be counterparts of the 3 unmarked chromosomes in WMT, our multiple tester stock, leaving at least 6 but not more than 9 Tripsacum chromosomes which do not carry dominant alleles of maize recessives. (2) The prehistoric wild maize from Tehuacan, Mexico, briefly mentioned in last year's News Letter and described in a recent article in SCIENCE (143:538-545), has characteristics which, if combined with those of Manisuris, could produce a plant quite similar to Tripsacum. Indeed, if we assume that one parent of Tripsacum was Manisuris, then among grasses now known the other parent could only have been wild maize or teosinte. Of the two, maize is more promising than teosinte as the putative parent.

The hypothesis that Tripsacum is a hybrid of Manisuris and wild maize is consistent with the data now available. Tripsacum resembles Manisuris or wild maize or is intermediate between them in 18 important botanical characteristics. There is evidence presented below that, with respect to their effects, there may be two types of Tripsacum chromosomes, "maizoid" and "manisuroid."

W. C. Galinat

4. Maizoid and manisuroid effects of Tripsacum chromosomes added to maize.

Tripsacum chromosomes having "maizoid" (see previous item) effects in the direction of archaeological wild maize or primitive races of living maize include the homeologs of maize chromosomes 2 and 9. The chromosome 2 homeolog produces longer internodes in the rachis associated with shorter ears bearing only eight rows of grain. The homeolog of chromosome 9 produces a staminate tipped ear, a characteristic of wild maize. These changes are not associated with increases in glume or rachis induration as they might be had they been caused by either teosinte or Manisuris germplasm. All of the maizoid isolates from Tripsacum have been completely female fertile and at least partially if not completely male fertile.

In sharp contrast are the effects of the "manisuroid" isolates which have extra chromosomes not markable by any of the seven marker genes from WMT maize and which, if Manisuris is one parent of Tripsacum, may represent the chromosomes originally from Manisuris. The manisuroid isolates tiller more profusely, have narrower leaves, smaller spikelets, smaller kernels, increased induration of rachis and glumes, less specialization between the staminate and pistillate glumes and are partially female sterile and usually completely male sterile.

W. C. Galinat
P. C. Mangelsdorf
R. S. K. Chaganti

5. Pollen fertility and type of Tripsacum chromosome.

Pollen studies of maize hyperploid for a Tripsacum dactyloides chromosome (Maguire 1956) with genes corresponding to maize chromosome 2 suggested the presence of a dominant "gene" on that chromosome for pollen sterility when present in the maize nucleus. Such a factor for pollen sterility did not exist in the corresponding chromosome which we have independently isolated from our stock of T. dactyloides from Manhattan, Kansas. In fact the average size, range, and distribution of sizes and starch accumulation in the pollen of plants with this Tripsacum homeolog to maize chromosome 2 is essentially identical to that of isogenic plants not bearing this extra chromosome. Although pollination tests for male transmission of this chromosome have not been made, the normal appearance of the pollen suggests that it would have equal male and female transmission as did the Tripsacum homeolog to maize chromosome 4. The indication is that stocks bearing the markable or "maizoid" Tripsacum chromosomes are male fertile while those bearing the nonmarkable or "manisuroid" Tripsacum chromosomes are essentially male sterile. The latter have smaller, usually empty pollen grains and anthers do not extrude from the spikelets.

W. C. Galinat
P. C. Mangelsdorf
R. S. K. Chaganti

6. Consistent transmission rate for Tripsacum homeolog to maize chromosome 2.

The transmission rate for the Tripsacum chromosome marked by Lg_1, which was lowest (23.9%) among the seven dominantly marked Tripsacum chromosomes (av. 32.2%) segregating from the backcross hybrid (WMT maize X T. dactyloides) X WMT maize as we reported in the last News Letter, has remained constant in two subsequent backcross generations. This rate of transmission is scored as percent of liguled plants grown from ears of addition monosomics for this extra chromosome which had been backcrossed to chromosome 2 tester maize. The data are as follows:

Year	Backcross Generation	No. Tripsacum Chromosomes	Lg %	N
1962	1	18	23.9	92
1963	2	3	18.0	54
F1963-4*	3	1	22.2	54

*Pooled data from 4 ear-row lines grown in the winter crop near Homestead, Fla.

W. C. Galinat
P. C. Mangelsdorf
R. S. K. Chaganti

7. Equal male and female transmission rates for Tripsacum homeolog to maize chromosome 4.

In this case the Tripsacum chromosome was derived from the Florida tetraploid of T. dactyloides following crossing with Alexander's tetraploid sugary golden maize. After a second backcross to diploid su gl_3, some of the ears were self-pollinated while the remainder were backcrossed a third time to the chromosome 4 tester of maize. A comparison of the frequency of starchy kernels in ears from these two types of pollinations shows that the Tripsacum homeolog to maize chromosome 4 has approximately equal transmissability through male and female gametes. The data:

Year	Backcross Generation	No. Tripsacum Chromosomes	Backcross			Self		
			Su %	N	No. Ears	Su %	N	No. Ears
1962	1	18	11.0	285	1	--	--	--
1963	2	2	8.1	1182	4	19.4	1060	4
1963	2	1	6.0	1188	3	10.2	1623	5

The percentages of nonsugary seeds on the selfed ears, 19.4 and 10.2, agree closely with the percentages expected, 15.5 and 11.6, if the male transmission is the same as the female, 8.1 and 6.0, respectively.

W. C. Galinat
P. C. Mangelsdorf
R. S. K. Chaganti

8. Variable transmission for Tripsacum homeolog to maize chromosome 9.

In contrast to the consistent female transmission rate observed for the Tripsacum homeolog to chromosome 2 of maize, the Tripsacum homeolog of maize chromosome 9 showed a sudden reduction in its transmission on the female side after separation from the rest of the Tripsacum genome. This transmission is scored as percent of nonwaxy kernels on ears of addition monosomics for this extra chromosome which had been backcrossed to chromosome 9 tester maize. The data are as follows:

Year	Backcross Generation	No. Tripsacum Chromosomes	Wx %	N
1962	1	18	37.0	92
1963*	2	1	12.3	1688

*Pooled data from 7 ears.

On the male side, the transmission of this extra chromosome may be much higher. When 202 pollen grains of one of these addition monosomics (63-470-1) were scored, 116 or 57.4% were classified as nonwaxy.

W. C. Galinat
P. C. Mangelsdorf
R. S. K. Chaganti

9. Transmission of Tripsacum chromosomes in the progeny of a maize-Tripsacum dactyloides hybrid derivative.

Segregation of Tripsacum chromosomes in the progeny of a maize-Tripsacum dactyloides hybrid derivative with three extra Tripsacum chromosomes was studied in backcross progenies. One of the three chromosomes also carried the dominant allele for the lg_1 (chromosome 2) gene of maize. The transmission frequency of the Lg_1 carrying chromosome is reported in a different entry (see No. 6). The observed and random-expected segregation of the three chromosomes on the female side is shown in the following table.

Table 1
Segregation of Chromosomes in the Backcross Progeny of 62-588-89

Pedigree	Number of Chromosomes			
62-588-89 x lg_1 gl_2 B v_4 (23 chs. in ♀)	20	21	22	23
Plants observed	32	15	1	0
Plants expected with random segregation	6	18	18	6

As in the case of the segregation of Tripsacum floridanum chromosomes in the progeny of the triploid hybrid reported above, here also the distribution is nonrandom. However, a study of chromosome numbers in the microspores of 62-588-89 itself showed that the 10, 11, 12, and 13 chromosome classes are randomly distributed as shown in the following table.

The observed nonrandomness of segregation of the Tripsacum chromosomes on the female side could then be due to either preferential segregation on the female side or to gametic or zygotic lethality.

Table 2
Distribution of Chromosome Numbers in the Microspores of 62-588-89

	Number of Chromosomes			
	10	11	12	13
Microspores observed	3	9	12	3
Microspores expected with random distribution	3	10	10	3

Raju S. K. Chaganti
Uma S. Tantravahi

10. Intra- and intergenomic affinities of maize and Tripsacum chromosomes.

In order to assess the significance of chromosome association in maize-Tripsacum hybrids, intragenomic synaptic relationships of maize and Tripsacum were studied. The frequencies of chiasmatic associations and side by side associations of chromosomes (which probably represent homologous or homeologous pairing, Person, Canadian Jour. Bot. 33:11-30, 1955; Kimber and Riley, Bot. Rev. 29:480-531, 1963) at meta-anaphase of meiosis in a haploid maize plant and at metaphase in the haploid genome of Tripsacum from the triploid hybrid [(maize x T. floridanum) x maize] were scored. If the association frequency found in the hybrid (chiasmatic and side by side) resulted to a large extent from intergenomic pairing, then such association should be in excess of the sum of the individual pairing frequencies of the haploid genomes of the constituent species. If, on the other hand, the pairing in the hybrid is autosyndetic or predominantly so, then the association in the hybrid should be equal to or less than the sum of the pairing in the haploid genomes of the parental species. The mean per cell of chiasmatic association in haploid maize, haploid genome of Tripsacum, and the F_1 hybrid was 0.06, 0.20, and 2.28 respectively while the mean per cell of side by side association in the three materials respectively was 0.28, 0.20, and 0.69. It can at once be seen that the pairing in the hybrid is much higher compared to the sum of mean pairing in the haploid genomes of maize and Tripsacum (2.28:0.26 chiasmatic and 0.69:0.48 side by side associations). Thus a significant amount of pairing in the maize-Tripsacum hybrids is intergenomic and involves maize and Tripsacum chromosomes.

Raju S. K. Chaganti

11. Nonrandom segregation of Tripsacum floridanum chromosomes in the progeny of the triploid hybrid [(maize x T. floridanum) x maize].

In order to study the mode of segregation of the Tripsacum chromosomes on the female side of the triploid hybrid, the distribution of chromosome numbers in a progeny population of 150 plants obtained by backcrossing the triploid hybrid by the maize parent was studied. The data are presented in the following table.

Random segregation of Tripsacum chromosomes would follow a distribution obtained by expanding the binominal $(1/2 + 1/2)^{18}$. The above data show clearly that the distribution is extremely skewed toward the side of the

Table 1
Segregation of Chromosomes in the Progeny of the Triploid Hybrid [(maize x T. floridanum) x maize]

Chromosome Number	Number of Extra Tripsacum Chromosomes	Observed Frequency	Frequency Expected on Random Segregation of Chromosomes
10	0	1	0.00
20	0	29	0.00
21	1	46	0.00
22	2	21	0.15
23	3	13	0.45
24	4	18	1.80
25	5	7	4.95
26	6	7	10.65
27	7	2	18.15
28	8	1	25.05
29	9	2	27.75
30	10	1	25.05
31	11	0	18.15
32	12	1	10.65
33	13	0	4.95
34	14	0	1.80
35	15	0	0.45
36	16	1	0.15
37	17	0	0.00
38	18	0	0.00
Total		150	

low chromosome classes (0-5). It appears that gametic or zygotic combinations involving high numbers of Tripsacum chromosomes are systematically eliminated.

Raju S. K. Chaganti

12. Transmission frequencies and phenotypic effects of two Tripsacum floridanum chromosomes in addition monosomics of maize.

In the summer of 1962 several maize plants which are addition monosomics for T. floridanum chromosomes were isolated from the progeny of the triploid hybrid [(maize x T. floridanum) x maize]. Synaptic relations, transmission frequencies and phenotypic effects of two of these, identified at pachytene as Tripsacum chromosomes 5 (length, 39.96 microns; arm ratio, 4.1:1.0) and 11 (length, 22.04 microns; arm ratio, 4.0:1.0) are studied and reported here. Neither of these chromosomes showed any synaptic relations with any of the maize chromosomes at pachytene or other stages of meiosis. At pachytene, however, their terminal knobs were usually seen to be sticking with the knobs on the maize chromosomes. This sometimes persisted to diakinesis where configurations consisting of associations of three chromosomes were found.

In backcross progenies the two addition monosomics showed transmission frequencies of 32.0% (chromosome 5) and 29.2% (chromosome 11).

In order to study the phenotypic effects of these chromosomes on maize, measurements were made on tem morphological characters (plant height, number of tillers, number of leaves, leaf length, leaf width, leaf length/leaf width, number of days to silking, number of ear shoots, number of days to anthesis, and length of the central spike) in the backcross progenies of the addition monosomics and means for 20- and 21-chromosome plants compared. In the case of chromosome 5, the 21-chromosome plants were significantly shorter, had shorter and narrower leaves and their leaf length/leaf width ratio was higher compared to the 20-chromosome plants. In the case of chromosome 11, the 21-chromosome plants were also significantly shorter, had narrower leaves, and exhibited a higher leaf length/leaf width ratio compared to the 20-chromosome plants. They were also later in silking. It seems quite likely that all the observed effects are the consequence of aneuploidy rather than due to specific genes residing on the extra chromosomes.

Raju S. K. Chaganti

13. Chromosome synapsis in an interspecific Tripsacum hybrid.

A hybrid between T. floridanum Simm., and T. dactyloides L., was produced by one of us (W.C.G.) and its cytology with reference to pachytene synapsis has been studied in order to understand the chromosomal relationships of the two species. At pachytene the chromosomes of the two species showed good synapsis for most of their lengths. However, the hybrid was found to be heterozygous for the following differences: (1) a duplication of 3.5 microns on the short arm of chromosome 7, about 3 microns removed from the proximal end; (2) a duplication of about three microns on the nucleolar arm of the nucleolus organizing chromosome immediately following the nucleolus organizer; (3) a terminal knob on chromosome 12. Besides the above, several other differences were found in the synaptic relationships. They are: (1) an intercalary unpaired region in the short arm of chromosome 1 about three microns in extent and about 3.6 microns removed from the distal end; (2) variable (total to none) failure in the long arm of chromosome 15; (3) fusion of the terminal ends of the two arms of chromosome 7 and the nucleolus organizer chromosome giving "ring shaped" bivalents at pachytene.

Diakinesis pairing was normal and 18 bivalents were always formed. Metaphase was extremely clumped and there was some bivalent lagging at anaphase.

From the above description it can easily be seen that there are no major differences between the karyotypes of the two species. Except for the two small duplications the chromosomes of the two species are more or less identical in their morphology. The present data support other unpublished evidence that the two species are closely related.

Raju S. K. Chaganti
Walton C. Galinat

14. Mutation load accumulated after six generations in isolation by a teosinte introgressed population of A158.

Previous sampling of an isolation block of intercrossing teosinte derivatives of A158 showed no significant change in yield but a drop in shelling percentage after four generations (MNL 36). Because the teosinte chromosomes in this material are known to be mutagenic, especially when heterozygous (MNL 34), we sampled 504 self-pollinated ears from the sixth generation and scored them for mutation load of seed and seedling abnormalities. Forty-one percent of the seedling mutations had effects on the mature plant. Many mutations having phenotypes expressed in the mature plant alone were probably missed because a sample of 35 of the more tripsacoid ears grown to maturity in the field yielded 7 or 20 percent mutations which would not have been detected in sand flat cultures.

Stock	No. Ears Scored	Percent with Abnormalities		
		Seed	Seedling	Mature Plant
General Population	469	2.7	6.7	No data
Highly Tripsacoid Cobs	35	2.8	-	20.0

In addition to defective seed and dwarfs of various sorts, such well known mutants as glossy, virescent, albescent, albino, adherent, narrow leaf, pale green, and golden occurred. The presence of adherent (ears 63-353 and 63-370) is of interest because this is the only one out of eleven variable characters obtained from the Maize Coop collection which has not previously associated with tripsacoid cobs in an earlier study (MNL 37).

W. C. Galinat
P. C. Mangelsdorf

15. Lignin content as a new measure of introgression.

Small sections of various maize cobs and individual rachis segments of maize relatives were immersed in 72% sulphuric acid for six hours at room temperature. In the process of acid erosion, cellulose and other polysaccharides are removed leaving behind lignin and a small amount of ash.

While definite conclusions cannot yet be drawn from these data, there is evidence that lignin determination is a refinement in evaluating introgression. This method and the relation between lignin content and internal cob morphology are being studied in detail.

The objective of this work is to investigate the heterotic effects of introgression in Corn Belt inbreds and perhaps to establish one basis for predicting the combining ability of inbreds.

Table 1
Percent Lignin Content of Cobs and Rachis Segments of Various Maize Lines and Maize Relatives

	Percent		Percent
Tripsacums		Maize Inbreds	
floridanum	54	WF9	68
dactyloides (2n)	48	I11A	68
Teosintes		L317	64
Amercameca	72	C103	63
Chalco	64	0h07	60
Honduras	32	W22	58
Maize-teosinte Hybrid		38-11	56
A158 x Florida teosinte	79	0h51A	56
Maize Varieties		0h40B	55
Parker's Flint	58	0h45	54
Wilbur's Flint	56	0s420	49
Gourdseed	53	I11Hy	42

G. S. Johnston

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1. A high frequency of hybrids between certain genetic stocks of maize and Illinois Tripsacums.

An effort was made in 1963 to cross certain heterozygous translocation stocks with plants of T. dactyloides (2N + 4N), collected from different locations in Illinois. Although not all the data have been summarized, nor has the hybrid nature of all progeny been confirmed cytologically, the results merit reporting and further detailed analysis. No special crossing techniques were used other than the "shortened silk" technique of Mangelsdorf and Reeves. Embryo culture was not used to obtain any of the hybrids. However, the pericarp was removed and the "poorly developed" seeds were germinated about 30 to 40 days after pollination. The following table summarizes the data for crosses with certain maize stocks.

The data illustrate the high number of hybrids produced by certain pollinations and, also, the fact that certain female parents give a larger number of hybrids than others. One pollination (15) produced 22 hybrids (3.27%) with a diploid Tripsacum from Harvel, Illinois. The authors are not aware of any reports in the literature indicating that this many hybrids have been produced from one pollination. In the classical work reported by Mangelsdorf and Reeves only 29 hybrids were obtained when 185,000 silks on 382 ears were pollinated with Tripsacum pollen (.01%). Several factors, such as sterility of the female parent,

Pollination	Pedigree	Total number of ovules	Number of hybrids	Percent hybrids
1.	T1-6c(+/T) x Acc 685 (Horse Shoe Lake) 4N*	1144	11	.96
2.	T1-6c(+/T) x Acc 685 (Horse Shoe Lake) 4N	912	12	1.31
3.	T1-6c(+/T) x Acc 685 (Horse Shoe Lake) 4N	608	13	2.13
4.	T1-6c(+/T) x Acc 684 (Horse Shoe Lake) 4N	660	0	--
5.	T1-6c(+/T) x Acc 687 (Horse Shoe Lake) 4N	688	18	2.61
6.	T1-6c(+/T) x Acc 687 (Horse Shoe Lake) 4N	936	4	.49
7.	T1-6c(+/T) x Acc 676 (Horse Shoe Lake) 3N	1120	0	--
	Total	6050	71	1.17
8.	T6-9(+/T) x Acc 687 (Horse Shoe Lake) 4N	864	2	.23
9.	T6-9(+/T) x Acc 687 (Horse Shoe Lake) 4N	714	2	.28
10.	T6-9(+/T) x Acc 685 (Horse Shoe Lake) 4N	742	9	1.21
11.	T6-9(+/T) x Acc 685 (Horse Shoe Lake) 4N	828	0	--
12.	T6-9(+/T) x Acc 684 (Horse Shoe Lake) 4N	882	0	--
13.	T6-9(+/T) x Acc 372-2 (Emerson) 2N**	532	0	--
	Total	4562	13	.28
14.	T4-10b(+/T) x Acc 694 (Freeman Spur) 4N	792	2	.25
15.	T4-10b(+/T) x Acc 662 (Harvel) 2N	672	22	3.27
	Total	1464	24	1.63
16.	T5-9(+/T) x Acc 694 (Freeman Spur)	756	8	1.05

* Refers to designation, location, and ploidy level of Tripsacum parent.

**Refers to a clone obtained from R. A. Emerson's garden at Ithaca, New York.

(See article 1 for explanation of table.)

	Phenotypes		X^2	P
	Resistant	Susceptible		
1. (Oh43 x Guatemala 125) BC2 ⊗ (Ht/ht) ⊗	88	24	0.847	0.30-0.50
2. (Oh43 x Guatemala 125) BC2 ⊗ (Ht/ht) ⊗	78	32	0.891	0.30-0.50
3. (Oh43 x Guatemala 125) BC3 (Ht/ht) x (ht/ht)	59	47	1.358	0.20-0.30
4. (WF9 x Guatemala 125) BC3 (Ht/ht) x (ht/ht)	37	34	0.127	0.70-0.80
5. (B14 x Guatemala 125) BC3 (Ht/ht) x (ht/ht)	59	47	1.358	0.20-0.30

(See article 2 for explanation of table.)

Tripsacum plant used and others, could account for the high frequency of hybrids. A more detailed study employing a number of different Tripsacum plants and certain maize stocks is being planned in an attempt to answer several questions posed by the results.

R. J. Lambert
E. R. Leng

2. Monogenic resistance to Helminthosporium turcicum extracted from teosinte.

In the 1962 Florida winter nursery plants of Guatemala teosinte were found to be highly resistant to northern leaf blight under natural epiphytotic conditions existing that year. The resistant plants were crossed to the susceptible inbred Oh43. Seedlings of the F_1 hybrid were tested in the greenhouse. All plants showed the typical phenotype for resistant lesions similar to the dominant (Ht) gene reported by Hooker in the 1963 M.N.L. In subsequent generations the material was backcrossed to the susceptible inbred for two generations and resistant progeny selected for backcrossing in each generation. A number of backcross generations were used to make the material more adapted to corn-belt conditions.

During the summer of 1963 backcross-two resistant plants (Ht/ht) were selfed and also backcrossed again to the susceptible recessive parent (Oh43, ht/ht). The backcross plants were also test crossed onto the susceptible inbreds B14 and WF9. The selfed and testcross seedlings were tested in the greenhouse. The limited data, presented in the following table, indicate a single dominant gene will explain the resistance observed. The phenotype of the resistant plants is similar to that found in GE 440 and 'Ladyfinger' popcorn by Hooker. Further tests are necessary to determine if this gene is allelic to the Ht alleles found in these two stocks. Should the gene be nonallelic to the Ht gene of Hooker, it may be possible to incorporate a greater degree of resistance to northern leaf blight than each gene separately.

R. J. Lambert
A. L. Hooker

3. A possible cause for unreduced female eggs in corn-Tripsacum hybrids.

The occurrence of unreduced megasporocytes in the F_1 hybrid between corn x T. dactyloides has been reported by Mangelsdorf and Reeves (Texas Bull. 574). Galinat (M.N.L. 1961) found similar results for corn x T. floridanum hybrids. Several mechanisms could account for the unreduced eggs in these hybrids. One of these could be the failure of cell wall formation in either mitotic or meiotic divisions. Failure of cell wall formation during mitosis should give rise to somatic sectors of tissue carrying the doubled number of chromosomes. This should result in fertile eggs which are either "clustered" on the pistillate inflorescence or occur in a certain pattern.

In backcrossing corn x T. dactyloides hybrids (female parent) with the corn parent, "normal seeds" resulted from 61% of the pollinations.

From the 1089 ovules pollinated, 107 seeds were obtained (9.82%). This is a much higher frequency than would be expected for random assortment of chromosomes. Cytological counts of root tip cells of a limited number of these individuals show them to be the result of unreduced eggs from the female parent. Extra care was taken to insure that all silks of the corn-Tripsacum hybrid were receptive when pollinated by the corn parent. When those pollinations which produced more than one seed are diagrammed, with respect to position of the seeds in spikelets of the rachis, evidence was obtained which may explain the occurrence of unreduced eggs. The following table lists the position of the seeds on the pistillate rachis. The spikelets were numbered consecutively starting at the base of the rachis. All even numbered spikelets will occur on one side of the rachis and odd numbers on the other.

Spikelet Number on Rachis

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Number of seeds
										x	x	x	x					4
					x	x	x	x										4
	x	x																2
		x					x	x	x									4
												x	x	x				3
								x	x									2
		x						x	x									3
		x	x															2
	x				x	x												3
						x	x	x	x				x					5
									x	x	x			x	x			5
															x		x	2
				x		x				x								3
		x		x														2
x		x												x				2
			x		x													2
		x												x				2
	x																x	2
										x		x						2
x								x				x				x		4
											x		x					2
												x		x				2
					x					x								2
x		x				x			x				x		x			6

Eleven pollinations out of twenty-four had seeds which were in adjacent spikelets on a rachis. In addition, eleven pollinations had sseds on only one of the two sides of rachis. Only two pollinations produced seeds on both sides of the rachis and not in consecutive order. If all progeny from these seeds in consecutive order should be the result of unreduced eggs then 45% of the individuals could have occurred as the result of somatic chromosome doubling. This doubling would have resulted in "islands" of tissue carrying 36 Tripsacum and 20 corn chromosomes. These sectors could be expected to produce megasporocytes with 18

Tripsacum and 10 corn chromosomes. If the cases where seeds were set on a specific side of the rachis are also the result of a more "localized" somatic sector, then 91% of the individuals occurred from unreduced eggs resulting from somatic doubling. If further analysis should show that somatic doubling is the cause of unreduced eggs, the event must occur with a high frequency. This mechanism could allow for backcrossing of corn-Tripsacum hybrids to corn parent under natural conditions.

R. J. Lambert
E. R. Leng

4. Maize X Tripsacum lanceolatum (Ruper. ex Fourn.) hybrids.

Attempts to hybridize maize with Tripsacum lanceolatum,* on a limited scale, have been very successful. From five pollinations, using three different maize stocks as female parent, 20 hybrids were obtained. Thirteen of these hybrids were obtained using translocation stock Tl-6c as female. Seven of the hybrids were obtained with inbred W153R as female. Table 1 summarizes the results for the five pollinations.

Table 1

	Number of seeds set	Total number of ovules	Number of hybrids
W153 x T. lanceolatum	31	420	7
Tl-6c x T. lanceolatum	39	700	13
Tl-6c x T. lanceolatum	19	500	--
c sh wx gl_{15} y R x T. lanceolatum	3	526	--
c sh wx gl_{15} y R x T. lanceolatum	35	675	--

The percentage of hybrid plants obtained was similar to that reported by other workers (.07%) for intergeneric crosses of this type. However, the author is not aware of any similar reported crossing experiments where the percentage of hybrid individuals has been as high as 1.85% for an individual pollination.

No special technique was used to obtain the hybrids other than the "shortened silk" technique of Mangelsdorf and Reeves. The seeds obtained appeared poorly developed; however, some germinated after the removal of the pericarp. The seed was germinated about 40 days after pollination.

Cytological analysis of a limited number of microsporocytes of two hybrid plants showed cells with 46 chromosomes. Most of the cells had 18 Tripsacum bivalents and 10 corn univalents; however, some cells did not have this number of chromosomes. No cells were found where pairing occurred between corn and Tripsacum chromosomes. A more detailed cytological analysis is necessary before conclusions on chromosome number or pairing relationships of all hybrid plants are known. Sterility of

*Plant used as pollen parent obtained from seed collected by D. E. Alexander near Taxco, Mexico, in 1959.

these two hybrid plants appears to be high. Pollinating 167 ovules of these two hybrid plants with pollen from the maize parent resulted in no seed. This would indicate female sterility for these two hybrid plants.

A number of species of Tripsacum have been hybridized with maize by different workers (Mangelsdorf, Farquharson, Galinat). It is interesting to speculate which species was involved in the tripartite hypothesis of Mangelsdorf and Reeves.

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1. Additional sources of chlorotic lesion resistance to Helminthosporium turcicum Pass.

A dominant gene resistance to northern corn leaf blight caused by H. turcicum and expressed in the form of chlorotic lesions supporting limited fungus reproduction was reported from our laboratory in the 1963 M.G.C.N.L.

This past year data have been obtained from other corn selections showing this type of resistance. In addition, field data were obtained for F_2 populations involving W37A shown to have a single dominant gene for resistance on the basis of greenhouse seedling tests in our previous report. Two sweet corn inbreds EES647 and EES650, the dent corn inbred W37A, and the pop corn inbred 35 (a white rice type and distinctly different in plant and ear type from Ladyfinger popcorn) were crossed with inbreds expressing susceptible-type lesions. These hybrids were advanced to the F_2 generation. With the Pop 35 hybrids, backcross populations were also tested.

The following data obtained from inoculated field plots or from inoculated seedling tests in the greenhouse indicate that resistance is due to a single dominant gene in each of the 4 sources. The data further suggest that homozygous plants can be distinguished from heterozygous plants.

The parental resistant inbreds, F_1 hybrids, and susceptible inbreds gave highly resistant, resistant, and susceptible reactions, respectively, in these tests.

Segregations for Lesion Type to H. turcicum in the Field

Cross	Observed number			P values	
	Highly Res.	Res.	Susc.	1:2:1	3:1
W37A x H52. F_2	24	50	26	0.95-0.98	0.80-0.90
W37A x 187-2 F_2	16	58	25	0.05-0.10	0.95-0.98
B14 x EES647 F_2	15	53	24	0.10-0.20	0.80-0.90
187-2 x EES647 F_2	14	48	29	0.05-0.10	0.10-0.20
B14 x EES650 F_2	24	49	25	0.98-0.99	0.90-0.95
187-2 x EES650 F_2	20	48	21	0.70-0.80	0.70-0.80

Segregations for Lesion Type to H. turcicum in Corn Seedlings

Cross	Observed number			Expected ratio	P value
	Highly Res.	Res.	Susc.		
R168 x Pop 35 F_2	21	54	16	1:2:1	0.10-0.20
W153R x Pop 35 F_2	24	41	28	1:2:1	0.30-0.50
B14 x Pop 35 F_2	13	51	17	1:2:1	0.05-0.10
(B14 x Pop 35) B14	0	34	31	0:1:1	0.70-0.80
B14 (B14 x Pop 35)	0	42	56	0:1:1	0.10-0.20
(B14 x Pop 35) Pop 35	49	46	0	1:1:0	0.70-0.80
(R168 x Pop 35) Pop 35	55	55	0	1:1:0	> 0.99
(W153R x Pop 35) Pop 35	45	48	0	1:1:0	0.70-0.80

In addition to the selections reported, other corn types have been located which express chlorotic lesions when infected by H. turcicum. Inheritance studies of these are underway as well as tests to determine if the genes present in any of the selections can be distinguished from the gene Ht.

A. L. Hooker

2. Additional gene loci for resistance to Puccinia sorghi.

Rust resistant inbreds 178 and 191 were crossed to the rust susceptible inbreds B14 and R168. The single crosses were advanced to the F_2 generation and backcrossed to the susceptible inbred. The following seedling data indicate that each line has a single dominant gene for resistance to culture 901aba P. sorghi.

Cross	F_2 generation			Backcross generation		
	R	S	P value (3:1)	R	S	P value (1:1)
178 x B14	81	13	0.01-0.02	57	55	0.80-0.90
178 x R168	89	35	0.30-0.50	55	65	0.20-0.30
191 x B14	83	25	0.50-0.70	71	78	0.50-0.70
191 x R168	92	36	0.30-0.50	75	75	> 0.99

Line Mex 185 having a single dominant gene for resistance (Pages 53-54, 1961 M.G.C.N.L.) was crossed with lines Syn A having gene $\underline{Rp_1}^c$ and NN14 having gene $\underline{Rp_3}$ for resistance to P. sorghi. Line Mex 185 was also crossed with line 178. Lines 178 and 191 were crossed with BY Dent. BY Dent has gene $\underline{Rp_1}^c$. These single crosses were advanced to the F_2 generation and also crossed with susceptible inbreds (test-cross populations). The following seedling data for reaction to P. sorghi culture 901aba were obtained:

	F_2 populations			Test-cross populations		
	R	S	P value (15:1)	R	S	P value (3:1)
Mex 185 x Syn A	100	12	0.05-0.10	95	36	0.50-0.70
Mex 185 x NN14	118	6	0.50-0.70	82	19	0.10-0.20
Mex 185 x 178	67	5	0.80-0.90	67	28	0.30-0.50
178 x BY Dent	127	9	0.80-0.90	87	37	0.20-0.30
191 x BY Dent	436	0	< 0.01	894	9	< 0.01

These data suggest that the gene in Mex 185 is not at $\underline{Rp_1}$ or $\underline{Rp_3}$ and therefore is at a new locus. It is suggested that this locus in line Mex 185 be designated as $\underline{Rp_4}$. The locus in line 178 assorts independently of $\underline{Rp_1}$ and $\underline{Rp_4}$. We do not know yet if the gene assorts independently of $\underline{Rp_3}$. The gene in line 191 is linked to $\underline{Rp_1}$ with a recombination value of 0.01. It is suggested that the locus in 191 be designated $\underline{Rp_5}$.

A. L. Hooker
W. L. Hagan

3. Location of genes determining resistance to Puccinia sorghi in lines Mex 185 and 178.

Translocation stocks with waxy or sugary marker genes were used in linkage tests with the dominant genes for resistance to P. sorghi in the corn inbreds Mex 185 and 178. A list of the translocation stocks used, breakage points, and methods of study were given in the 1961 M.G.C.N.L. pages 55-58. In all cases except for the chromosomes listed below, the linkage was negative or inconsistent.

Translocation	Number of seedlings				χ^2 values
	Normal Starch		Mutant		
	R	S	R	S	
	(Mex 185 crosses)				
T1 - 4a (su)	178	39	26	117	88.309**
T4 - 8a (su)	128	30	19	115	129.553**
	(178 crosses)				
T3 - 9c (wx)	113	13	19	129	160.971**

These data were confirmed with field tests of other progenies. It appears that the gene $\underline{Rp_4}$ in Mex 185 is on chromosome 4 and that the gene in 178 is on chromosome 3.

A. L. Hooker
W. A. Russell

4. Higher population studies of the $Rp1$ locus for resistance to P. sorghi.

Previous studies at this laboratory have shown that the corn inbred lines GG208R, Cuzco and Mex 212 carry a single dominant gene that conditions resistance to a specific biotype (901aba) of corn rust Puccinia sorghi Schw. Further work showed that genes in GG208R and Cuzco were allelic and were respectively designated $Rp_1{}^a$ and $Rp_1{}^d$. The gene in Mex 212 appeared to be either a member of this allelic series or a gene very closely situated to Rp_1. The data from the following test crosses support the latter view.

Crosses	Number of seedlings observed		Expected		Freq. of Susc.
	Res.	Susc.	Res.	Susc.	
(GG208R x Mex 212) x R168 ($Rp_1{}^a$ $Rp_1{}^?$) x rp rp	6175	8	1:0		13×10^{-4}
(GG208R x Cuzco) x R168 ($Rp_1{}^a$ $Rp_1{}^d$) x rp rp	4168	2	1:0		4.8×10^{-4}

The very low frequency of susceptibles arising in cross number 2 can be due to deletion or mutations. The F_1 generations are currently being studied to measure mutation and deletion frequencies. The frequency of susceptibles arising in cross number 1 involving Mex 212 is almost 2.7 times larger than that in cross number 2. The difference is highly significant. It appears then that in test cross number 1 the frequency of susceptibles also includes crossover events. If this is true, then corn chromosome number 10 has a region bearing several genes close to one another conditioning resistance to biotypes of corn rust.

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1. Protogyny and male sterility in maize.

A large collection of maize germ plasm from India, North Central and South America, Caribbean and other maize growing regions of the world has been collected under the Coordinated Maize Breeding Scheme at this center. Inbred lines developed from such wide germ plasm have been screened and are being used for production of various hybrids. During a study of this material, it was discovered that some inbred lines

frequently showed the presence of some protogynous plants in their progeny. These included among others, Cos 302-A2-1-#-2-#-# and Cau 303-1-f-f, developed from the Colombian Varieties Costeno Blanco and Cau 303; G733-A130-6-#-# developed from Funks Hybrid G733 of U.S.A.; and some S_1 lines developed from the advanced generation of a released double cross, Ganga Hybrid Makka 101.

The protogynous plants of Cos 302 and Cau 303 lines when crossed with G733-A130-6-#-# produced a large number of male sterile plants in the progeny. The actual count of male sterile to male fertile plants in the progeny of one such cross (Cau 303-1-f-f x G733-A130-6-#-#) was 30 Male Sterile:11 Male Fertile. Such male sterile plants obtained earlier in the progeny of Cos 302-A2-1-#-2-#-# x G733-A130-6-#-# (designated as $\underline{Ms}_x$) were crossed back to the pollinator inbred line, an unrelated single cross and some of them were allowed to open-pollinate. The counts of male sterile and male fertile plants obtained in each progeny are given in the table below:

Pedigree	Male Sterile	Male Fertile
Ms_x open-pollinated	50	20
Ms_x x unrelated single cross	43	7
Ms_x x G733-A130-6-#-#	15	9

Similarly male sterile plants were observed in the progeny of the protogynous plants from two S_1 lines isolated from Ganga Hybrid Makka 101, i.e. GHM 101-37 and GHM 101-44. Open-pollinated progeny from these male sterile plants showed three kinds of plants--male sterile, protogynous as well as protandrous. Some selfed progenies of protogynous plants, however, gave only protogynous and protandrous plants.

As early as 1924 J. H. Kempton reported an inherited protogynous condition in a pop corn variety from Spain and also found the presence of male sterile plants in the segregating generation following a cross with a protandrous variety of maize. Kempton further remarked, "It seems probable that proterogyny in maize is the result of a variable expression of a male sterile condition, the variability being brought about through the interaction of modifying factors."

Recently Noble and Russell (Crop Science, 1963) have reported that in backcross progeny and in recovered "$\underline{Rf}$ $\underline{Rf}$" lines with T type cytoplasm it is frequently observed that the pollen shedding is delayed relative to silking date. Similarly, in single crosses with T type cytoplasm of "$\underline{Rf}$ $\underline{Rf}$" lines the interval between pollen shedding and silking has been noted to be decreased (i.e., extent of protandry is decreased) as compared with crosses of these lines with normal cytoplasm. This is due to a delay in pollen shedding rather than earlier silking and sometimes may even result in pollen shedding taking place after silking has occurred in hybrids with sterile cytoplasm.

These results indicate either an incomplete dominance of the Rf, fertility restoring factor (Noble and Russell, 1963) or as Duvick reported (Genetics, 1956) there may be modifying or minor fertility restoring genes which affect the fertility restoration in such a way that pollen shedding would be delayed.

The observations described by the present authors seem to indicate that in nature sterile cytoplasm can be detected, as expressed in the form of protogynous condition where a complete set of fertility restoring genes and the modifiers are not present to affect normal and timely development of pollen. The pollen development is therefore delayed and results in a protogynous condition. The protogynous marker is worth being explored and may be a valuable tool for identification of sterilizable cytoplasm. Experiments are in progress to throw more light on the exact nature of gene-cytoplasm interaction resulting in the protogynous condition, and to confirm the above hypothesis.

R. L. Paliwal
T. R. Dayani

2. Primitive maize in Sikkim.

Primitive maize types were reported from the hill country of Assam and Burma by Anderson (1945), and Stonor and Anderson (1949). More recently, certain peculiar varieties, occurring in Central Nepal, have been described by Ono and Suzuki (1952). The Indian Agricultural Research Institute has been conducting a survey of maize germplasm in the northeastern Himalayas and the mainland of India. These studies have revealed that a wide spectrum of genetic variability in maize exists in the northeastern Himalayas, while the northern plains and peninsular India are characterized by a lack of such genetic divergence.

Among the collections from these regions, those from Sikkim present strikingly primitive features. A detailed morphological study has been made of two such types; physiological, genetical and cytological investigations are in progress. The distinctive morphological characteristics of Sikkim Primitive 1 (SP 1) are presented below:

Plant Characters: SP 1 is a pop corn. In its native habitat the plants attain a height of 130 cm to 200 cm. Each plant has a central stem and two to four tillers. Each stem or tiller terminates in a drooping tassel and bears from four to six ears. The lowermost four to five internodes are highly condensed and are from 2.2 cm to 5.0 cm. in length. The main stem bears about 13 leaves. The leaf bearing the best developd ear has a length and width of 57.0 cm and 5.6 cm, respectively. The venation index is about 2.5.

Tassel Characters: The tassel is drooping and has on an average five primary and seven secondary branches. The condensation index is 1.05. The basal end of the lowermost one to two primary branches bear from 10 to 20 functional pistillate flowers. These ultimately develop viable seeds.

Ear Characters: The four to six ears on each stem or tiller are borne at successive nodes, starting with the node immediately below the flag leaf node. The uppermost ear is about 5 cm long and terminates in a well developed spike, bearing functional male flowers. The successive ears downwards, gradually increase in size and the corresponding male spike decreases, till the lowermost ear is about 7.5 cm to 10.0 cm long with an incipient male spike. Ear diameter ranges from 1.5 cm to 2.3 cm. The kernel rows are not regular and range from 8 to 10 per ear. The number of husk leaves per ear are five to seven, thin and partially open upon maturity, thereby partly exposing the ear. The glumes are fine and soft, and cover the kernel up to about half its length. The kernels are very small, round, hard and pop upon heating.

N. L. Dhawan

3. The role of cytoplasm in the manifestation of heterosis and other traits in maize.

During the past several years maize germplasm from the Americas and India has been studied for the exploitation of heterosis and for the improvement of yield and other agronomic traits of maize in India. While considerable practical benefits have resulted from the release of highly productive hybrids, yet these studies have posed a number of fundamental problems that are now being investigated. Briefly stated these problems are: (1) what is the maximum heterosis that can be attained, under very high soil fertility and keeping in view the agro-climatic conditions prevalent in India, (2) the extent of genetic divergence needed in the parental races in order to obtain maximum heterosis in hybrid combinations, (3) the role of cytoplasm and hybrid nucleus in the manifestation of heterosis and other quantitative traits, and (4) the role of cytoplasm as an isolating mechanism, thereby serving as a barrier to natural crossing during the evolutionary divergence of maize races.

In the studies relating to item (3) above, all possible reciprocal combinations, between primitive and highly advanced races are being investigated in the F_1, F_2 and backcross generations. This material was selected in order to ensure that divergent cytoplasm, if present, was utilized along with a high degree of hybridity in the nucleus.

The results obtained from such reciprocal inter-racial crosses reveal that cytoplasm also plays an important role in the manifestation of heterosis and other quantitative traits. Data from the reciprocal crosses between the primitive type, Sikkim Primitive 2 (SP 2), and a highly evolved race, Colorado (Col) from U.S.A. are presented below:

Pedigree	Yield kg/ ha.	Yield % Col	Yield % SP 2	Pt. Ht. cm	Pt. Ht. % of Col	Pt. Ht. % of SP 2	Days to Silk
SP 2	615	45	100	108	61	100	74
Col	1375	100	224	177	100	164	51
SP 2 x Col	639	46	104	140	79	130	68
Col x SP 2	2972	216	483	215	122	199	56

Grain Yield: The cross Col x SP 2 gave 116 per cent more grain yield, and the reciprocal cross 54 per cent less yield than the Col parent. When compared to the SP 2 parent, the cross Col x SP 2 yielded 383 per cent more and the reciprocal cross four per cent more. The SP 2 cytoplasm inhibited the expression of the hybrid nucleus.

Plant Height: The differences between the reciprocal crosses were also striking, but not marked to such an extent as in the case of grain yield. The cross Col x SP 2 manifested 22 per cent more, and the reciprocal 21 per cent less plant height than the Col parent. When compared to the SP 2 parent, Col x SP 2 was 99 per cent taller, whereas the reciprocal cross was only 30 per cent taller.

Days to 75 Per Cent Silking: The cross Col x SP 2 (56 days) showed the earliness of the earlier parent Col (51 days), the reciprocal cross (68 days) was 12 days later in silking and approached the late parent SP 2 (74 days).

Additional data from several reciprocal crosses between primitive and advanced races are being analyzed, and a number of quantitative traits are being studied. The indications are that the degree of inhibition exercised by the cytoplasm on the expression of the hybrid nucleus varies with different races so as to give a range from complete masking to little or no masking. It appears that in the study and exploitation of heterosis one should not only look for superior hybrid nuclei but also for superior sources of cytoplasm. Fleming et al (Agronomy Journal, 1960) and Brown (Iowa Academy of Science, 1961) have presented preliminary data relating to this phase of study.

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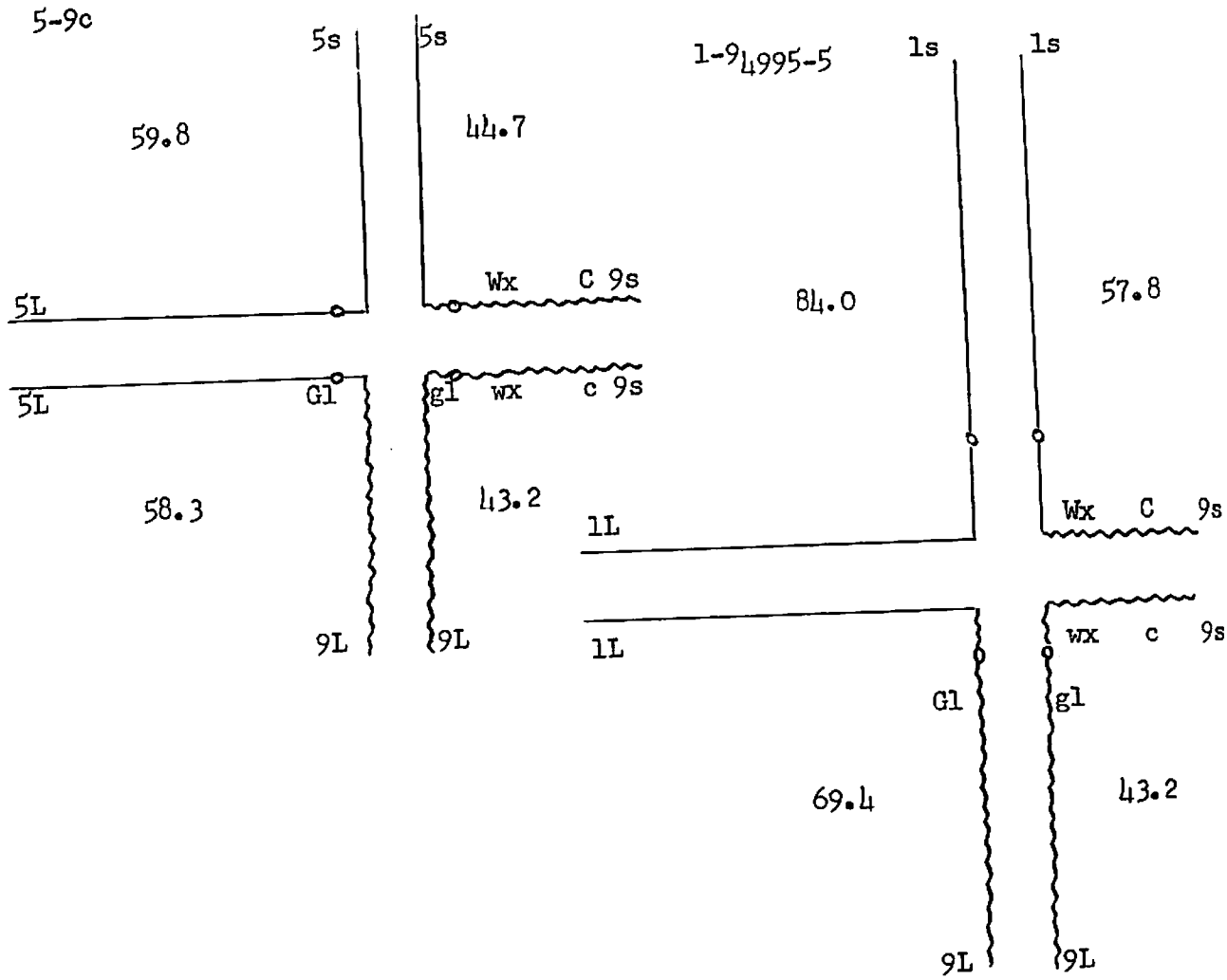
1. Cytological location of gl_{15}.

Linkage studies of gl_{15} (Coe, MNL 32:100) have shown that it gives 7% recombination with wx. The order in chromosome 9 is sh-wx-gl, but the centromere position with respect to wx and gl was not clear. A test of the location of gl was made by a genetic analysis of plants heterozygous for this gene and for translocations 5-9c, 3-9c, $1\text{-}9_{4995\text{-}5}$ and 7-9a with breakpoints in chromosome 9 at 9L .1, 9L .12, 9S .20, and 9S .07 respectively. The results from studies with two of these translocations will be discussed. Diagrams of chromosome pairing in the translocation heterozygotes and the postulated gene locations are included in this report. The relative length of each chromosome in microns is also given, as determined from Longley's chromosome measurements and the reported breakpoints.



Data from the BC1 and BC2 generations for the two translocations have been combined and are presented below:

	C Wx Gl	C Wx gl	C wx Gl	C wx gl	c Wx Gl	c Wx gl	c wx Gl	c wx gl
T 5-9c	650	94	0	170	171	14	21	483
T $1\text{-}9_{4995\text{-}5}$	590	163	5	110	114	24	39	581

Since the C Wx Gl plants were consistently used as female parents in the backcrosses, transmission of n + 1 gametes is possible and the inclusion of tertiary trisomics in the population must be considered. Certain trisomics appear as crossover types and therefore the true recombination between genes located in different arms of the translocation cannot be obtained unless all trisomics are identified by chromosome counts or by progeny tests.

In the backcross data from T 5-9c and T $1\text{-}9_{4995\text{-}5}$ the discrepancy in the C Wx gl versus c wx Gl classes indicates that the C Wx gl class

includes both trisomics and Wx-Gl crossovers. Additional evidence of trisomics comes from a comparison of the c Wx gl and C wx Gl classes from these translocations. The c Wx gl class could arise as a tertiary trisomic following a single crossover between C and Wx. This would account for the greater size of this class as compared to the C wx Gl class which comes from double crossovers. Progeny tests were made on a few suspected trisomics. In the T 5-9c backcross, 10 C Wx gl plants with pollen classified as normal or low sterile proved to be trisomic. When these plants were used as pollen parents on c wx silks, the transmission of C was 13.0% and of Wx, 3.4%. A few c wx Gl plants with intermediate pollen sterility from both the T 5-9c and T $1\text{-}9_{4995\text{-}5}$ populations also were trisomic. Three c wx Gl plants from the T 1-9 backcross progeny were self-pollinated and gave 63 Gl: 46 gl, indicating a Gl/gl/gl constitution. Thus, two of the four possible kinds of trisomics have been identified. The genetic data indicate that gametes with $5 + 9 + 9^5$ are more frequent than those with $5 + 9 + 5^9$, and $1 + 9 + 1^9$ more frequent than $1 + 9 + 9^1$.

The identification of trisomics of C Wx gl phenotype in the T 5-9c backcross indicates that Wx and Gl are in different arms of the translocation and that Gl must lie beyond 9L .1. Thus, the order in chromosome 9 is Wx-centromere-Gl.

Ellen Dempsey
Victor Smirnov

2. Linkage of du and oy.

A backcross of plants heterozygous for the du and oy mutants on chromosome 10 gave 488 individuals distributed as follows:

Du Oy	Du oy	du Oy	du oy
48	219	165	56

The du-oy recombination value is 21.3%, which agrees well with the value of 18-19% obtained from F_2 data (MNL 37). Since oy does not show linkage with R and R-du is about 20% (Kramer), oy is probably located in the short arm of chromosome 10.

Ellen Dempsey

3. Linkage studies with the Ms factor of KYS sterility.

An attempt was made to locate the Ms factor of KYS sterility. The F_1 of Mangelsdorf tester (ms ms S S) and a pale green stock (Ms Ms S S) was crossed with a KYS male parent (ms ms s s). The progeny consisted of 39 plants with normal pollen (ms ms S s) and 22 plants with partly filled pollen grains (Ms ms S s) and no completely male sterile plants. All were selfed and tested for segregation of bm_2, lg_1, su, y, gl_1, wx, and g. If ms is linked with one of the genes in the Mangelsdorf tester, most of the plants with normal pollen should segregate for that particular factor, while most of the plants with partially filled grains should

not segregate. No indication of linkage was found between Ms and any of the above markers.

Ellen Dempsey

4. Recovery of a chromosome which fails to enter the telophase I nucleus.

Plants heterozygous for T6-9b, in which the 6^9 chromosome consists of 6S, a small portion of 6L and the distal .6 of 9S, were studied cytologically in order to follow the behavior of the 6^9 chromosome through microsporogenesis. This chromosome was marked with wd and Wx and gave normal transmission of these alleles through the male gametes. However, at metaphase I it occurs as a univalent in about 30% of the cells and it is frequently excluded from the interphase nuclei altogether. Examination of anaphase I, telophase I, and interphase stages showed that the 6^9 chromosome seldom divides equationally in the first meiotic division; it is generally found on the plate at early telophase I and when the daughter nuclei are about to be formed, it moves slightly toward one pole. At interphase it is found lying in the cytoplasm as a round vesicle with chromatin somewhat dispersed. Droplets resembling nucleolar material often collect around the 6^9 chromosome. Condensation of the 6^9 chromosome occurs as the prophase II chromosomes become shorter and more distinct. After the nuclear membrane disappears, the 6^9 chromosome rejoins the other chromosomes and there is no evidence of discarded chromatin in the cytoplasm at metaphase or anaphase II or in the quartets. In a few metaphase II cells it was possible to identify the 6^9 chromosome; it was slightly apart from the other chromosomes and was a little more condensed and shortened. The 6^9 chromosome is apparently unaffected by its exclusion from the nucleus.

A similar behavior has been postulated for a univalent chromosome in monosomic wheat (Sears, Chromosoma 1952 and Sanchez-Monge and MacKey, Hereditas 1948), but their results were complicated by the occurrence of misdivision and the frequency of male transmission could not be ascertained because male gametophytes lacking this chromosome are usually non functional.

In MNL 37 it was suggested that the low transmission of translocated 6^9 chromosomes through the ovules was caused by a loss of the 6^9 chromosome in the inner two megaspores following an equational division at anaphase I. It now appears more likely that the 6^9 chromosome fails to be included in any of the megaspore nuclei and is permanently discarded in the cytoplasm. The difference in behavior in male and female flowers may be due to the orientation of the second division spindles at right angles to the first division spindle in microsporogenesis. A cytoplasmic fragment at telophase I is thus strategically located near the future site of the equatorial plate, whereas in megasporogenesis it occupies the future position of one of the poles and is less likely to move onto the plate.

Ellen Dempsey

5. Studies on preferential segregation involving In 3b and evidence of pseudo high negative interference.

It has been argued that preferential segregation caused by abnormal 10 takes place at the second meiotic division only when heteromorphic dyads (one chromatid knobbed and the other knobless) are present as a consequence of crossing over between the knob and the centromere. The data obtained previously have been consistent with this hypothesis. If a structural aberration reduced crossing over, a lesser degree of preferential segregation should occur since there would be fewer heteromorphic dyads. Studies where a piece of 3L was transposed to 9S and which resulted in marked reduction in crossing over in 9S, indicated that lower crossing over did indeed produce lower preferential segregation for loci in 9S. However, it was felt that further confirmation on this point was desirable. Therefore, plants heterozygous for K 10 and also heterozygous for In 3b were produced. The breakpoints of In 3b are in the long arm of 3 at positions .25 and .75. The loci $\underline{gl}_6$, $\underline{lg}_2$ and $\underline{A}_1$ were the marker genes in 3L, but only the Lg locus is included within the inverted segment. The following data were obtained when plants heterozygous for abnormal 10 and for a structurally normal chromosome 3 with a knob in 3L and In 3b were test crossed as the female parent.

K 10/k 10 In 3b gl Lg k A/ N3 Gl lg K a X gl lg a

(1)	(2)	(0)	(1-2)	(1-2)	(0)	(2)	(1)	
Gl	Gl	gl	gl	Gl	Gl	gl	gl	
Lg	lg	Lg	lg	Lg	lg	Lg	lg	
A	A	A	A	a	a	a	a	
10	120	985	63	44	1005	95	21	Σ = 2343

Gl-Lg = 5.4% Lg-A = 13.3%

% Gl = 50.3 % lg = 51.6 % a = 49.7

There is little indication of preferential segregation for the $\underline{lg}_2$ and $\underline{a}_1$ alleles carried in the knobbed chromosome. The proximal breakpoint of In 3b lies in that portion of the arm in which crossing over is normally low and few crossovers in the proximal uninverted segment occurred to form heteromorphic dyads. The observed results are in agreement with the hypothesis that heteromorphic dyads are essential for preferential segregation.

An interesting feature of the above data is the coincidence value for double crossovers in the Gl-Lg and Lg-A intervals. In structurally normal plants heterozygous for the Gl, Lg and A loci a coincidence value of approximately .8 has been found, but in the above data the coincidence is 5.7. This might be mistaken for a case of high negative interference were it not known that we were dealing with a heterozygous inversion. The high percentage of double crossover strands is due solely to the fact that all single exchanges within the loop give deficient crossover chromatids while the double crossover chromatids (from 2 and 3 strand doubles) give viable spores. It may be surmised that some of the reported examples of high negative interference have a basis in cryptic inversion heterozygosity.

Sister plants of the above but lacking abnormal 10 were test crossed and gave the following data:

k 10/k 10 In 3b gl Lg k A/ N3 Gl lg K a X gl lg a

(1)	(2)	(0)	(1-2)	(1-2)	(0)	(2)	(1)	
Gl	Gl	gl	gl	Gl	Gl	gl	gl	
Lg	lg	Lg	lg	Lg	lg	Lg	lg	
A	A	A	A	a	a	a	a	
3	46	463	3	7	493	45	1	Σ = 1061

Gl-Lg = 1.3% Lg-A = 9.5%

% Gl = 51.7 % lg = 51.2 % a = 51.4

Here again one could conclude that there was high negative interference since the coincidence value is 7.8 but, as with the K 10 data, the high percentage of observed doubles is due to the elimination of all single crossover chromatids within the inversion.

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1. Research for "Restorers" among Moroccan corn populations.

Corn populations collected from different parts of Morocco were crossed to the male-sterile single hybrid M 13 R^T MS x W 64 A, the tester being pollinated with a mixture of pollen from 10 plants per population. Eighty-four crossings were effected and their progenies examined for fertility of tassels. These were arranged in three groups: sterile, semi-sterile and fertile. The results of these observations are indicated in Table 1.

Table 1
Results of the Crosses

Population number	Localities	Progenies			
		Fertile	Partially sterile	Total plants	Percent sterile
1	Berrechid	28	9	102	63.7
3	Rabat	4	12	107	85.0
4	Salé	13	15	104	73.0
6	Basse-Moulouya	10	17	107	74.7
8	Rharb	0	1	50	98.0
9	Basse-Moulouya	24	27	108	52.7
10	Doukkala	33	23	110	49.0
12	Fès-Meknès	7	4	50	78.0
14	Taza	34	16	112	55.3
17	Ouezzane	20	7	106	74.5

Population number	Localities	Progenies			
		Fertile	Partially sterile	Total plants	Percent sterile
18	Rabat	5	19	77	68.8
19	El Menzel	27	21	110	56.3
20	El Menzel	25	9	50	32.0
22	Settat	18	6	50	52.0
23	Settat	15	17	105	69.5
24	Settat	9	5	50	72.0
25	Guercif	19	34	103	48.5
26	Guercif	1	6	108	93.5
27	Taza	0	5	107	95.3
28	Safi	14	18	104	69.2
30	Safi	38	21	110	46.3
32	Moyen-Atlas	10	21	50	38.0
33	Moyen-Atlas	3	29	50	36.0
34	Moyen-Atlas	3	6	47	80.8
35	Zagora	0	1	50	98.0
37	Tétouan	1	3	49	91.8
39	Tétouan	1	8	105	91.4
40	Tétouan	10	11	50	58.0
42	Tétouan	5	9	50	72.0
44	Tétouan	6	10	50	68.0
50	Ouarzazate	1	6	50	86.0
51	Ouarzazate	11	13	50	52.0
52	Ouarzazate	1	5	103	94.1
53	Al Houceïma	0	4	105	96.1
54	Al Houceïma	0	4	50	92.0
55	Souss	1	28	106	72.6
56	Souss	0	5	110	95.4
57	Souss	0	4	50	92.0
58	Souss	0	4	108	96.2
59	Souss	2	8	104	90.3
60	Souss	1	10	50	78.0
63	Souss	2	0	50	96.0
64	Souss	1	6	110	93.6
65	Souss	0	3	110	97.2
66	Souss	1	8	109	91.7
67	Souss	3	6	50	82.0
68	Souss	0	7	108	93.5
69	Souss	0	2	58	96.5
70	Souss	0	0	109	100.0
71	Ben Slimane	15	21	96	62.5
72	Berrechid	0	2	111	98.1
73	Souss	2	13	96	84.3
75	Tafilalet	0	6	50	88.0
76	Tafilalet	6	8	50	72.0
77	Tafilalet	0	3	50	94.0
78	Tafilalet	3	21	111	78.3
80	Tafilalet	0	3	110	97.2
83	Tafilalet	4	29	114	71.0
85	Beni-Ahmed	2	12	117	88.0

Population number	Localities	Progenies			
		Fertile	Partially sterile	Total plants	Percent sterile
86	Beni-Ahmed	4	16	114	82.4
87	Beni-Ahmed	2	7	108	91.6
88	Chaouen	0	1	49	97.9
89	Chaouen	0	9	111	91.8
90	Moyen-Atlas	1	10	108	89.8
93	Moyen-Atlas	0	4	50	92.0
94	Moyen-Atlas	0	7	50	86.0
95	Moyen-Atlas	1	8	50	82.0
97	Moyen-Atlas	5	23	121	76.8
98	Moyen-Atlas	0	10	38	73.6
99	Moyen-Atlas	0	0	50	100.0
101	Tafilalet	0	14	105	86.6
102	Tiznit'	0	0	50	100.0
103	Tamanar	12	9	50	58.0
105	Oued Massa	4	5	27	66.6
108	Souss	0	3	50	94.0
109	Tamanar	0	2	50	96.0
115	Sefrou	0	2	50	96.0
116	Sefrou	0	17	109	84.4
117	Sefrou	0	3	110	97.2
118	Fès-Meknès	6	35	112	63.3
119	Fès-Meknès	3	11	50	72.0
123	Tanger	0	8	50	84.0
124	Chaouia	0	4	50	92.0
126	Doukkala	2	28	50	40.0

The results are very variable but some of the populations restore male fertility to a considerable degree: 7 give fewer than 50 per cent sterile plants and 17 fewer than 66 per cent. These populations are highly heterozygous and will have to be made homogeneous before they can be used; they will, however, be good sources of restorer genes.

A. Cornu

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1. The survey of maize factors (supplement I).

The following is the first supplement to the compilation of our work from 1963. The factors are listed alphabetically.

Numerical order	Mark	Denomination	Number of genes	Number of non-included symbols
7		amarello endosperm	1	
29	bu	burned leaf	1	
34	Ce	controlling element of y^m	2	
40		corrugated leaf	1	
51	Ds	dissociation	1	
57	el	elongate chromosome	1	
60		expanded glumes	1	
63		fired	1	
65	fn	phenol colour	1	
68		fungoid	1	
70	fz	frazzled leaves	1	
73	gc	glucostactous	1	
75	gi	giant plant	1	
78		green mosaic	1	
81	ha	high amylose	2	1
88		chocolate pollen	1	
108	me	mealy endosperm	1	
109	mg	miniature germ	1	
110	mi	midget	1	2
111	mn	miniature seed	1	
112	Mp	modulator of P^v	2	2
113	mr	midrib	1	
114	ms, Ms	male sterile	22	5
115	Mt	mottled aleurone	1	
116	na	nana	2	
117	nc	necrotic	1	
118	nl	narrow leaf	2	1
119		necrotic	2	
120		new starchy	1	
121	o, O	opaque endosperm	3	1
122	og, Og	old gold stripe	1	1
123	or, Or	orange endosperm	2	
124		orobanche seedling	1	
125	oy	oil yellow	2	
126	P	pericarp and cob colour	32	1
127		pale aleurone	1	
128	pa	pollen abortion	1	
129	pb, Pb	piebald	5	1
130	Pc	purple coleorhiza	4	
131	pd	paired spikelets	1	
132	pe	pubescens hairy sheath	1	
133	pg, Pg	pale green seedling	12	5
134	Ph	purple husks	1	
135	pi	development of secondary pistillate florets	1	
136	pk	polkadot leaves	1	
137	pl, Pl	purple plant colour	1	
138	pm	pale midrib	1	

Numerical order	Mark	Denomination	Number of genes	Number of non-included symbols
139	pn, Pn	papyrescent glume	1	
140	po, Po	polymitotic	1	
141	Pp	pseudopod	1	
142	pr, Pr	red aleurone	2	
143	ps	panicula specialis	1	
144		pink scutellum	1	
145	pt, Pt	polytypic	1	
146	Pu	purple plumule	2	
147	py, Py	pigmy	2	
148	r, R	aleurone and plant colour	18	6
149		ragged seedling	1	
150	ra, Ra	ramosa ear	3	
151	rd	reduced plant	1	1
157	rp, Rp	rust susceptible (resistance)	4	2
166	sd, Sd	striped	1	
170	si, Si	silky ear	3	
171	sk	silkless	1	
182		target spot	1	
200	wd, Wd	white deficiency	1	
219	Summary		600	254

We have attempted to compile a comprehensive collection of the factors of maize, which we submit to our colleagues for their kind consideration.

Supplements and amendments will be published.

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1. The dominant mutable V^m mp-1817.

This dominant mutable expressed in the mature plant has virescent older leaves with dark green mutant stripes. A difference is observed in the virescent background of plants originating from crosses of the original green-striped plant with very white older leaves to diverse stocks. This virescent expression varies from extremes of near white to a near green and is caused by genetic modifiers in these diverse stocks. This has been confirmed by recrossing particular types distinguished by the background to lines with the known modifiers. Each line shows its distinctive effect on the expression. The relationship of the lines showing this effect, as well as linkage tests, are now being studied.

This virescent expression of $\underline{V^m}$ mp-1817 is associated with an additional feature--namely, the severe etching of the seed. It was originally not recognized since it is only detected when the mutant is used as a female and all the original crosses were made using it as a male. It may be an expression of this same mutant since the etching has not been separable from the plant character in preliminary tests.

The frequency and size of stripes also vary and are similar to the patterns of the En system. In tests of $\underline{a}^{m(r)}$ seeds giving rise to mutable plants, it is evident from the non-mutability of the $\underline{a_1}^{m(r)}$ kernels that En is absent, suggesting that the mutable is not under the control of En, but rather of another mutable system.

Peter A. Peterson*

2. Maleic hydrazide-induced chromosome breakage and its relation to differing knob number.

The roots of germinating seedlings were treated with 10^{-3} m solutions of maleic hydrazide. The chromosome breakage measured indirectly by counting anaphase bridges was studied, utilizing differing knob numbers of 0, 4, 6, 7, 8, 12 and 24. Comparisons could be made between 2 lines containing identical knobs but different knob numbers by utilizing the homozygote and the heterozygote (derived by crossing the individual strains to knobless flint).

The results show that a direct relation does not exist between knob number and chromosome breakage. For example, two different strains with 12 knobs were compared. One homozygous strain had a breakage frequency of 34.8% while the heterozygote derived from another strain, also with 12 knobs, had a frequency of 22.1%. These values were significantly different at the 1% level.

It would seem that the strains themselves as well as the particular knobs involved are important in determining the frequency of chromosome breakage. When identical knobs could be compared, it was found that the expected difference in breakage frequency was not realized. This would suggest that strain differences such as their influence on the physiology of the cell would be a significant feature influencing chromosome breakage. Additional studies to analyze the determinants involved are in progress.

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1. The production of new A-B translocations by crossing over with reciprocal A translocations.

The A-B translocations first produced by Roman have been very useful tools in genetic studies. In spite of their obvious usefulness, no new A-B translocations have been induced since Roman's initial series. This is probably due, in part, to the immense amount of work necessary to induce, isolate, and characterize such translocations. A theoretically possible alternative means of producing new A-B translocations is through crossing over between an A-B translocation and a reciprocal A translocation. We have results which seem to indicate that we have succeeded in producing several new A-B translocations in this way.

In one instance, TB-1b and T1-2c (carrying $\underline{sr}_1$) were involved. TB-1b has its break point at 1S.05 and the break points of T1-2c are at 1S.77 and 2L.33. Plants carrying TB-1b were pollinated by homozygous T1-2c plants. It is important that the crosses be made with the A-B translocation plants as females. If they are used as males, non-disjunction will give F_1 plants that are hyperploid for the chromosomal segment translocated to the B centromere. Such hyperploid segments will pair together most of the time instead of pairing with the 1-2c translocation, and thus, the necessary crossover will not be possible. If the cross is made with the A-B translocation plants as female, there will only be one B^1 chromosome present and it will thus be forced to pair with the 1-2c translocation. The pairing expected in the F_1 is shown in Figure 1. A crossover anywhere in chromosome one in the region distal to the TB-1b break point and proximal to the break point of T1-2c will produce an A-B translocation that will consist of the short arm of chromosome one from S.05 to S.77 and the distal 2/3 of the long arm of chromosome two. Such a crossover followed by adjacent I segregation will produce a balanced gamete with the new "hybrid" TB-1S, 2L.

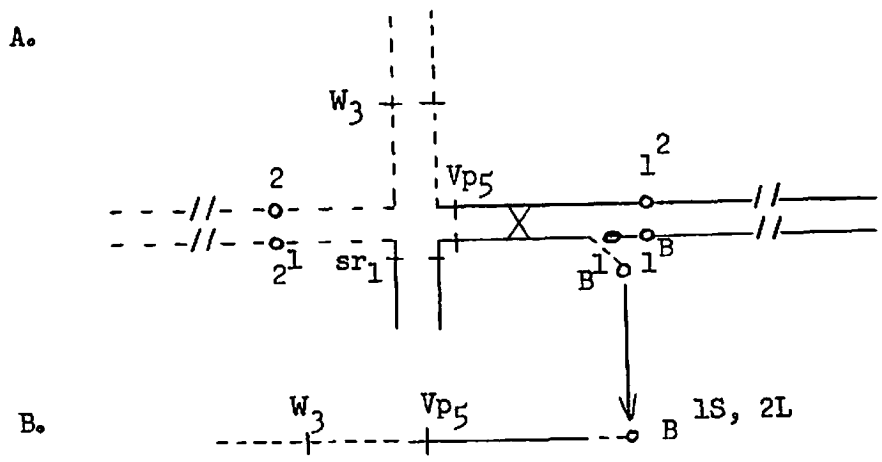


Figure 1. Expected chromosome pairing in plants heterozygous for TB-1b and T1-2c (A) and new A-B translocation produced by crossing over (B). (Gene loci discussed in the text are indicated. Positions are only approximate.)

To test for the production of such a new A-B translocation, the F_1 plants were used as pollen parents in crosses with plants carrying $\underline{w}_3$. This is a white endosperm albino-viviparous mutant located well out on the long arm of chromosome two. If the new A-B translocation was produced by crossing over, non-disjunction of the B centromere should result in the appearance of some $\underline{w}_3$ mutants on the test ears. If the deficient sperm fertilized the polar nuclei, and the hyperploid sperm fertilized the egg nucleus, white seeds will be produced with dormant embryos that give green seedlings. The reciprocal fertilization would give yellow seeds with albino-viviparous seedlings.

In tests made last summer, 8 ears were found that had white-dormant and/or yellow-albino-viviparous seeds. There were a total 51 white-dormant seeds with the number per ear ranging from 1 to 14. Approximately half of these have been planted in the greenhouse for our winter planting and these have all given green plants. There were a total of 16 yellow-albino-viviparous seeds found on these 8 ears with the number per ear ranging from 1 to 6. These figures probably do not represent the total number of deficient (hypoploid) embryos since $\underline{w}_3$ is not always viviparous. Thus, it is expected that some of the dormant yellow seed will give albino seedlings also. Theoretically, when the plants from white seeds (hyperploid plants) are selfed or sib pollinated, most of the F_2 offspring that are homozygous for the normal chromosome arrangement will also be homozygous for $\underline{w}_3$ which is carried on the normal chromosome. The remaining offspring will be heterozygotes or homozygotes for this complex rearrangement. Thus, most of the surviving plants with normal pollen will be homozygous for this new A-B translocation.

Another translocation $T1\text{-}2_{4464}$ (1S.53, 2L.28) heterozygous with TB-1b was tested on $\underline{w}_3$ stocks with results similar to those for T1-2c. Translocation $T1\text{-}2_{5376}$ (1L.77 and 2L.08) in combination with TB-1a (1L.20) when tested on $\underline{w}_3$ stocks also produced dormant-white seeds.

In order to determine if similar results could be obtained when a different A-B translocation and a different gene locus were involved, the combination of $T2\text{-}3_{6270}$ (2S.46, 3L.60) and TB-3a (3L.10) was tested against the white endosperm albescent gene found near the end of the short arm of chromosome two. In these tests, a total of 21 dormant white seeds were found. Five of these were planted in the greenhouse and all gave green plants.

The four reciprocal A translocations used in these preliminary tests were chosen so that there would be a long distance between the A-B translocation break point and that of the reciprocal A translocation. This was done to provide the maximum possibility for crossing over. Because of the long distance involved, there is a considerable piece of the chromosome arm involved in the parent A-B translocation still present in the new "hybrid" translocation. Thus, when these "hybrid" A-B translocations are used to locate unplaced genes, a positive test will mean it is carried on one or the other of the two segments involved. However, if the parent A-B translocation used to produce the new "hybrid" A-B translocation does not uncover the gene, then it must be in the segment transferred to the B centromere by crossing over. Now

that it appears that such "hybrid" A-B translocations are possible, reciprocal A translocations can be selected that will minimize the amount of the original chromosome arm incorporated in the new A-B translocation.

Not only would such hybrid A-B translocations be useful in placing new genes to chromosome arms not previously covered by A-B translocation, but they could be utilized to subdivide the regions of the present A-B translocations. If a new hybrid A-B translocation is tested with a gene that is uncovered by the original parent A-B translocation, the new A-B translocation will only uncover the gene if it is proximal to the break point of the reciprocal A translocation involved. For example, $\underline{vp_5}$ is uncovered by TB-1b. Crossover studies have shown it to be very close to the break point of T1-2c. If it is proximal to this break point, then the "hybrid" A-B translocation produced by crossing over with this translocation will still have attached to the B centromere the segment of chromosome one which carries the $\underline{vp_5}$ locus. Thus, $\underline{vp_5}$ should be uncovered by the new "hybrid" translocation. However, if $\underline{vp_5}$ is proximal to the break point of 1-2c, the $\underline{vp_5}$ locus will be carried in the 2^1 chromosome and it will not be uncovered by the new translocation.

D. S. Robertson

2. Additional data on the genetics of TB-9b.

In last year's News Letter I reported upon the segregation in plants hyperploid for TB-9b (i.e., 9 $9^{B}B^{9}B^{9}$). Hyperploid plants with the genetic and cytological constitutions shown in Figure 1 were pollinated by pollen from homozygous $\underline{c}$ $\underline{sh_1}$ $\underline{wx}$ plants.

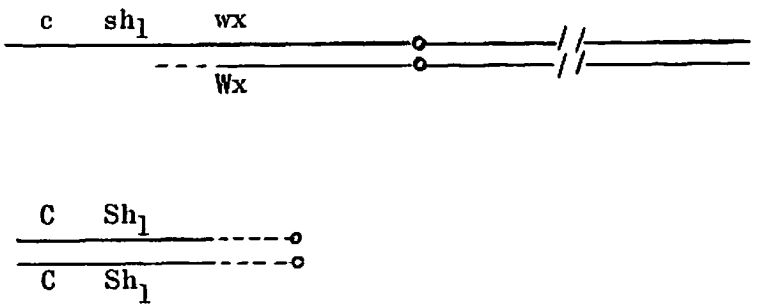


Figure 1. Genetic and cytological constitution of embryos of plants hyperploid for TB-9b.

Table 1 summarizes the testcross data and a suggested cytological configuration and genotype for each testcross phenotype observed.

Table 1

Testcross Data of Plants Hyperploid for TB-9b (c sh$_1$ wx/C Sh$_1$-/C Sh$_1$-/--Wx x c sh$_1$ wx/c sh$_1$ wx) and Suggested Cytology and Genotype for Each of the Observed Testcross Phenotypes

Testcross phenotypes and frequencies	Suggested cytology and genotype of observed testcross phenotypes
C Sh Wx Freq. 1310 % 47.83	c sh wx$_o$-//— C Sh Wx$_o$-//— $_o$
c sh wx Freq. 102 % 3.72	c sh wx$_o$-//— c sh wx$_o$-//—
C sh wx Freq. 0 % 0	
c Sh Wx Freq. 4 % 0.15	c sh wx$_o$-//— c Sh Wx$_o$-//— $_o$
C Sh wx Freq. 1315 % 48.01	c sh wx$_o$-//— c sh wx$_o$-//— C Sh $_o$
c sh Wx Freq. 7 % 0.26	c sh wx$_o$-//— c sh Wx$_o$-//— $_o$
C sh Wx Freq. 0 % 0	
c Sh wx Freq. 1 % 0.04	c sh wx$_o$-//— c sh wx$_o$-//— c Sh $_o$

Last year, directed segregation of the B and 9 centromeres, after crossing over in the c-sh region, was suggested to explain the presence of four c Sh Wx seeds in the absence of the reciprocal crossover class (C sh wx) and the extremely rare occurrence of the c Sh wx class. Additional data reported in Table 2 do not support such an explanation.

Table 2

Summary of Colorless Classes in Testcrosses of Plants Hyperploid for TB-9b (c sh_1 wx/C Sh_1-/C Sh_1-/--Wx x c sh_1 wx/c sh_1 wx)

Phenotypes	Frequency	Percentages
c sh wx	446	2.7
c Sh Wx	34	0.2
c sh Wx	63	0.4
c Sh wx	21	0.1
Total colored	15,805	96.6
	16,369	

The presence of 21 c Sh wx seeds suggests that crossing over between the B^9 and the normal 9 chromosomes in the c-sh region does not affect the independent assortment of the two pairs of chromosomes (i.e., the 9 and 9^B pair and the pair of B^9 chromosomes). If such independent assortment occurs, half the time the crossover B^9 chromosomes will end up in the same nucleus as 9^B chromosomes at the end of the first meiotic division. When this happens, one of the second meiotic division products of this nucleus will be C Sh Wx (non-crossover) and the other will be c Sh Wx (crossover). The c Sh wx class would be expected if the crossover B^9 chromosome went to the same first telophase pole as the crossover 9 chromosome. However, the c Sh wx class would be expected in only half the frequency of the c Sh Wx class since half the time the crossover B^9 chromosome will be aligned on the second metaphase spindle so that the crossover B^9 chromatid and the crossover 9 chromatid will go to the same pole resulting in a phenotype that cannot be distinguished from the non-crossover C Sh wx phenotype. The other 50 per cent of the time, when the chromosome alignment on the second metaphase spindle is such that the crossover B^9 chromatid and the non-crossover 9 chromatid go to the same pole, half of the second division products will be c Sh wx. In summary, a crossover in the c-sh region followed by independent assortment of the chromosomes involved will result in two alignments of first metaphase chromosomes. The first, when the two crossover chromosomes go to opposite poles, will result in $\frac{1}{4}$ of the products being c Sh Wx. The other alignment in which the two crossover chromosomes go to the same pole will result in 1/8 of the products being c Sh wx. Thus, the c Sh Wx class is expected to be twice as frequent as the c Sh wx class as the data indicate. If the foregoing explanation of the origin of the c Sh Wx classes is correct, plants from these seeds when used as pollen parents should show the typical non-disjunction of plants carrying an A-B translocation. When plants from c Sh Wx seeds were crossed as pollen parents to plants carrying yg_2, which is located near the end of the short arm of chromosome nine, 13 of the 14 F_1 ears produced segregated for yg_2, thus confirming the cytology suggested in Table 1. The same plants when used as females for crosses involving yg_2 pollen did not segregate any yg_2 seedlings. As would be expected, reciprocal crosses of 12 plants of the c Sh wx to plants carrying yg_2 did not segregate for this mutant.

The C sh wx class, which is the reciprocal of c Sh Wx, is rare (from the material summarized in Table 2, only one C sh wx seed was observed), because this class would be the result of two rare events: (1) a crossover in the c-sh region followed by (2) the non-disjunction of the B^9 centromeres.

In our 1963 report, three possible explanations for the c sh_1 Wx class were suggested. The most likely explanation involved a crossover between the sh_1 locus and the translocation point putting c and sh_1 on the B^9 chromosome (see Table 1). When upon independent assortment this crossover chromosome goes to the same pole as the 9^B chromosomes, one-fourth of the products will be c sh Wx. However, if the crossover B^9 chromosome ends up at the same pole as the crossover chromosome 9, the crossover products will form combinations that result in phenotypes classified as non-crossovers (i.e., c sh wx and C Sh wx). If the foregoing explanation of the origin of the c sh_1 Wx class is correct, these plants will be heterozygous for TB-9b and should show the typical non-disjunction when used as a pollen parent. Such plants were also reciprocally crossed to plants carrying yg_2. No yg_2 plants were found in the F_1 progeny when 26 c sh Wx plants were used as females. However, when these same plants were outcrossed as males to plants carrying yg_2, 18 of the F_1 ears obtained segregated for yg_2 seedlings while two did not. These two may be the result of the failure of B centromere to undergo non-disjunction or they could have been produced by a crossover in the region between sh-wx proximal to the translocation break point. This would yield a chromosome 9 of the genotype c sh Wx. Such a crossover event accompanied by non-disjunction would result in seeds of the c sh Wx phenotype that would have only normal chromosomes and thus would give only normal seedlings when crossed reciprocally with plants carrying yg_2.

Previously, we had suggested that the C Sh wx class resulted from the presences of a normal chromosome nine (c sh wx) and a B^9 chromosome (C Sh) in the same megaspore. However, there is a possibility that such a phenotype could be produced by a crossover in the region proximal to Sh and distal to the translocation point. Such an event if followed by either normal disjunction or non-disjunction of the B^9 chromosomes could produce some C Sh wx phenotypes. If the non-crossover explanation is correct, then on testcrossing C Sh wx plants, less than 50% C seeds should be observed. If the C Sh wx class was produced by crossing over followed by non-disjunction of B centromere so that the B^9 chromosomes did not end up in the same megaspore as the crossover C Sh wx chromosomes, then 50% of the testcross seeds should carry C. If this class was produced by crossing over followed by normal disjunction of the B^9 centromeres, then the C Sh wx seed would have 2 normal chromosomes nine, one carrying c sh wx and the other C Sh wx (C.O.) and a B^9 chromosome carrying c sh (C.O.) or C Sh. Testcrossing the plants from such seed should yield ears with slightly under 50% C seeds or considerably more than 50% C depending upon the genotype of the B^9 chromosome. In summary, if the C Sh wx class was produced by crossing over, testcrosses of this class would be expected to yield approximately 50% or more C seeds. Table 3 is a summary of testcross data in which C Sh wx plants were used as male and female parents. It will be noted that the total

Table 3

Summary of Testcrosses of C Sh wx Plants Used as Males and Females

	Male parent				Female parent			
	C Sh	C sh	c Sh	c sh	C Sh	C sh	c Sh	c sh
Number	509	21	5	2962	1719	5	12	4420
Per cent	14.56	00.60	00.14	84.70	27.92	00.08	00.19	71.80

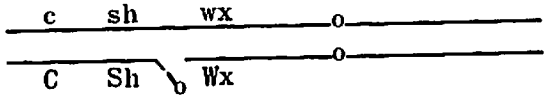
percentage of C in both sets of data is considerably below the 50% level. In the individual ear data, the highest percentage of C seeds observed in the male data was 32.39% and in the female data 35.26%. These data support the non-crossover origin of the C Sh wx class.

Both Rhoades (Genetics 21:491-502, 1936, Genetics 25:483-520, 1940) and Maguire (Genetics 49:69-80, 1964) have studied plants which were hyperploid for duplications similar to those carried by the C Sh wx plants. The genetic results would suggest that segregation in the hyperploid C Sh wx plants most closely resembles that described by Rhoades for plants which were hyperploid for a telocentric short arm of chromosome 5.

Plants of the C Sh Wx phenotype from the testcross progeny of original 9 $9^{B}B^{9}B^{9}$ hyperploid plants were testcrossed as females. These should be heterozygous for TB-9b and have the genotype c sh wx/C Sh Wx with the dominant alleles carried on the translocated chromosomes. A summary of this testcross is given in Table 4.

Table 4

Summary of Testcross Data for Plants of the Constitution



	C Sh Wx	c sh wx	C sh wx	c Sh Wx	C Sh wx	c sh Wx	C sh Wx	c Sh wx
Number	784	1054	18	19	812	66	1	19
Per cent	28.27	38.01	0.65	0.69	29.28	2.38	0.04	0.69

The striking thing about these data is the high frequency of the C Sh wx class (29.28%). This value approximates those for the C Sh Wx and c sh wx classes. The most reasonable explanation for such a distribution would be that the B^{9} chromosome in meiosis I moves at random with respect to chromosome 9 and 9^{B} so that the following distributions are produced in equal frequency 9 : $9^{B}B^{9}$: 9 B^{9} : 9^{B}. Since 9^{B} gametes do not function, 1/3 of the non-crossovers would be expected to be C Sh Wx, 1/3 c sh wx and 1/3 C Sh wx.

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1. Mendelian characters in Italian maize.*

Self pollination has been carried out in plants of 220 further samples of Italian maize provided by the "Stazione di Maiscultura" of Bergamo.

The following mutants have been detected in a total of 1,448 progenies.

Character	No. of cases exhibiting a ratio of 3:1	15:1	Character	No. of cases exhibiting a ratio of 3:1	15:1
A. Seed Traits			B. Seedling traits (in greenhouse)		
Defective seed	107	4	Abnormal growth	41	38
Small seed	3	-	Allium type	-	2
Pregermination	6	1	Albescent	2	4
Germless	16	1	Albino	9	10
Floury endosperm	6	-	Fine stripe	12	9
Lemon	15	2	Glossy	56	6
Opaque	5	-	Green mottled	16	4
Shrunken	6	-	Booster color	20	6
Waxy	2	-	Japonica	4	5
White	10	-	Yellow green	20	5
"Orange skin"	2	-	Lutescent	10	2
			Luteus	21	4
C. Plant character (in field)**			Liguleless	5	-
			Dwarf	12	3
Zebra type	18	-	Albino (lemon seed)	1	-
Brown midrib	5	-	Pale green	69	12
Golden stock	4	-	Pale luteus	4	-
Striped	14	-	Virescent	43	14
Argentea	1	-	Green striped	3	1
Golden	1	-	Sun red	1	-
Opposite leaves	1	-	Open coleoptile	2	-
White leaf base	1	-			
Adherent	3	-			
Tassel seed	1	-			
Ramosa	1	-			
Brachitica	3	-			
Crinkly leaf	1	-			

**These mutants refer to a reduced sample of the studied material.

*Work subsidized by the Rockefeller Foundation, New York.

Appropriate allelism tests and/or crosses of these mutants as well as those reported in previous MNL issues are underway.

Preliminary results from such experiments, for a part of these mutants, are summarized as follows:

All the waxy types are allelic to the known mutant on chromosome 9; on the contrary, most of the opaques turn out to differ from one another. Also the sugary mutants are all allelomorphic to $\underline{su}_1$.

Several shrunken stocks contain $\underline{sh}_2$ alleles, but others are different. One of them appears "uncovered" by TB-4. Incidentally, in the same chromosome region involved in the TB-4 stock a shrunken type has been obtained following artificial mutagenesis experiments.

Among the seedling traits, the program has made substantial progress mainly thanks to the work of Dr. F. Salamini, whose results are condensed in the following table:

	gl_1	gl_2	gl_3	gl_4	gl_6	gl_7	Total
Number of gl allelic to known mutants	57	11	20	1	2	1	92
Number of gl possibly allelic to known mutants	4	2	10	1	2	1	20
Number of gl not sufficiently studied							21
Mutants examined							133

The glossy types have been also crossed by TB-A stocks. Out of a total of 346 ears from such crosses not a single case has been found "uncovered" with the exception of TB-7 and TB-3 (6 and 2 ears respectively). The latter, however, were cases in which alleles of $\underline{gl}_1$ and $\underline{gl}_6$ were involved, and these are known to be located in the distal part of long arm of chromosome 7 and 3, respectively. A somewhat analogous finding has been obtained in artificial mutagenesis; while, it is possible with ethyl methansulphonate to obtain chlorophyll mutations in the chromosome regions involved in the TB-A stocks, not a single case of glossy has been detected making appropriate use of TB-3, TB-4, TB-9, and TB-10.

Three liguleless types turned out to be alleles of the $\underline{lg}_1$ locus.

Most of the dwarf plants are semilethal or sterile. One case is in the chromosome region involved in the TB-1a stock.

Among the chlorophyll mutations, the following are reported: two pale green in the translocated region of TB-9a; one pale green in the translocated region of TB-7; one yellow green in the translocated region of TB-3; one yellow green in the translocated region of TB-7; two yellow

green in the translocated region of TB-9b; one yellow stripe in the translocated region of TB-7.

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B. Borghi
C. Lorenzoni
M. Pozzi
F. Salamini

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1. Crossing-over in the A_2-Bt-Pr region.

Recombination data for markers of chromosome 5 in different genetic backgrounds are reported in the following table (backcross of the multiple recessive to heterozygous seed plants possessing T cytoplasm):

Genetic backgrounds	Kernel classes					
	A Bt Pr	A bt pr	A Bt pr	A bt Pr	a Bt	a bt
A 158	1725	76	335	59	139	1905
W 22	1320	59	383	31	69	1648

From these data the following recombination frequencies and standard errors may be calculated:

	Region A-Bt	Region Bt-Pr	Double recomb.
A 158	6.5 ± 0.4	17.9 ± 0.8	3.3 ± 0.4
W 22	4.5 ± 0.1	23.0 ± 0.6	2.3 ± 0.4

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M. G. Petruccioli

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1. Another isolation of the En-Spm system.

Two pale green plants with sectors of dark green were present in a 13 plant progeny of a second generation self in a corn breeding nursery in 1959. The unstable pale green plants were outcrossed as males to available silks in an inbred nursery. The unstable pale green phenotype again appeared in the F_2. Pollen from these unstable pale green plants

was used to pollinate three different tester stocks each sensitive to a different controlling element, viz: (1) C Ds (no Ac), (2) a_1^m (no En) and (3) a_1^{m-1} (pale aleurone, no Spm).

The kernels from the cross with the C Ds tester would be expected to show a chromosome breakage pattern in the F_1 if the pale green stock carried recessive c as expected and an Ac-like element. They did not show such a pattern.

The second and third crosses had to be carried to the F_2 to detect mutability at the a-locus because the unstable pale green plants carried a dominant A_1 allele. In both crosses a few dotted seeds were present on the F_2 ears as one would expect if A and a^m were segregating along with C-c, R-r and if a controlling element were present as well. One F_1 with the pale aleurone a^{m-1} stock was backcrossed to the a^{m-1} parent and produced an ear which segregated 1/2 purple to 1/2 dotted as expected where A and a^{m-1} were segregating in the presence of C- and R-. The fact that all of the non-purple class were dotted is puzzling.

It thus appears that my unstable pale green stock activated mutable a_1 alleles which had previously been described as part of the En and Spm systems. This is additional confirmation of Dr. Peterson's report that his En element is the same as Dr. McClintock's Spm (MNL 37:72).

The origin of this new isolation of a mutable system can be traced to the hybrid of three dent corn stocks, viz: (1) C0111, an inbred line produced at the Central Experimental Farm, Ottawa, Canada; (2) a line received in 1952 from the Eastern States Farmers Exchange which had come originally from the Bishop open pollinated variety; and (3) J.H.L.E., a long eared variety received from Dr. W. L. Brown in 1954. Evidence of a mutable phenotype had not been observed in these three lines prior to combining them into a three-way hybrid, nor in the first two lines which have been grown every year since. No unstable pale green plants were observed in the first segregating generation either, although the progeny consisted of only 13 plants, as did the next selfed generation in which the two original mutable pale green plants were found.

R. I. Brawn

2. Mutation of P^{cw} to P^{wr}.

Further observations are available on the origin of the colorless pericarp-red cob inbred Pa W703 which apparently arose by mutation from the red pericarp white cap-white cob inbred Q703 (see MNL 36:50 and 37:109). The F_1 from the cross Q703 x Pa W703 was both selfed to produce an F_2 and testcrossed to the colorless pericarp-white cob allele P^{ww} as carried by inbred A171 to produce:

	F_2	$F_1 \times P^{ww}$
Red pericarp white cap - red cob	363	0
Red pericarp white cap - white cob	196	347
Colorless pericarp - red cob	160	329
Colorless pericarp - white cob	0	0

The F_2 results do not show a significant deviation from a 1:2:1 ratio while the testcross fits a 1:1 ratio consisting of parental classes only. Both populations suggest that the two inbreds differ at only one locus with regard to pericarp and cob color. It is suggested that the original inbred Q703 carried $\underline{P}^{cw}$ and the derived inbred Pa W703 has $\underline{P}^{wr}$. This would have involved a mutation of both the pericarp and cob color component of $\underline{P}^{cw}$, the pericarp component from dominant to recessive and the cob color component from recessive to dominant, to produce $\underline{P}^{wr}$.

R. I. Brawn

3. Tl-2c - P linkage.

Testcross data of a plant heterozygous for Tl-2c and $\underline{P}^{wr}$-$\underline{P}^{ww}$ indicates about 20 per cent crossing over between them:

Tl-2c P^{wr}	Tl-2c P^{ww}	+ P^{wr}	+ P^{ww}	Σ
11	48	41	10	110

R. I. Brawn

4. Brown pericarp and salmon silks with P^{vv}.

The gene combinations $\underline{P}^{vv}$ bp (brown pericarp) and $\underline{P}^{vv}$ sm (salmon silks) have been synthesized. The first has brown stripes on a clear background as expected. The silks with sm, however, are not pigmented, even when a sizeable area of red pericarp occurs on an ear. This is understandable since the red pigment of the pericarp on newly arisen red spots, and the stripes of medium variegated, do not seem to extend to the silk attachment region.

On the other hand the pericarp phenotype "dark crown", which has thus far defied genetic analysis, might be expected to interact with sm to produce pigmented silks. It is planned accordingly to examine the dark crown situation in the background of sm.

The principle reason for synthesizing these combinations is to study the residue at the P locus after the controlling element Mp has transposed away from the locus. It appears that the self-colored (red) pericarp mutants arising from $\underline{P}^{vv}$ following transposition of Mp are not all alike in color. In the background of sm and bp it may be possible to make more definitive observations.

R. I. Brawn

MAIZE RESEARCH STATION
Yousafwala (Montgomery), West Pakistan

Maize is an important summer season crop in West Pakistan where it is grown over an area of over one million acres every year for the production of grain. About as much area is grown for fodder. Maize being a

crop that provides subsistence for both human beings and cattle, considerable stress is being laid on its improvement in recent years. A Research Station equipped with necessary facilities has been started at Yousafwala (Montgomery) with the object to evolve and introduce high yielding maize hybrids suitable for the soil and climatic conditions available in this part of the country. A good collection of elite inbred lines has been collected from United States and elsewhere for direct use in the commercial hybrid seed production after ascertaining suitable cross combinations.

Some of the important problems tackled at this Research Station are briefly outlined below.

Evolution of maize hybrids for different cropping patterns.

In countries like Pakistan where agriculture has to face a fast increasing population, more intensive cropping systems have to be adopted. Different types of hybrids are required for introduction to various cropping patterns prevalent in West Pakistan. In submontaneous area districts short-duration hybrids are needed for growing in between two crops of potatoes while hybrids with medium maturity are required for wheat growers who want to take a crop of maize before planting their wheat crop. For sugar cane and tobacco growers maturity is no problem. They want high yielding hybrids that may take up to 120 days to mature.

To provide maize hybrids for different cropping patterns breeding material of different maturity periods is being developed.

A variety obtained from Nouran Valley in the northern areas of Pakistan that comes into flowering 30 days after planting and matures in 58-60 days has been included in the breeding program for evolving early maturing hybrids. It is being crossed to elite inbred lines viz: Pb7, M14, WF9, 38-11, Hy, L317, 52B and 20P2. The resulting material will be divided into different maturity groups and desirable lines drawn for commercial production.

Studies on borer resistance in maize.

Corn borer (Chilo-zonellus Swin.) is a serious pest of maize in West Pakistan. It inflicts heavy losses every year to the corn industry in this country. With a view to developing breeding material that can withstand the attack of this devastating pest a few exotic hybrids known to be resistant to European corn borer (Pyrausta nubilalus HBN) were imported through the courtesy of United States Operations Mission in Pakistan. Advanced generations of these hybrids were subjected to the attack of the pest during the spring season. Artificial liberation of larvae was also tried. It is gratifying to note that quite a number of plants in the progeny of each hybrid stood up. These plants were interpollinated and their progeny grown during the regular crop season, i.e., late in summer. Selected plants from this synthetic were again interpollinated and the resulting plants again subjected to the attack of borer in the next spring season.

It has been observed that in every advanced cycle generation the number of plants that withstand the attack of borer is increasing significantly. It is hoped that after a few more cycles of recurrent selection sufficient resistance will be developed in the material to withstand the attack of the pest.

This corn borer is active from March until July, thereby relegating the growing period of maize to the fag end of the season. The limited growing season has forced the farmers to grow short duration varieties with the result that acre yields of maize are very low in this country. Efforts to develop borer resistant maize hybrids will enable the farmers to grow a long duration crop and thereby increase their yield.

Evergreen maize hybrids.

The maize crop is raised both for grain and green-fodder in the canal colonies of West Pakistan. The stalks of the crop usually dry up at the time of maturity and as such cannot be utilized for forage purposes. Under the circumstances the necessity for the evolution and introduction of a maize strain that matures its grain while the plants are still green had long been felt. One of the breeding lines at Yousafwala was found to be segregating for the evergreen character. The plants bearing this character remain perfectly green for about one month after the cobs are matured and harvested. Hybrid combinations from this material will provide the farmer at once grain for his family as well as green fodder for his cattle. There is a general shortage of green fodder at the time when the maize crop is harvested, as the summer season fodder crops are over and winter season crops are not yet ready for harvesting.

In view of great economic value of the evergreen character for the farmers in West Pakistan, a regular breeding program has been undertaken to transfer this habit into important inbred lines viz: Pb7, M14, WF9, L318, 38-11, WM13R, 54AP1 and 20P2 to incorporate it in the commercial hybrids.

A. Ghafoor Bhatti

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1. Chromosome numbers in maize root tissue culture.

During the past year and a half we have maintained continuous cultures of corn root callus on a modified basic White's media containing 2-4D. Before attempting to utilize such a tissue culture technology in genetic studies it was important to first determine the stability of the chromosome complement. Toward this end, and after nine months of continuous culturing, chromosome counts were made on nine cultures derived from seven independently initiated ones representing marked genetic stocks in two inbred backgrounds.

Eight of the nine cultures proved to yield cells with only the normal diploid 20 chromosomes. In such a population of chromosome counts cells are found with chromosome number estimates other than twenty (18, 19, 21, 22); these are rare and are found also in intact root cells scored as controls. The one exception to the diploid genome which has been encountered is a presumed diploid-tetraploid chimera, in that cells from the same culture yielded 20 and 35-40 chromosome counts.

Again at twelve months after initiation of the cultures chromosome counts were made on part of the material assessed at nine months. The same diploid chromosome number was found. The diploid-tetraploid chimera culture was not re-evaluated due to its poor rate of growth at that time and since. It is not, however, the only culture we have been experiencing growth rate problems with.

As far as can be determined by comparative observation between chromosome squashes of callus and intact root cells, no intrachromosomal aberrations are apparent.

Irwin M. Greenblatt
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1. Two rings of 10 chromosomes each.

Plants having $2^{\circ}10$ were obtained from the cross of 1-5-6-7-8 by 3-2-4-9-10/3-2-4-9. A few seeds were obtained by backcrossing these as ♀ with pollen of 1-5-6-7-8. The plan is to establish a stock that is homozygous for both groups of interchanges. This will be irradiated to combine the two rings.

2. Chromosome identification set of interchanges.

Interchanges: 1-2a, 2-4d, 3-7c, 5-7c, 8-9a and 8-10b, backcrossed to the A188 inbred; but segregating 1 heterozygous:1 homozygous interchange are available.

3. Crosses between interchanges involving the same chromosomes.

Preliminary results have been obtained. In 2-6(8786) x 2-6c(2S.90, 6S.77 x 2L.37, 6L.25). There were 61 cells with an association of four at diakinesis and 10 with 10 pairs. In all cells with 10II, only one was associated with the nucleolus. This indicates that the end segments of the "pairs" from the interchange complex are paired homologously; the mid-segments in these same "pairs" are non-homologous. This agrees with the observation by Tabata (Cytologia 28:278-292, 1963).

In 2-6f x 2-6(8786) (2L.79, 6L.87 x 2S.90, 6S.77) there were 28 cells with an association of four chromosomes and 10 with 10 "pairs." In the latter, there were either two nucleoli, each associated with a "pair" of chromosomes, or a single nucleolus with two "pairs" attached. In these "pairs" the mid-segments are homologous, and the end segments are non-homologous. In this cross the total mid-segment length was about 5 times that of the end segments. In the cross described in the first paragraph the two were about equal.

Other combinations of 2-6 and 1-5 interchanges will be grown for further studies.

C. R. Burnham
assisted by Ron Phillips
Don Buck
Ted Lorch

UNIVERSITY OF MISSOURI
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1. Tetrasporic embryo sac development in trisomic sectors.

A reconsideration of the apparent grouped crossovers and trisomics from the heterozygote $\underline{\alpha}$ $\underline{a}$ $\underline{sh}/\underline{a}^{m}$ $\underline{Sh}$ reported in the Maize News Letter No. 31 has revealed an interesting explanation. When the above heterozygote which was prepared with 4 different alleles in the $\underline{a}^{m}$ position was crossed by an $\underline{a}^{s}$-$\underline{sh}$, $\underline{Dt}$ pollen parent, a number of crossover types occurred as would be expected if $\underline{a}^{m}$ paired with either $\underline{\alpha}$ or $\underline{a}$ and normal exchange took place. The frequencies were low therefore the cases as expected usually occurred as single seeds; however, there were a few in groups of two or more including one with 5 dilute dotted nonshrunken seeds arranged so that there was little likelihood of their being due to coincident occurrence of single rare events. Hence, the earlier conclusion of groups of crossovers. On test it was discovered that the dilute nonshrunken cases ($\underline{\alpha}$ $\underline{a}^{m}$ $\underline{Sh}$, $\underline{\alpha}$ $\underline{a}$ $\underline{Sh}$ and $\underline{\alpha}$-$\underline{Sh}$) included in addition to the usual crossovers some that had trisomic or parental type embryos. Furthermore the 5 seeds in the sector described above had some of both. The number of these noncrossovers (Table 1) was 48 trisomics, 40 $\underline{\alpha}$ $\underline{a}$ $\underline{sh}$ parentals, and 42 $\underline{a}^{m}$ $\underline{Sh}$ parentals or roughly 1:1:1.

Explaining the occurrence of dilute nonshrunken endosperms with trisomic embryos is easy if we invoke nondisjunction and the production of trisomic and monosomic daughter cells in the germ line. However, this will not explain noncorresponding embryos unless we admit further nondisjunction in the embryo sac developed from an n + 1 megaspore. If this does occur it provides at best 2 tetrasomic : 1 $\underline{\alpha}$ parental : 1 $\underline{a}^{m}$ parental embryo which is not what we observed.

If we assume occasional trisomic megasporocytes with an extra chromosome 3 we can explain the noncorresponding embryos by further assuming

Table 1

Distribution of All the Dilute Nonshrunken (a Sh) Cases from the Heterozygote a a sh/a^m Sh Pollinated by a^s sh

Allele Tested	Seeds Examined	Total a Sh	Dilute Nonshrunken Cases Analyzed							
				Crossovers			Non Crossovers			
								Parentals		
			Total	a a^m Sh	a a Sh	a-Sh	Trisomic	a a sh	a^m Sh	Lost
a^m-1	312057	251	155	20	35	61	13	11	15	96
a^m-3	131448	32	28	0	0	5	10	5	8	4
a^m-4	40501	26	24	0	5	6	3	5	5	2
a^s	307090	287	166	0	43	68	22	19	14	121
Total	791096	596	373	20	83	140	48	40	42	223

that all four meiotic products are included in the development of the embryo sac. If we work out the frequencies and types of endosperms and embryos from tetrasporic embryo sac development we find that we may expect dilute dotted nonshrunken endosperms associated with 4 $\underline{\alpha}$ $\underline{a}^m$: 1 $\underline{\alpha}$ $\underline{\alpha}$: 1 $\underline{a}^m$ $\underline{a}^m$: 3 $\underline{\alpha}$: 3 $\underline{a}^m$ embryos. This of course is not our expected 1:1:1 ratio; however, it was recognized that when the data were collected $\underline{\alpha}$ $\underline{\alpha}$ $\underline{a}^s$ or $\underline{a}^m$ $\underline{a}^m$ $\underline{a}^s$ trisomics would not be distinguished from $\underline{\alpha}$ $\underline{a}^s$ or $\underline{a}^m$ $\underline{a}^s$ parentals and would be classified as such in the tables. A recheck of the remaining test ears of the parental cases confirmed that some were indeed misclassified trisomics. By combining these groups we again have a 1:1:1 ratio. The data provide a good fit as shown by a χ^2 of .8000 and a P value of .68.

From this we conclude that when nondisjunction for chromosome 3 occurs in mitotic divisions of the germ line to produce a trisomic sector, this provides a condition where tetrasporic embryo sac formation occurs with the resulting production of tetrasomic endosperms and trisomic and noncorresponding parental type embryos.

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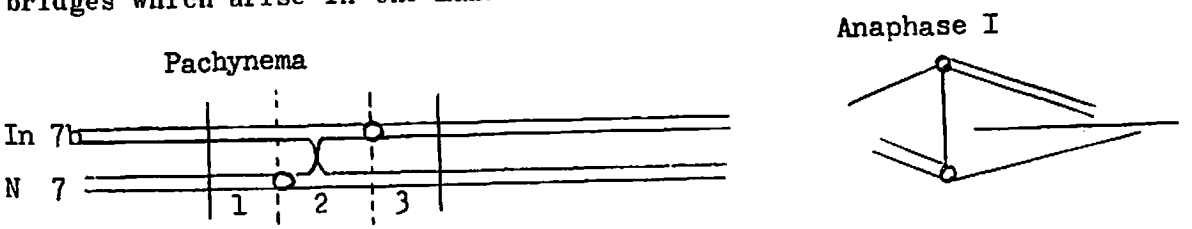
1. The detection of non-homologous crossing over.

In the 1961 News Letter it was suggested that the occurrence of non-homologous crossing over could be detected by the use of pericentric inversions which exhibit a high frequency of non-homologous pairing. Non-homologous crossing over may be detected by the observation of anaphase bridges which arise in the manner shown below:



A crossover in region 2 will lead to the formation of a bridge and a fragment at the first anaphase of meiosis. This is something not normally expected in a pericentric inversion heterozygote. Since anaphase bridges may also arise from short heterozygous paracentric inversions which may be present in the material and may be difficult to detect cytologically, it is necessary to examine normal sibs of the pericentric inversion heterozygotes and to determine if there are bridges present without the pericentric inversion. In Table 1 data are presented for In 7b (S.32-L.30).

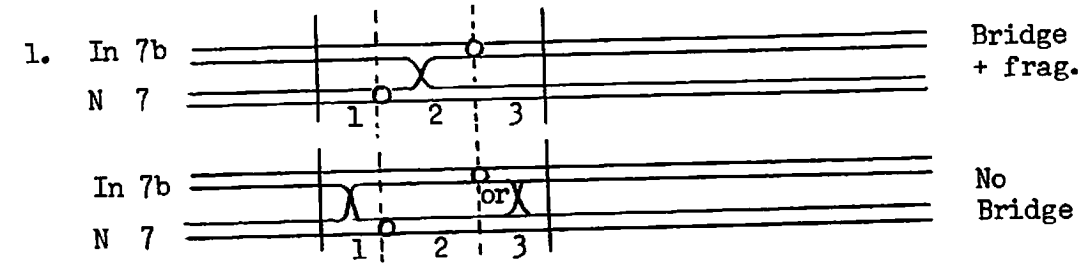
Table 1

Anaphase Configurations of In/N and N/N Plants

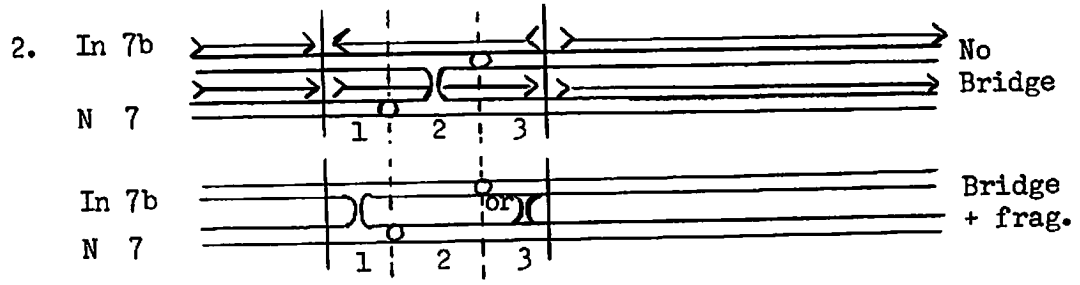
In/N Plant no.	Number of cells			N/N Plant no.	Number of cells		
	No Bridge	Bridge + frag.	Bridge, no frag.		No Bridge	Bridge + frag.	Bridge, no frag.
- 3	532	3	1	- 4	478	0	0
- 9	515	4	0	-16	538	0	0
-10	628	6	1	-18	401	0	1
-13	576	1	1				
Total	2251	14	3	Total	1417	0	1

There were 14 cases of bridge and fragment formation out of a total of 2,268 cells examined or 0.62%. There were no cases in the controls. When a bridge is found without a fragment it is possible that these are not true bridges but rather they may represent chiasmata which have not been resolved. Consequently, they will be disregarded.

These results are fairly good evidence for the occurrence of non-homologous crossing over. How the non-homologous crossing over takes place is not clearly established as there are three hypotheses which could explain it. The first has been suggested in the diagram on the preceding page; it is the simplest--namely that crossing over proceeds as it does with homologous paired segments.

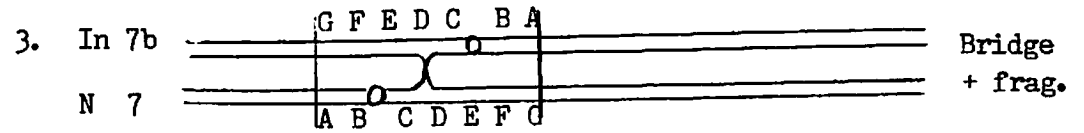


The second hypothesis takes note of the possibility that the chromosomes may be polarized and therefore crossing over may take place in the manner shown below.



The third hypothesis is based on the fact that in the middle of the non-homologously paired segment there is a site perhaps which is homologous. Crossing over could take place at this site. It would be then actually homologous crossing over. What this site where the

homology meets (coming in opposite directions) consists of is a matter of some interest as it would cast some light on the nature of the pairing code. It may be a pair of chromomeres or a pair of nucleotides.



Which hypothesis is correct is difficult to determine. In case of hypotheses #1 and #3, the fragment is always the same size--it consists of the equivalent of one chromatid. On the contrary, under hypothesis #2, the size of the fragment would be variable, sometimes very small from a crossover in region 1 and sometimes larger than a one chromatid equivalent from a crossover in region 3. The observed fragments were uniformly large, so hypothesis #2 is probably not correct.

The use of other pericentric inversions is planned. If anaphase bridges are found in inversion heterozygotes where the two break points are equidistant from the centromere, then hypothesis #2 may be valid. Or if one break point is close to the centromere and there are a considerable number of anaphase bridges formed, this would tend to invalidate hypothesis #2.

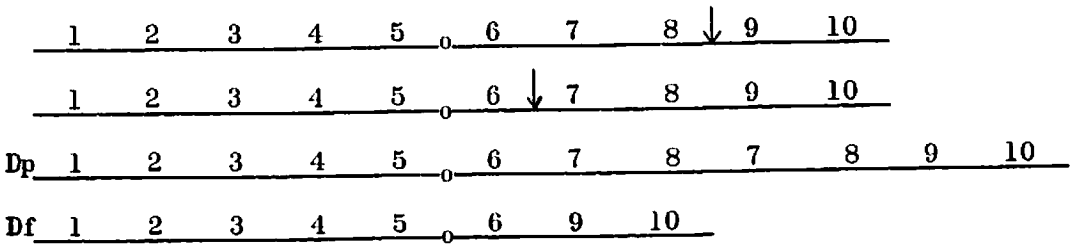
Also, it is possible to isolate and examine some of the products of non-homologous crossing over if they do not cause inviability or if they do, a trisomic culture can be used.

The occurrence of non-homologous crossing over is relevant to a number of cytogenetic problems--such as whether the translocations found in the progeny of monoploids arise solely from crossing over in duplicated segments and how chromosomal aberrations are formed under natural conditions.

G. G. Doyle

2. <u>The formation of duplications by the induction of translocations between homologous chromosomes and by the transposition of chromosome segments to non-homologous chromosomes</u>.

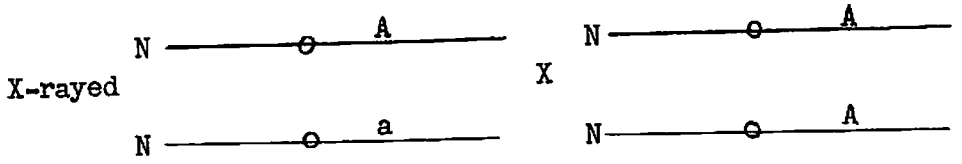
Translocations between homologous chromosomes will produce chromosomes with duplicated segments in tandem (and concurrently--chromosomes with deficiencies), as shown in the diagram below.



Translocations between homologous chromosomes should not be uncommon. Theoretically they should occur with 1/ (n-1) times the frequency of translocations between non-homologous chromosomes, where n is the haploid chromosome number. In maize this frequency should be 1/9.

Undoubtably many such duplications have been produced in the past by X-irradiation. However, it is very difficult to detect such duplications since they would cause no pollen sterility and would in most cases have no readily observable effect on the phenotype of the plant.

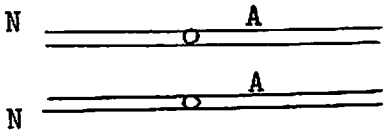
There is a method by which these duplications can be isolated. Kernels or other diploid tissue, such as young ears, which are heterozygous for gene markers are irradiated with the result that duplication-chromosomes containing both a recessive gene and its dominant allele are formed. This irradiated material is crossed as the female with homozygous dominant plants and then crossed in the next generation to recessive testers. The duplication carrying plants can be identified by the fact that their progeny will be mostly of the dominant phenotype, but with a low frequency of recessives. The rationale of this method is explained below:



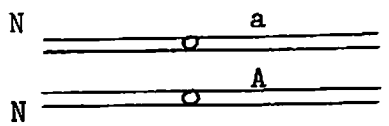
X_1 Progeny

Testcross Progeny

If translocation between homologues did not occur

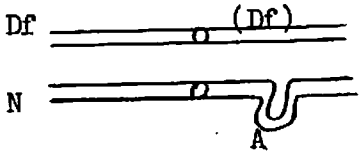


1/2 X aa — all A/a



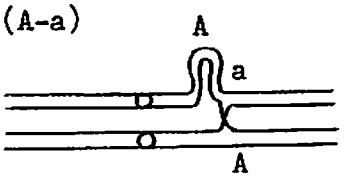
1/2 X aa — 1/2 A/a, 1/2 a/a

If translocation between homologues did occur



X aa — 1/2 A/a, 1/2 a/Df

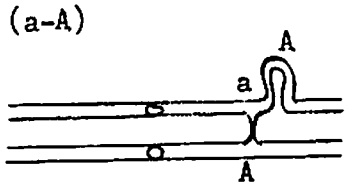
Dp (A-a)



(1/2) A/a, (1/2) A-a/a,
(?) a/a, (?) A-A/a

---

or - Dp (a-A)

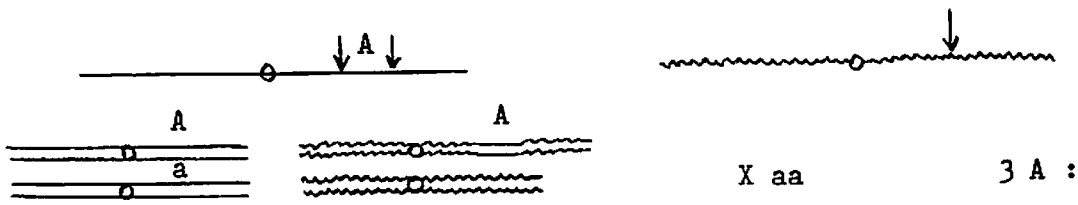


(1/2) A/a, (1/2) a-A/a,
(?) a/a, (?) A-A/a

If the Df chromosome does not result in the inviability of the megaspore and the ensuing haploid generation arising from it, then its presence cannot be detected when the testcross is made using it as the female as was done in the experiment to be described.

The presence of the Dp chromosome may be detected in the testcross progeny by the occurrence of a small number of recessives which are formed in the manner diagrammed above and also may arise from intrachromosomal pairing and crossing over--an event believed by Laughnan and Peterson to occur at the $\underline{A}^{b}_{1}$ locus, which is a small tandem duplication.

The genetic marking system also permits the detection of another type of duplication event--the transposition of a genetically marked segment to a non-homologous chromosome. This is a three-break aberration and probably is uncommon. It is shown diagrammatically below:



It may be detected genetically by a 3:1 ratio (instead of a 1:1 ratio) in a testcross progeny. It should be noted that the transposed chromosome segment must carry the dominant gene if it is to be detected.

One hundred kernels of a hybrid between Kys and Mangelsdorf's tester ($\underline{bm}_2$, $\underline{lg}_1$, $\underline{a}_1$, $\underline{su}_1$, $\underline{pr}$, $\underline{y}$, $\underline{gl}_1$, $\underline{j}$, $\underline{wx}$, and $\underline{g}_1$) were treated with 10,000 r and the plants crossed as the female with Kys. Eighty ears were obtained. Forty-four of the ears showed semi-sterility; in many of the ears the semi-sterility was in longitudinal sectors. A sample of 10 kernels was taken from around midsection of each of the eighty ears, from both fertile and semi-sterile sectors. Thus 800 kernels were

planted and these plants were used as the female in a cross with Mangelsdorf's tester. This resulted in 448 ears which were large enough to be classifiable. The results are given in Table 2.

Table 2

Genetic Tests for the Presence of Duplications

Gene seg.	No. of ears with ratios of 1:0	1:0	(aberrant)	Gene* seg.	No. of ears with ratios of 1:0	1:1	(aberrant)
Su:su	219	229	0	Lg:lg	68	57	2 (c,d)
Y:y	205	242	1 (a)**	Gl:gl	48	79	0
Wx:wx	221	225	2 (a,b)	G:g	51	71	5 (e-i)

*Sandbench test on seedlings.
**Letters to designate families with aberrant ratios--will be described in text.

The genes bm_2 and j_1 produce mature plant characters and it is not worthwhile to check their gene segregation. The Mangelsdorf's tester used was not an a_1 tester and therefore the genes a_1 and pr were not tested. Of the six genes used in this study, four gave aberrant ratios. Some cases, however, are known to be spurious. All the suspects must be checked cytologically before we can say that a duplication has been produced.

The first case (a) was an ear with 34 Y Wx, 2 Y wx, 5 y Wx, and 1 y wx kernels or 36 Y:6 y and 39 Wx:3 wx. Since it is unlikely that two genes have been duplicated at the same time, it is suspected that this is a contaminant ear. The second case (b) is an ear with 67 Wx:14 wx, this perhaps is a case of transposition--the χ^2 for a 3:1 ratio is 2.4. The ear (c) segregated 1:1 for a form of vivipary--apparently a newly induced dominant mutant--and gave 63 Lg plants and 2 apparently ligule-less ones in the sandbench. The seed from this ear was separated into two classes, normal appearing kernels and kernels which had embryos that bulged outward (the embryos apparently late in going into dormancy). The first class when planted in the greenhouse gave 59 Lg:1 "lg"; the second class gave 36 Lg:3 "lg". The "lg" plants were found to have normal ligules on the third or fourth and later true leaves. Therefore this is not a valid case of a duplication. The ear (d) gave 90 Lg and 1 lg. Not enough seed remained to run a test in the greenhouse. In the case of golden-1, five aberrant ratios were found out of total of 127 cases. There are (e) 69 G:1 g, (f) 87 G:1 g, (g) 34 G:1 g, (h) 94 G:1 g, and (i) 60 G:11 g. Golden is not too good a seedling marker in the background used so some or all of the cases may be spurious.

The segregation of Wx:wx can also be observed in the pollen. Tassel samples were collected from 240 plants (3 from each of the eighty families) and the pollen was stained with iodine and examined with the microscope. The results were: all Wx--105 plants, 1 Wx:1 wx--126 plants, and 8 plants which had mostly Wx pollen but with a small percentage of wx. These aberrant plants had the following ratios of Wx:wx (585:6)*, (517:58), (520:7), (514:17)*, (1584:122), (577:20)*, (540:13)*, and (510:8). There seems to be an association with semi-sterility in the pollen and the formation of wx types in these 8 plants. The asterisk following the ratio indicates that it was a semi-sterile plant.

The frequency of plants with semi-sterile pollen was 8.75%, 21 out of 240. One plant was completely sterile.

The occurrence of translocations between non-homologous chromosomes was thus very low if we accept the value of 8.75% as an estimate. Consequently, the frequency of translocations between homologous chromosomes must be very low--1/9 x 8.75% or ca. 1%. No attempt was made to select kernels preferentially from the semi-sterile ears or semi-sterile sectors of the X_1 ears. This would increase the frequency of translocations between non-homologues in the population, but it is probably true that translocations between homologues frequently do not produce semi-sterility--particularly those ones which are especially desired such as those producing duplications of the wx locus. It is known that chromosomes deficient for much of the short arm of chromosome 9 are functional through the megagametophyte.

This method of obtaining duplications needs further examination. There is probably some difficulty in duplicating genes which are close to the centromere, such as y, since a proximal break is required.

G. G. Doyle

3. Chromosome 9 mapping.

Enough 3-point testcrosses and 2-point data are finally available to order the loci provisionally. See Newsletters 33:78 and 32:100 for earlier data. Table 1 presents new 2-point testcross data, combinations with earlier samples, and information from 3-point testcrosses. Table 2 presents new 3-point data. Unquestioned orders are Wx-D_3-Pg_{12}-Ms_2-Gl_{15}-Bk_2-Bf-Bm_4 and Wx-D_3-Ar-V-Bk_2; Wx-Ms_2-Ar is indicated in some sketchy experiments. With addition of data for Wc (Burnham, Newsletter 33:74), the most logical complete map is as follows:

Dt	Yg_2	C	Sh	Bz	Bp	Wx	D_3	Pg_{12}
0	7	26	29	31	44	59	62	66

Ms_2	Ar	V	Gl_{15}	Bk_2	Wc	Bf	Bm_4
67	70	71	74	83	108	138	142

Several intervals and orders are still in doubt because of difficulties in isolating 3-point testers in these short intervals. The most uncertain placement is that of Ar and V in relation to Gl_{15}. Although Gl_{15} is easily classifiable, recombination tests with this marker have been very erratic; no definite pattern that would explain the variation has been seen.

Coincidence data suggest that the centromere may be to the right of D_3, near Pg_{12}. This would place D_3 in the short arm, with centromere placement somewhere between the limits of Anderson and Randolph (2-3 units from Wx, Genetics, 1945) and Rhoades and Dempsey (10-11 units, Newsletter 30:42, 51).

Table 1.

Recombination Data from Testcrosses for 2-point Intervals in Chromosome 9

X	Y	Phase	X Y	X y	x Y	x y	Total	Recombinations Number	Recombinations Percent	3-point Sum
Ar	Bk_2	CB	248	69	66	242	625	135	21.6±1.6	
Ar	V	CB	1	220		---	221		0.9±0.9	
Ar	Wx	CB	291	26	19	289	625	45	7.2±1.0	
Bf	Bk_2	RB	125	229	198	92	644	217	33.7±1.9	
Bf	Bm_4	RB	11	343	276	14	644	25	3.9±0.8	
Bk_2	Bm_4	CB	185	138	102	219	644	240	37.5±1.9	38
Bk_2	Wx	CB	233	81	77	234	625	158	25	29
		RB	6	43	36	7	92	13	14	15
							717	171	23.9±1.6	
D_3	Gl_{15}	CB	58	1	4	57	120	5	4	
		RB	0	99	65	1	165	1	1	
							285	6	2.1±0.8	

Table 1

Recombination Data from Testcross for 2-point Intervals in Chromosome 9 (Cont'd)

X	Y	Phase	X Y	X y	x Y	x y	Total	Recombinations Number	Recombinations Percent		3-point Sum
D_3	Ms_2	CB	427	10	7	309	753	17	2	*	
		RB	5	102	75	3	185	8	4	*	
							938	25	2.7±0.5*		
D_3	V	RB	7	99	96	1	203	8	3.9±1.4		
D_3	Wx	CB	825	23	28	649	1525	51	3		
		CB	533	11	13	381	938	24	3	*	
							2463	75	3.0±0.3		
Gl_{15}	Ms_2	RB	5	265	254	5	529	10	1.9±0.6*		
Gl_{15}	Pg_{12}	RB	1	79	70	2	152	3	2.0±1.1		
Gl_{15}	V	RB	0	20	16	1	37	1	2.7±2.7		
Gl_{15}	Wx	CB	170	12	14	187	383	26	7		
		CB	69	13	10	65	157	23	15		15
		CB	228	42	50	209	529	92	17	*	18
		RB	9	136	163	9	317	18	6		6
							1386	159	11.5±0.9		

*F_1 used as male; heterofertilizations resolved.

Table 1

Recombination Data from Testcross for 2-point Intervals in Chromosome 9 (Cont'd)

X	Y	Phase	X Y	X y	x Y	x y	Total	Recombinations Number	Recombinations Percent	3-point Sum
Ms_2	Pg_{12}	RB	4	182	224	0	410	4	1.0±0.5*	
Ms_2	Wx	CB	418	16	21	298	753	37	5 *	5
		RB	75	450	530	69	1124	144	13 *	14
							1877	181	9.6±0.7*	
Pg_{12}	Wx	CB	68	3	6	75	152	9	6	
		CB	203	25	17	165	410	42	10 *	
							562	51	9.1±1.2	
V	Wx	RB	10	109	111	10	240	20	8	9
		CB	913	146	146	891	2096	292	14	
							2336	312	13.4±0.7	

*F_1 used as male; heterofertilizations resolved.

Table 2

3-Point Testcrosses in Chromosome 9

F_1	Parental		Reg. 1		Reg. 2		1-2		Total
$\frac{+\ +\ gl_{15}}{wx\ pg_{12}\ +}$	67	73	6	3	1	2	0	0	152
	140		9		3		0		
			5.9 ± 1.9		2.0 ± 1.1		c = 0		
$\frac{+\ +\ +}{wx\ d_3\ gl_{15}}$	55	50	7	3	1	4	0	0	120
	105		10		5		0		
			8.3 ± 2.5		4.2 ± 1.8		c = 0		
$\frac{+\ +\ gl_{15}}{wx\ d_3\ +}$	96	63	2	3	0	1	0	0	165
	159		5		1		0		
			3.0 ± 1.3		0.6 ± 0.6		c = 0		
$\frac{+\ +\ v}{wx\ d_3\ +}$	96	94	2	3	7	1	0	0	203
	190		5		8		0		
			2.5 ± 1.1		3.9 ± 1.4		c = 0		
$\frac{+\ +\ +}{wx\ ar\ bk_2}$	230	226	16	18	61	63	3	8	625
	456		34		124		11		
			5.44		19.84		1.76		
			7.2 ± 1.0		21.6 ± 1.6		c = 1.1		
$\frac{+\ +\ +}{wx\ d_3\ ms_2}$	418	298	11	9	10	7	0	0	753
	716		20		17		0		
			2.7 ± 0.6		2.3 ± 0.5		c = 0		
$\frac{+\ +\ ms_2}{wx\ d_3\ +}$	100	73	2	2	5	3	0	0	185
	173		4		8		0		
			2.2 ± 1.1		4.3 ± 1.5		c = 0		
$\frac{+\ ms_2\ +}{wx\ +\ gl_{15}}$	227	207	47	38	3	4	1	2	529
	434		85		7		3		
			16.07		1.32		0.57		
			16.6 ± 1.6		1.9 ± 0.6		c = 1.8		
$\frac{bk_2\ +\ bm_4}{+\ Bf\ +}$	219	184	124	92	10	14	0	1	644
	403		216		24		1		
			33.54		3.73		.16		
			33.7 ± 1.9		3.9 ± 0.8		c = 0.1		

F_1	Parental		Reg. 1		Reg. 2		1-2		Total
+ + ms_2	200	165	17	24	3	0	1	0	410
wx pg_{12} +	365		41		3		1		
			10.2 ± 1.5		1.0 ± 0.5		c = 2.4		
+ + v +	220		--	--	1	--	--	--	
wx ar + bk_2	(110)				1				111
					0.9 ± 0.9				

Ar is between T1-9a and T1-9c (9L.15 and 9L.22); Bk_2 is proximal to TB-9a (9L.5); Bf is distal to $T4\text{-}9_{5788}$ (9L.82) and probably to $T5\text{-}9_{7205}$ (9L.90) according to duplication-deficiency tests.

E. H. Coe, Jr.

4. Deletions of B' and chromosome 2 markers.

Pollen of + B' + was x-rayed (1,000-2,000 r) and used on marked B and b (gl_2+/gl_2sk). Zygotes from the hybrids + B' + x gl B sk and + B' + x gl b sk were x-rayed (1,000-2,000 r) at 24 to 52 hours after pollination. The resulting individuals were examined for exceptional plant color and loss of Gl_2. Exceptions were classified for all markers, checked for pollen sterility, and progeny-tested when possible. Hemizygotes for Gl B have a distinctive morphology (compact, club-like tassel and zigzag culm) that helped to distinguish exceptions.

Cross	Irradiated	Recognized Loss					Examined
		Gl B'	Gl B' Sk	Gl	B'	'	Number
B x B'	pollen	23	4	4	0	0	3200
b x B'	pollen	20	1	3	0	0	1100
B' x B	zygotes	11	2	6	0	0	1700
B' x b	zygotes	5	0	2	0	0	350

Loss of B' is invariably accompanied by loss of Gl (distal to B). Loss of Gl is usually accompanied by loss of B' (exceptions are morphologically distinct from Gl B hemizygotes and are attributable to breakage between Gl and B'). Zygotes of B'/B constitution do not show conversion of B up to 52 hours after pollination. B' is refractory to x-rays except by deletion.

B' must be entirely chromosomal. The conversion or paramutation event is not immediate at fertilization; it may be as late as meiosis.

E. H. Coe, Jr.

5. Somatic mutation to B'.

Evidence from plants bearing sectors with new B' mutations shows that mutation can occur in B B or B b tissues, that B' is largely or completely cell-limited, and that conversion can occur well after tassel-branch differentiation, though it may not be restricted to late stages.

E. H. Coe, Jr.

6. Meiosis in haploids.

Agreement was found with the well-documented pattern of pairing and chromosome behavior typical of haploids in general and of maize haploids in particular (see review by Kimber and Riley, Bot. Rev. 1963) in meiosis of four haploids. Unexpectedly, however, a normal-appearing organized nucleolus was present in almost all microspore nuclei, whether small or large, even when a quintet or more of spores was formed. The meiotic behavior of the haploid microspore mother cells resulted in typical conspicuous shortages of chromatin in the spores and can be assumed to have produced only two chromosomes 6 for distribution among the spore set from each mother cell. Whether this unexpected nucleolus formation is unique to haploids of this origin (Coe's stock 6), or is a general phenomenon, an explanation is not immediately apparent.

A. E. Longley
J. B. Beckett
E. H. Coe, Jr.

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1. A scheme for simultaneous detection of nondisjunction, nonreduction and androgenesis.

In order to obtain information about factors involved in these phenomena from the same set of experiments, the following scheme has been adopted.

Ears of plants of the constitution $\underline{A_1}\ \underline{sh_2}/\underline{a_1}\ \underline{SH_2}$ subjected to various experimental conditions are pollinated by pollen from plants homozygous for $\underline{a_1}$ and $\underline{sh_2}$. Results expected under different situations are given below.

Phenotypes of kernels	Probable events	Remarks
1. $\underline{a_1}\ \underline{Sh_2}$	(a) nondisjunction of chromosome 3, (b) nonreduction of eggs, (c) mutation	These can be distinguished by chromosomal counts.
2. $\underline{a_1}\ \underline{sh_2}$	(a) androgenesis, (b) mutation	Examination of parental traits is necessary.

Phenotypes of kernels	Probable events	Remarks
3. $\frac{A_1\ sh_2}{a_1\ Sh_2}$ or	normal meiosis, gametogenesis and fertilization not involving any of these phenomena (nondisjunction, non-reduction and androgenesis)	

Since any one of the ten chromosomes has an equal chance of being involved in nondisjunction, only one-tenth of all nondisjunctional events can be detected by this method. However, the method will be useful for comparative studies of effects of different factors on this phenomenon.

S. K. Sinha*

2. A search for the sugary phenotype in Orissan maize.

As a part of a program for breeding sweet corn suitable for the maize-growing tracts of Orissa--an eastern state of India, samples of maize collected from different parts of the state have been screened to isolate, if possible, sugary mutants from the local varieties.

A few sugary kernels have been found on two ears from two different places, out of the present collection comprising more than 500 ears mostly from hilly districts.

The search is being further extended and the breeding potential of these few seeds at hand is being tested.

L. N. Mahapatra
S. K. Sinha*

3. Cytogenetic studies of maize varieties of Orissa.

Studies along the following lines have been undertaken in maize collected from different parts of Orissa: (a) pachytene analysis for cytological characterization of varieties; (b) change in chiasma frequency consequent upon selfing; (c) study of meiosis to detect any evidence for chromosomal aberrations, if involved in varietal differentiation; (d) search for the presence of genes affecting meiosis and gametogenesis; (e) embryological studies to assess as far as possible the extent of teosinte-introgression and other interesting peculiarities, if any.

Amongst the results obtained, mention may be made of evidences for (1) the presence of desynaptic genes in some varieties, (2) absence of any chromosomal aberration, (3) reduction in chiasma frequency consequent upon selfing, and (4) the possibility of teosinte-introgression in many varieties. All varieties examined so far are free from B-chromosomes.

L. Mangaraj
S. K. Sinha*

4. A search for fertility-restorer genes for S/T cytoplasm in maize varieties of Orissa.

Necessary crosses have been made involving several local inbred lines and open pollinated varieties and strains carrying S/T cytoplasm obtained from the U.S.A. through the courtesy of Drs. I. P. Trotter and J. M. Poehlman.

L. Mahapatra
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1. Long seeded flint corns from shallow seeded crosses.

In tropical and subtropical regions, flint corns are often used in those areas where storage insects and diseases are troublesome. As a rule they yield less than dent corns in terms of weight of grain per unit area. It should be possible to use a delayed backcross method e.g. (flint x dent) dent; self and select flint segregates; repeat (flint x dent) dent; self and select flint segregates, to increase the yield of these flints while preserving their superior storage qualities. However the theoretical yield expected could be modified (a) downward by the low shelling percentage of some shallow seeded flints or (b) upward by capturing the prolific tendencies of some long seeded flint popcorns, provided no deleterious gene systems are introduced by the recurrent dent parent. (Selection following the first backcross cycle may be prolonged.)

Will the shallow seeded flints give rise to long seeded segregates? Flints of several sources (Table 1) were crossed with long seeded "Country Gentleman" sweet corn. The F_1 was grown and selfed in 1962; the starchy F_2's were grown in 10 ft. row samples and selfed in 1963. At harvest the ears were broken and visually classified into 3 categories from cross cob observation. Yields in 1962 and 1963 were severely reduced by excessive drought so that balanced sampling was impossible. A single ear sample of a "Country Gentleman" self was classified as intermediate.

The results (Table 1) indicate that some flints dominate seed shape more than others but in the crosses with a common long seeded variety, selection of long seeded flint segregates is not too difficult.

C. C. Wernham

Table 1

F_2 Flinty Segregates from Flint x Country Gentleman Crosses

Flints Used		F_2 Seed Shape			Ears Sampled		Selected F_2 Seed Available	
		Round	Intermediate	Long	F_2	F_1	Long	Intermediate
P. I. 200, 308	Yugoslavia	4	2	2	8	2	1	
P. I. 201, 555	Yugoslavia	14	6	4	24	4	1	1
P. I. 204, 830A	Turkey	57	24	9	90	10	5	
Reciprocal		31	20	7	58	7	4	1
P. I. 213, 802	N. Dakota	60	12	0	72	8		1
P. I. 213, 804	N. Dakota	29	10	5	44	6	4	1
P. I. 213, 807	N. Dakota	25	20	10	55	6	6	
P. I. 213, 808	N. Dakota	7	3	0	10	1		3
P. I. 214, 123	Bolivia	3	9	4	16	3	4	
P. I. 214, 196	Canada	5	8	4	17	3	3	
P. I. 214, 274	Canada	6	5	1	12	2	1	
P. I. 217, 462	New York	3	2	1	6	1	1	
P. I. 226, 581	Ethiopia	8	22	11	41	5	10	
P. I. 245, 132	U.S.S.R.	21	15	5	41	7	4	
P. I. 245, 133	U.S.S.R.	23	16	6	45	6	3	
P. I. 245, 135	U.S.S.R.	92	11	2	105	11	2	
Totals		388	185	71	644	82	49	7

2. When do maize leaves appear to be wide?

In many cases "brachytic" inbreds of maize have unusually wide leaves--in excess of 5 inches. This appears to be a pleiotropic relationship since segregates of broad leafed brachytic x narrow leafed normals do not yield wide leafed normals.

The severe drought of 1962 severely stunted many inbreds in our disease nursery so that they had a pronounced brachytic appearance. Some stunted lines had unusually wide leaves--in excess of 4.5 inches. Selfed seed of six such cultures were selected for greenhouse increase. In the greenhouse two of these had narrow leaves, which seemed to indicate that leaf width was an environmental response. Four cultures continued to produce wide leaves. These were selfed or intercrossed as maturity permitted. Under irrigation in 1963, these inbreds and their intercrosses produced again unusually wide leaves--5 to 7 inches with 6 inch leaves (at ear level) very common. The hybrids are striking looking plants which are exceedingly attractive. They do not appear to roll or wilt more than ordinary corn under hot dry conditions. Breeders interested in silage corn are impressed.

The leaves of these plants are short as well as wide. Lack of long leafed inbreds prevents us at present from making crosses to determine if one can also have wide (in excess of 5 inches) long leafed (36 inches or more) plants.

C. C. Wernham

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1. Cyclic hydroxamate content of maize seedlings II.

Segregation for the presence or absence of a cyclic hydroxamate or its 2-glucoside in maize seedlings (MNL 36:71-72, 37:110) is presently being scored using waxy-marked translocation stocks. The trait has been recovered from crosses of Pa54 and Pa423 with mutant selections from the Gehu flint in which the trait was first noted. Tolerance of the mutant to atrazine has been reported together with a pedigree of related Gehu material (Weeds 12:27-30, 1964). Of the 30 plant introductions evaluated as 0-1, 0-2 or 0-3 in the tabulation of tested seedlings in MNL 37, inbred material indicates potential segregation of a similar nature in three of the introductions. Selfed plants from P.I. 179573, 179576 or 195757 yielded seedling progeny consisting of both plus and 0 ("minus") individuals.

Robert H. Hamilton
William D. Bell

2. A new lutescent trait in maize.

Among recently-collected, chlorophyll-deficient traits, an expression similar to the tomato lutescent (Rick and Butler, Advances in Genetics 8:267-382, 1956) has been observed. This maize lutescent appears as a pale green leaf color in the seedling stage followed by a yellowing but persistence of the older leaves, hence the tentative designation lutescent. It is apparently not equivalent to Bianchi's lutescent term in MNL 37.

Expression of the new maize lutescent suggests nitrogen-deficiency symptoms. Preliminary experiments conducted by Mr. David Shortess of this laboratory indicate that changes in nitrogen nutrition strongly influence expression.

William D. Bell

3. Mutant nomenclature.

The growing assemblage of cataloged mutants in maize, tomato, barley, soybean, tobacco, potato, Arabidopsis and other vascular plants points out the need of a standard taxonomy of genetic traits. I have found little correlation in comparing the names given to traits from one genus to another. Moreover, inconsistencies exist within naming systems.

Investigations of gene action are obviously complicated by the differentiated cells and tissues of higher organisms. However, few of the names given to mutants in higher plants give any indication of gene action although differences in metabolism have been elucidated in a number of cases. I hesitate to suggest a renaming program for cataloged mutants, but a standardization of nomenclature seems to be an inevitability in the near future.

William D. Bell

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1. Introgression in Corn Belt maize.

It has previously been shown (News Letters 35, 37) that introgression in maize, from its relatives teosinte and "Tripsacum", can be recognized on the basis of the morphological effects produced by the introgressed germ plasm on the component parts of the pistillate spikelet and its associated rachis internode. Since Corn Belt maize possesses numerous tripsacoid characters, a morphological study of a sample of the Corn Belt material was undertaken so as to recognize the introgressed components responsible for the tripsacoid characteristics. About 30 inbreds and a few typical flints and dents were employed in the present studies. The northern flints, it seems, are comparable to teosinte introgressed

A158 maize derivatives in most of the tripsacoid features of the pistillate spikelet, such as up-curved lower glume and inclined rachilla (teosinte component 4 modification), elongated rachis internodes (teosinte component 9 modification), whereas, the southern dents do not show many of the obvious effects of the teosinte introgressed components mentioned above. However, they do possess highly indurated and extremely thick rachis segments--the tripsacoid characters which are probably imparted to a certain extent by teosinte components 3 and probably 4+. Most of the inbreds fall within the two extremes of flints and dents giving further evidence that Corn Belt maize originated by the hybridization of northern flints and southern dents. It is interesting that some of the inbreds (C103, 099, 526, F14), although having different genetic background than the experimental teosinte introgressed A158 derivatives previously reported (News Letter, 37), still compare closely for spikelet characteristics with the A158-derivatives modified by individual teosinte components Florida 4+ and Nobogame 4B. Other inbreds, 695, 029, 291, and Oh 43, for example, showed the dilute effects similar to those expected from the introduction of more than one teosinte component. Still others, like 334, appeared to be even more tripsacoid than any of the teosinte introgressed derivatives of A158 or the flint and dent varieties.

S. M. Sehgal
W. L. Brown

2. Tripsacoid characters and combining ability.

If flints and dents have different types of introgressed components and if these components are heterotic (Sehgal, 1963), it would be logical to expect the inbreds with high combining ability to possess tripsacoid components from both blint and dent varieties and therefore to be probably more tripsacoid than either flints or dents. Examination of the internal cob morphology of a number of inbreds shows that this is true for many of them (C103, 705, 336, 385) and especially so for inbred 334. Inbred 334 possesses numerous tripsacoid features (strongly inclined, short and thick rachilla; extremely horny and highly indurated rachis segment) and appears to be more tripsacoid than the other inbreds studied or the experimental introgressed types. This is also one of the best general combiners in our cultures.

S. M. Sehgal
William L. Brown

3. Recovery of extreme segregates.

If Corn Belt maize possesses various degrees of teosinte germ plasm in its genetic constitution as most of the available evidence suggests, then it should be possible to recover segregates comparable to the experimentally introgressed teosinte derivatives by inbreeding the O.P. varieties. Such segregates, although not very frequent, do sometimes appear in F_2 and subsequent generations and have been observed in inbred progeny of the varieties Krug, Lancaster, and Midland. Some of the extreme segregates have many tripsacoid features of both plant and ear, and a few even exhibited a tendency toward single spikelets.

William L. Brown

4. Unique characters in maize.

Cultivated maize is unique among cereals in many ways, for example, in possessing the pistillate inflorescence in the form of an ear, in being one of the most heterotic organisms, in possessing peculiar mutagenic systems, and in throwing extreme segregates on inbreeding. There is evidence from our studies, and from those of Mangelsdorf and his associates, that these unique properties of maize result, in part at least, due to introgression from teosinte and possibly even from Tripsacum.

S. M. Sehgal

5. Evidence for transposition in maize.

In single crosses between some of the teosinte and "Tripsacum" derived lines of inbred A158, segregation into normally tripsacoid ears and extremely tripsacoid ears was found (Sehgal, 1963). The internal morphology of the two types of cobs supports the previous assumption that the extremely tripsacoid ears are homozygous for the introduced germ plasm, and the normally tripsacoid ears are heterozygous. Furthermore, the extremely tripsacoid ears show numerous differences in internal morphology when compared to the parental derivatives, thus suggesting that these are not the result of accidental selfing. The homozygosity of the introgressed segments is tentatively attributed to the transposition of the chromosomal segments from one chromosome to another.

S. M. Sehgal

6. Crossing inbred A158 and its modified derivatives with Florida teosinte.*

In a previous News Letter (No. 36), I reported the results of crossing original A158 and its teosinte and "Tripsacum" derivatives, with Nobogame teosinte. The same group was studied in the summer of 1962 in crosses with Florida teosinte. The pistillate inflorences in the F_1's remained in their initial stage of development till late October due to the long day environment in which they were grown and therefore could not be studied. The staminate inflorescences, although late, were well developed and were employed for various observations. The hybrids between A158 and Florida teosinte showed a well developed compact central spike with polystichous arrangement of the spikelets, whereas the hybrids between modified derivatives x Florida teosinte, fell into one of the following categories:

(1) Lax central spike with polystichous arrangement of spikelets	Florida 3A Florida 3B Florida 9 Mexico Honduras

*This work was done at the Bussey Institution of Harvard University.

2. Lax central spike, primarily distichous arrangement of spikelets	Durango 1,7,9 Florida 4+ Nobogame 4A Bolivia Argentina Paraguay
3. Absence of central spike in some individuals of the F_1 population	Nicaragua

S. M. Sehgal

7. Immunological studies of corn kernel proteins.

The double diffusion agar method (Ouchterlony) has been used to study precipitin reactions of salt-soluble proteins extracted from germs of mature corn kernels. Several precipitin lines, each presumably representing a different protein or group of proteins, have been identified. Two of these lines are most easily produced and have been studied intensively. They are labeled "A" and "B". Most inbred lines contain antigen necessary to produce both lines. However, a few inbred lines (all tracing back to one source) lack the antigen necessary to produce precipitin line "A". One other inbred lacks the antigen necessary to produce precipitin line "B". Single kernel analyses of F_2 and BC_1 populations, and of P_1, P_2 and reciprocal F_1 crosses show that each antigen is inherited as a dominant, single gene character. The fit to a two-factor ratio was good when a small F_2 population segregating for both "A" and "B" was tested, indicating that they are probably not linked. Variations in intensity of reaction indicate that modifiers probably affect the amount of each antigen produced in a kernel. It is also possible that homozygous recessive individuals do not really lack the antigen, but merely have it in concentrations so low that the test, as employed, does not detect it. Further studies will, it is hoped, answer some of these questions.

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1. Heritability of radiation induced alterations of paramutation.

Last year experiments were described in which the radiosensitivity of $\underline{R}^r$ and the $\underline{R}^{st}$, $\underline{R}^{mb}$ components of paramutation was investigated (MNL 37:133-134). When $\underline{R}^r$ is irradiated prior to crossing, about 50% of the time the paramutation expression was altered. The classification can be summarized into two categories: no paramutation and segregating for paramutation. When the $\underline{R}^{st}$ and $\underline{R}^r$ components were irradiated, about 25% of the time paramutation expression was altered. An additional category of increased paramutation alteration after $\underline{R}^{mb}$ irradiation occurred along with the no paramutation and segregating for paramutation classes.

A second testcross of the $\underline{R}^{r:mb}$ $\underline{r}^{g}$ or $\underline{R}^{r:st}$ $\underline{r}^{g}$ genotypes to $\underline{r}^{g}$ $\underline{r}^{g}$ was made of representatives in each of the groups and each of the categories within groups. Seeds were selected to represent the variability of intensity within ears and planted in sequence of intensity within rows. Plants were numbered so the harvested ears could be arranged in the same sequence as the intensity of seeds. Two samples were taken for the segregating for paramutation category: one from seeds similar to normal paramutation; one from seeds similar to no paramutation.

The sequence of increasing intensity between ears within a row does not follow too closely the order in which they were planted. In general the variability within a category is not transmitted. The no paramutation classes (dark purple) gave rise to rows in which all ears were segregating 50% dark purple and indicated there was no paramutagenic change in $\underline{R}^{r}$. The segregating for paramutation categories gave rise to two distinct groups: one very similar to the "normal" paramutation class and one similar to "no" paramutation. There was no overlap in these classes although they come from the same testcross ear. There is a segregation of the effect within the ears. The "increased" paramutation category was reflected by groups of ears distinctly lighter than "normal" paramutation in the second testcross. They were as light as and probably lighter than the degree of alteration induced by $\underline{R}^{st}$. The radiation induced alterations in paramutation expression have all been carried a second generation and they maintained their identity.

Duane B. Linden

2. On growing Corn Belt inbreds in Puerto Rico.

Experimental plots of corn have been planted on thirty-three different dates in the past two years. The majority of the material consists of genetic stocks used in the paramutation program and are in W22 background. They were originally obtained from Brink and had been backcrossed to W22 by him for several generations. The remaining materials are South American races and crosses of the races with W22 stocks.

As a general rule (with W22 material) pollinations are made 60 days after planting and ears harvested 30 days after pollination throughout the entire year. Plantings have been made in every month (except March by chance) and there may be a slight shortening of the cycle in plantings May-August but the difference is less than one week. Some of the South American races have longer growth periods with a few taking 110 days from planting to harvest. Some hybrids between South American races and W22 stocks were harvested 79 days after planting.

There have been two instances of crop failure. The very first planting was not harvested as insects got the material first. One planting in summer 1963 suffered from winds of the hurricane. There are several perils associated with genetic maize culture in Puerto Rico but each can be either controlled or tolerated. Insects are the major problem, but by routinely dusting with DDT we no longer have losses from them.

Helminthosporium turcicum is probably the major leaf disease. However, with the rapid maturation of the seed the disease may even work to advantage in assisting the drying of the plant. In any case the seed germination has been good in all plants if the only disease was H. turcicum regardless of severity. The only times difficulties have been encountered in seed germination have been when maturity corresponds to the summer, high rainfall months and premature sprouting of kernels occurs. This difficulty is reduced by husking the ears on the plants before maturity to assist drying and eliminate the water holding of the husks.

Many of the more serious problems associated with corn culture occur in the summer months. In the fall and winter plantings good seed with over 90% germination is now fairly routine. Pigment development in the aleurone is excellent except in heavily diseased ears even in the ears that matured 79 days after planting. Puerto Rico should be considered as a possible location for winter nurseries of experimental breeding and genetic programs.

Duane B. Linden

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1. New pale green and virescent genes on chromosome 3.

A pale green mutant and a virescent mutant were found segregating in progenies of intercrossed foreign introductions. The expressions are variable in both cases but classification is good. The pale green remains as such throughout its life cycle. A normal color is restored in the virescent in early seedling stage.

The mutants were crossed to a series of waxy-marked translocations involving all chromosomes and F_2 waxy and starchy seeds were examined separately. An association was indicated between each mutant gene and translocations involving chromosome 3. The data from the pale green are as follows: starchy seeds gave 268 normals: 74 pale greens; waxy seeds gave 81 normals: 14 pale greens. The data from the virescent are as follows: starchy seeds gave 125 normals: 71 virescents; waxy seeds gave 38 normals: 0 virescents.

These data indicate that both mutant genes are located on chromosome 3. Tentative designations of pale green-774 and virescent-686 were assigned. Further studies are foreseen in order to determine more precise locations.

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1. Non-disjunction of knobbed chromosome 10.

It has been reported that the presence of a terminal knob on the long arm of chromosome 10 increases the frequency of chromosome 10 non-disjunction (Emmerling, 1958 Jour. Hered. 49:203). The test described below was not made for the specific purpose of testing for non-disjunction; however, the data clearly corroborate the findings in the published report cited above.

A major modifier (M^{st}) of stippled aleurone is located six units distal to the R locus. The frequency of colored aleurone spots is increased when M^{st} is present. Data had indicated that M^{st} may be transposable and in the course of subsequent tests to verify the transposability of M^{st} the following cross was made:

$$\frac{r^r \; + \; K}{R^{st} \; M^{st} \; +} \; X \; \frac{R^{st} \; + \; +}{}$$

The endosperm phenotypes resulting from the mating are two well defined classes: dark stippled kernels or those with the higher frequency of colored spots ($R^{st} M^{st} +$ / $R^{st} M^{st} +$ / $R^{st} + +$), and light stippled kernels or those with the lower frequency of colored spots ($r^r + K$ / $r^r + K$ / $R^{st} + +$). The frequency of kernels in the two classes was not equal because of preferential segregation of the knobbed chromosome; 16,798 kernels from 68 ears were scored and 12,962 were classified as light stippled (77%) and 3,836 as dark stippled (23%).

The dark stippled kernels were germinated in a sand bench and the seedlings scored for the presence of anthocyanin pigment. Such seedlings were exceptional since they would have had to receive r^r from the female but still be associated with a dark stippled phenotype. The original basis for the experiment was that such exceptional seedlings would carry r^r and M^{st} either in linked positions because of a crossover, or independent because of a transposed M^{st}. The sole purpose of introducing K into the matings was to reduce the number of such exceptions attributable to crossing over, since K strongly suppresses crossing over in the region distal to R.

A total of 3,211 seedlings were scored and 16 with anthocyanin pigment were found. The pigmented seedlings were transplanted to pots in the greenhouse and the ears were pollinated with r^g pollen. Ears from only seven plants set seed and these all segregated three classes of kernels: dark stippled, light stippled, and colorless. Breeding tests from the three kernel classes from the seven ears were made in the next field season, and the tests showed that the seven exceptional plants were trisomic for chromosome 10. The plants carried both chromosomes 10 from the female parent, $r^r + K$ and $R^{st} M^{st} +$, and one chromosome 10 from the male parent, $R^{st} + +$.

If the total population of kernels (16,798) is adjusted for the proportion of dark stippled kernels scored for seedling color, 3211/3836, and for the proportion of red seedlings verified, 7/16, the frequency of female gametes carrying both chromosomes 10 was seven in 6,152 gametes tested, a rate of 11.4×10^{-4}.

R. B. Ashman

2. The regulatory nature of the waxy locus.

Evidence accumulated over the past year makes it probable that the waxy locus is regulatory in nature and not the structural locus for the nucleotide transferase. The most important evidence is the finding that starch granule preparations from the embryos of developing waxy seeds (16 day) have as high or higher transferase activity than similar preparations from non-waxy seeds. The low level of activity shown by starch granule preparations from whole seeds of waxy stocks when ADPG is used as a substrate is not due entirely or even largely to the presence of starch granules from the embryo. Starch granule preparations from waxy endosperms alone still have low activity. Comparative activities are given in Table 1. At 16 days, the embryos were 1.5 and 1.1 percent of the wet weight of the waxy and non-waxy seeds, respectively.

Table 1

mμM ADP Released Per Mg. of Starch Under Standard Assay Conditions.* All Preparations from Developing Seeds Frozen 16 Days After Pollination. 1962 Collections.

	+	wx
Starch granules from embryos alone	114	142
Starch granules from endosperms alone	30	3.3
Starch granules from whole seeds	35	3.7

*To 2.5 mg. of starch granule preparations (1 mg. if embryo is source) is added 25 microliters of a solution that contains 0.31 μM ADPG, 0.17 μM EDTA, 6.85 μM glycine, and is buffered at pH 8.4. After 15 minutes at 37° C, 25 microliters of 0.01M phosphoenolpyruvate solution and 25 microliters of a pyruvate kinase solution containing about 26 enzyme units per ml. are added, and reaction allowed to proceed for 15 minutes more before being stopped by the addition of dinitrophenyl hydrazine. Thus total reaction time is 30 minutes at 37° C.

It is clear from Table 1 that the low nucleotide transferase activity of the waxy endosperms is not characteristic of waxy embryos. Yet the evidence at hand points clearly to the dependence of endosperm transferase activity on the allelic state at the waxy locus. Thus it is improbable that the waxy locus is the structural locus for transferase and likely that it is regulatory in nature.

Corroborative evidence for this conclusion comes from a study of the transferase activity of 17 waxy mutants that occurred as separate mutational events. The results are given in Table 2. All the mutants have

Table 2

mμM ADP Released Per Mg. of Starch Under Standard Assay Conditions. All Preparations from Developing Seeds Frozen 16 Days After Pollination. 1963 Collections. Whole Seed Preparations.

Mutant	Origin	mμM ADP
C	Spontaneous	5.4
90	Spontaneous	3.8
Bear G	Spontaneous	6.0
Brink 1	Spontaneous	3.6
Brink 2	Spontaneous	4.2
Brink 4	Spontaneous	4.8
Brink 6	Spontaneous	3.8
Brink 8	Spontaneous	3.2
H21	Spontaneous	4.4
T4B	Presumptive Irradiation*	6.6
Q1R	Presumptive Irradiation*	3.4
S3G	Presumptive Irradiation*	6.6
N1R	Presumptive Irradiation*	4.8
N3Y	Presumptive Irradiation*	7.4
wx^{m-1}	Ds	5.0
wx^{m-6}	Ds	6.4
wx^{m-8}	Spm System	4.8
M14 (Non-waxy)		62

*From Dr. Caspar, Blandy Experimental Farm.

measurable activity and in a rather restricted range. To find that 17 different mutants (spontaneous, irradiation, and controlling element), were all hypomorphic and to about the same degree would be improbable if the waxy locus were the structural locus for transferase unless a special set of assumptions was invoked.

Oliver Nelson
Charles Tsai

3. Differential crossing over in male and female gametes of plants heterozygous for Dp 9.

Rhoades (MNL, 32) first reported reduced recombination between markers on the short arm of chromosome 9 in plants heterozygous for Dp 9. In attempting in 1961 to evaluate the effect of heterozygosity for Dp 9 on recombination within the wx locus, we observed a pronounced difference between male and female gametes in crossing over between markers on the short arm of 9.

The genotype of the heterozygous plants (15562) was $\frac{C\ Sh\ Dp\ +\ wx^{90}\ Gl_{15}}{c\ sh\ N\ wx^{c}\ +\ gl_{15}}$. Randomly selected plants were crossed as male parents onto a $\frac{c\ sh\ N\ wx^{c}\ +\ gl_{15}}{}$ tester and as females by the same tester stock. Table 1 presents the data for the c sh interval and Table 2 for the sh gl_{15} interval.

Table 1

15562 Plants as Male and Female Parents in Crosses with a c sh N wx^c + gl_{15} Tester

Plant		C Sh	C sh	c Sh	c sh	Σ	% Recomb.	% Fertilization Effected by Dp 9 Gametes
15562-3	♀	426	1	1	440	868	0.2	
	♂	500	37	4	1444	1985	2.1	25.4
15562-6	♀	442	2	0	401	825	0.2	
	♂	446	23	18	1069	1556	2.6	29.8
15562-8	♀	137	0	0	159	296	0	
	♂	318	49	5	848	1220	4.4	26.5
15562-15	♀	431	0	1	437	869	0.1	
	♂	356	24	0	851	1231	1.9	28.9
All	♀♀	1436	3	2	1437	2878	0.2	
All	♂♂	1620	133	27	4212	5992	2.7	27.5
Remaining 11 15562 Plants Used as ♀♀		2380	2	5	2337	4724	0.1	

Table 2

15562 Plants as Male and Female Parents in Crosses with a c sh N wx^c + gl_{15} Tester. Only sh Kernels Sampled.

Plant		sh gl_{15}	sh Gl_{15}	Σ	% Recombination
15562-3	♀	95	3	98	3.1
	♂	866	71	937	7.6
15562-6	♀	94	2	96	2.1
	♂	650	36	686	5.2
15562-8	♀	32	1	33	3.0
	♂	587	47	634	7.4
15562-15	♀	105	2	107	1.9
	♂	575	80	655	12.2
All	♀♀	326	8	334	2.4
All	♂♂	2676	234	2912	8.0
Remaining 11 15562 Plants Used as ♀♀		856	21	877	2.4

There is a significant difference between male and female gametes for both the c-sh and the sh-gl_{15} regions. The difference is most pronounced in the c-sh region where recombination in the male gametes is nearly normal. For the sh-gl_{15} region the rate of recombination in the male gametes is more reduced relative to normal values (8 percent versus 25 percent), and less difference is observed between male and female gametes.

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1. Effect of diplontic selection on estimates of change in radiosensitivity during seed germination.

Some recent studies on responses of germinating seeds to x-rays have involved comparisons of temporal changes in (a) somatic mutations and (b) inhibition of seedling growth. Maize seeds heterozygous for "wd", a terminal deficiency of chromosome 9 that conditions an albino phenotype when homozygous, were irradiated on a rotating turntable with their roots shielded by a disc of lead. Sectors of albino tissue in embryonic leaves (nos. 1-6, those present as discrete primordia in the embryo) of plants grown from irradiated seeds were used as an index of chromosome breakage. Plant height after 3 weeks of post-treatment growth furnished estimates of growth inhibition.

Changes in these two variables produced by 800r of x-rays showed positive correlation through an initial period of low sensitivity, 0-15 hours, and an abrupt rise in x-ray-induced injuries from 15-32 hours (Table 1). However, somatic mutation frequencies were maximal at 32 hours, then declined to a low at 40 hours (Table 1, column 4). Growth inhibition showed a similar sequence of changes but was not maximal until 40 hours (Table 1, column 6).

To gain insight into this apparent discrepancy between two widely used criteria of radiosensitivity, samples of seed were irradiated with one of three doses of x-rays, 200, 500, or 800r, at four points in time (Table 2, column 1) including the interval where changes in growth inhibition and frequencies of albino sectors showed opposite trends.

Changes in growth inhibition were the same at all doses (Table 2). There was a progressive increase from 31-41 hours after which time growth inhibition decreased.

Though changes in somatic mutation frequencies were somewhat more complex some definite trends could be distinguished. Albino sectors induced by irradiation with 800r showed the characteristic decline in frequency from 37-41 hours (Table 3, column 5). Subsequently their

Table 1

Ontogenetic Changes in Somatic Mutations and Growth Inhibition Produced by 800r of X-Rays

Hours Germinated	Rep	No. Plants Treated	Average No. Sectors/Leaf Leaf 5	Average Seedling Height (cm.)	Reduction in Seedling Height— Percent of Control
0	1	28	0.00	45.5	+1.9
	2	28	0.04	33.1	0.0
15	1	28	0.07	44.8	1.7
	2	28	0.04	32.6	1.5
24	1	27	0.26	35.8	20.6
	2	27	0.22	28.0	15.4
28	1	26	0.62	37.6	16.6
	2	28	0.46	27.7	16.3
32	1	27	0.74	35.1	22.2
	2	27	0.37	25.9	21.8
36	1	28	0.41	27.9	38.1
	2	28	0.43	20.6	37.8
40	1	24	0.38	22.2	50.8
	2	28	0.28	18.5	44.1
44	1	26	0.56	29.5	34.5
	2	26	0.40	19.4	41.4
48	1	27	0.65	27.2	39.7
	2	24	0.38	20.4	36.9
48 (Control)	1	28	0.07	45.1	--
	2	28	0.00	33.1	--

frequency was equal to or less than that induced with 500r. This trend was largely absent from the 200 and 500 r series (Table 3, columns 3, 4). Instead, somatic mutation frequencies tended to increase from 31-46 hours and thereafter remained relatively constant. These data suggest that chromosome breakage, like growth inhibition, was maximal from 40-45 hours and that the decline in sector frequencies from 37-41 hours reflected an increase in x-ray-induced lethality and differential survival of damaged cells. This suggestion draws support from the fact that in the 31 and 37 hour series of irradiations somatic mutations showed a progressive increase with dose (kinetics of dose-response curves showed a 2-hit component), whereas in the 41 and 48 hour series dose-response curves showed a saturation effect, maximum response being reached with 500r.

Table 2

Effect of X-Ray Dose on Seedling Growth

Hours Pregerminated	rep	Growth Inhibition (Per cent of Control)		
		200r	500r	800r
31	1	-1.0	8.4	23.8
	2	2.3	8.4	22.4
37	1	2.4	12.6	27.5
	2	-2.3	14.0	37.3
41	1	6.5	26.2	49.7
	2	7.8	25.3	41.9
46 (48)	1	6.5	23.6	47.1
51	2	2.6	14.0	29.9

Table 3

Effect of X-Ray Dose on Changes in Somatic Mutation Frequencies

Hours Pregerminated	rep	Average Number of Sectors/Plant					
		Leaves 5 & 6			Leaves 7-9		
		200r	500r	800r	200r	500r	800r
31	1	0.07	0.39	0.61	0.04	0.11	0.21
	2	0.04	0.25	0.58	0.00	0.11	0.43
37	1	0.11	0.14	0.64	0.07	0.14	0.43
	2	0.04	0.22	0.71	0.04	0.21	0.32
41	1	0.32	0.32	0.43	0.00	0.18	0.25
	2	0.15	0.32	0.44	0.11	0.25	0.15
46 (48)	1	0.25	0.39	0.36	0.00	0.32	0.50
51	2	0.25	0.40	0.38	0.07	0.19	0.30
48 (Control)	1	0.00	0.00	0.07	0.00	0.00	0.00
	2	0.00	0.04	0.00	0.04	0.00	0.04

Similar observations were recorded for albino sectors in leaves 7-9 (Table 3, columns 6-8), those derived from apical initial cells which do not initiate mitosis until after 72 hours germination. However, only the 41 hour series of treatments showed the saturation effect.

Dose-response curves for the 48 hour series (46 and 51 hour irradiations), like those for the 31 and 37 hour series, increased at a power of the dose greater than one. Hence, though onset of mitosis at ca. 38 hours in cells comprising embryonic leaf primordia undoubtedly influenced their capacity to recover from radiation injuries, the principal determinant of radiosensitivity was apparently some change in physiologic state that affected the entire shoot system.

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1. The effects of progressive alterations in chromosome constitution on pachytene and metaphase trivalent frequencies in 21 chromosome plants carrying maize-Tripsacum interchange chromosomes.

Three secondary exchange products have been recovered from a 21 chromosome stock carrying a pair of maize-Tripsacum interchange chromosomes. In effect, the secondary exchanges approximately halved the extent of the Tripsacum segment (in the 2^T chromosome), leaving either its distal region (carrying a terminal knob), or its intercalary region, as a substitution in chromosome 2, or returning its distal half to the T^2 chromosome. These secondary exchange products were identified both by means of genetic markers and by cytological morphology and synaptic behavior.

Plants carrying the secondary exchange products were then crossed in a variety of combinations to give (so far) an array of eleven constitutions differing in duplication or triplication of corresponding chromosome regions, extent and position of segments of maize chromosome 2 in the T^2 chromosome and also extent and position of segments of the Tripsacum chromosome in the 2^T chromosomes. Trivalent frequencies at pachytene and metaphase I have been studied in all of the available constitutions.

Estimates of these frequencies at pachytene were remarkably similar to metaphase estimates throughout the entire array of constitutions. Furthermore in the case of one plant with exceptionally clear pachytene cells, where it was possible to classify 90 percent of 116 cells for presence of a trivalent or univalent, 52/104 (or 50 percent) contained a trivalent. In the unlikely event that all 12 of the unclassified cells actually contained a trivalent, the pachytene trivalent frequency was 55 percent. Of 221 metaphase cells from this plant, 96, or 43 percent, contained a trivalent from which the minimum frequency of cells with at least one chiasma (in the appropriate arm) per pachytene trivalent is inferred to have been about 43/55 or 78 percent. If the trivalent frequency in the 12 unclassified cells did not differ greatly from the

rest of the sample, then the minimum frequency of cells with at least one chiasma per trivalent was about 43/50 or 86 percent. The extent of homologous pairing available for crossing over in the pertinent region of these trivalent configurations is estimated to have included a maximum of 29 genetic map units. This estimate of genetic length is probably an overestimation since it is based upon a uniform cytological distribution of the genetic map although it is known to be somewhat more concentrated distally. Therefore, on the basis of these calculations, a chiasma frequency of less than 58 percent was expected where an actual minimum of 78 percent was found.

Thus it appears that either crossing over precedes and is required for pachytene pairing, or crossing over always, or almost always, follows synapsis of the regions studied in this experiment, even when their genetic length is considerably less than 50 units.

When estimated frequency of trivalents at metaphase is plotted against estimated cytological extent of homology in the T^2 chromosome for regions present in either or both of the other chromosomes, points for the various chromosomal constitutions follow an interesting pattern of tight clusters. If the results are taken at face value, it appears that: (1) The frequency of trivalent formation is depressed by the presence of homologous regions in triplicate in a way which is relatively insensitive to the length of these triplicated regions. (2) The frequency of trivalent formation nevertheless increases with increase in the extent of homology in the T^2 chromosome to either or both of the other two chromosomes. It also appears that the location of the terminal knob is unimportant, that terminal or intercalary position of a triplicated region makes no important difference, and that common homology to the T^2 chromosome can be divided between the 2^T chromosome and a normal chromosome 2 without significant change in trivalent frequency.

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1. Genetics of tillering.

A Pawnee stock and a grassy-tillered stock from E. G. Anderson, as well as Argentine Pop, were added to the forms being studied. The first crosses are expected to be ready for analysis this coming season.

2. Effects of maleic hydrazide and indole butyric acid on nana-1.

Eight plants, suspected of being homozygous for na_1 but all 5 feet tall, were backcrossed to na_1 in 1962. Seed from each produced some tall plants, so the conclusion was that the treatment had not caused the increase in height. Repeated in 1963 on homozygous na_1 plants, internodal elongation did occur in three out of five plants treated with MH (100

micrograms daily) plus IBA (500 micrograms daily), 2 out of 5 plants treated with MH alone and 1 out of 5 plants treated with IBA alone. Heights below the peduncle thus were increased from an average value of 13 cm in control plants to anywhere between 20 and 43 cm. The greater heights occurred with the combination treatment.

3. Kn suppression.

Development of knots in 4 strains of Coop stock was completely inhibited by daily treatment with 500 micrograms of NAA. Flowering was also suppressed. Plants were only 1/2 to 2/3 the height of controls. Internal anatomical differences include reduction of leaf thickness to that of normal plants (Kn leaves may be up to 3 times thicker than normal sibs). The same effect, less pronounced, is also obtained by GA treatment. Brace roots are induced to form as flanges, joined to one another, internally, they have a single stele for the entire group.

4. Modification of expression of Vg.

Results of applying IBA daily in 500 microgram doses were variable. In the 5 stocks employed, some were unchanged, some were rendered fertile and others showed variable responses between these extremes. The suppressed ligule development (Laughnan, MNL 1956) by which Vg plants may be early identified was unaltered. Galinat has suggested that some of the many modifiers of Vg he has found may be responsible for the variable results.

5. Dry-weight studies on two genetic strains of milo.

As reported last year at Allerton, threefold increases were obtained using daily treatments of IBA at 500 micrograms. In 1963, 25-plant samples in a randomized field pattern were collected from a population of over 1,500 plants. Rates of application were varied from daily, 3-day, 6-day and 10-day spans. IBA-soaked seed showed no differences from unsoaked, or those soaked in water, regardless of subsequent treatment. Incomplete data show that the increase in dry weight is real, but that it is not a threefold one. The greatest increases come under 6-day applications. Using the known auxin antagonist tri-iodo benzoic acid in a comparable set of plants, root development was completely inhibited under daily treatment; further, up to 50 basal corms were produced in lieu of tillers. These were later shown to be viable and to grow into normal-looking sorghum plants in the greenhouse. Under 10-day treatments, root growth was actually increased over that of controls. Anatomical changes accompany these changes, but their extent and significance is not known.

6. Masking of expression of v_4 by growth substances.

This mutant is expressed more strongly under cool temperatures. A stock of silkless carrying v_4 showed, when treated with MH and one of the growth substances IAA, IBA, or NAA, no virescents except in controls. Later plantings failed to show any virescence, presumably because of the increase in growing temperature.

7. Use of atrazine.

A commonly-employed weed killer and suppressor, atrazine has been shown to remain detectable in the soil for periods measured in years. A comparison of 15 races, stocks and hybrids grown on treated versus untreated halves of the same rows was made. To test responses of the plants under these conditions, a part of every section of each row was treated with GA. Response to GA was not influenced by atrazine. Other features with atrazine: dwarf-1, reduced in height and tillering; corn-grass, intensified in expression; zapalote chico, average height increase of 6 inches; spancross, size and vigor increased; pioneer 349 and its immediate parents, greater seedling vigor; others made no noticeable responses.

8. Tests involving other growth-regulating substances.

A number of other compounds not previously tested in 500-microgram doses were applied to races, hybrids and inbreds as well as to genetic stocks including ra_1, ra_3, tsb, na_1 and na_2. These were based on comparisons of 10-plant samples and a 10-plant control. The most significant outcome of the study is that these chemicals, applied at the rather high dosage, did not seriously alter plant growth, anthesis, and ear formation. The numbers below refer to numbers of stocks tested.

Alpha-phenyl butyric acid--more growth in 7, 6 equal to controls.
Gamma-phenyl butyric acid--6 equal to, 3 larger, 4 less than controls.
Indole propionic acid--5 shorter, 2 equal to controls.
Maleic Hydrazide (100 ppm)—reduces color in 5, shorter in 4, taller with more color in 4.
2,4-D (100 ppm)--3 reduced in growth, 3 enhanced, 2 equal to controls.
Indole--heavier vegetative growth in 2, slower growth and shorter in 2.
Iso-butyric acid--1 shorter, 5 with more vegetative growth, 1 slowed in reproduction.
Normal butyric acid--5 retarded, 1 equal to controls.

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1. A second case of hybrid enzyme formation in heterozygotes.

The pH 7.5 esterases specified by the E_1 gene exist in the form of a dimer. In homozygotes the dimers are composed of two identical monomers (autodimers), while in heterozygotes three enzyme types are found: the two autodimers produced when the alleles are in homozygous condition and in addition a hybrid enzyme (allodimer), composed of two homologous but non-identical monomers. As expected, the migration rate of the allodimer is always intermediate between the two autodimers. In starch gel electrophoresis the SS autodimer, formed by the E^S allele, bands out at a characteristic slow moving position. However, even plants which lack the E^S allele show a weak esterase band at the SS position (constant S).

This esterase must be specified by another non-allelic gene since this gene does not segregate from the $\underline{E}_1$ alleles. For example, $\underline{E}_1^F/\underline{E}_1^F$ plants show two bands at the pH 7.5 esterase positions; an intense FF band and a weak band at the SS position. If such a plant is self-pollinated all of the progeny give identical zymograms and show both esterase bands. Further proof for the non-allelism comes from the observation that no hybrids are formed between the pH 7.5 esterases and the constant S. This point also argues against the possibility of duplicate genes since if the product of the two genes were the same, allodimers should be formed.

The gene controlling the synthesis of the "constant S" enzyme has been designated $\underline{E}_3$. We have recently picked up a mutant of this gene, $\underline{E}_3^S$, which produces an enzyme with an altered electrophoretic migration rate. Homozygotes for either allele form only a single $\underline{E}_3$ esterase band. However, as is the case with the pH 7.5 esterases, three bands are found in the heterozygotes. The hybrid band shows an intermediate migration rate and in diploid tissue its intensity is greater than that of the other two bands.

The $\underline{E}_1$ and $\underline{E}_3$ genes are not linked and segregate independently. Since the genotypes can be accurately determined by scoring the zymograms of seedling extracts it is possible to score all nine genotypes segregating in the F_2 progeny of the cross $\underline{E}_1^N/\underline{E}_1^N$, $\underline{E}_3^S/\underline{E}_3^S$ X $\underline{E}_1^F/\underline{E}_1^F$, $\underline{E}_3^F/\underline{E}_3^F$. The observed distribution closely fits the expected 1:2:1:2:4:2:1:2:1 distribution.

E_1^N/E_1^N, E_3^S/E_3^S - 46

E_1^N/E_1^N, E_3^S/E_3^F - 84

E_1^N/E_1^N, E_3^F/E_3^F - 47

E_1^N/E_1^F, E_3^S/E_3^S - 87

E_1^N/E_1^F, E_3^S/E_3^F - 186

E_1^N/E_1^F, E_3^F/E_3^F - 87

E_1^F/E_1^F, E_3^S/E_3^S - 49

E_1^F/E_1^F, E_3^S/E_3^F - 114

E_1^F/E_1^F, E_3^F/E_3^F - 50

Hybrid enzyme formation may be quite common in maize. Two cases have now been described where mutant alleles form enzyme types with altered charge, and in both cases ($\underline{E}_1$ and $\underline{E}_3$) hybrid enzymes are formed in heterozygotes.

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1. A plant color factor linked to the R locus.

From a stock of $\underline{R}^r$ Ecuador, a non-paramutable R allele of South American origin, a plant color factor, closely linked to R, was isolated. The linked factor has the plant color attributes of $\underline{R}^{ch}$—i.e., the production of pericarp color in the presence of Pl, concentration of anthocyanin at stem nodes, and production of pink silks. Another phenotypic effect of this factor is to produce red striping in leaves exposed to light, somewhat similar to the effect of B (sun red).

From 1440 kernels resulting from crosses of the type: $\underline{R}^{st}\underline{M}^{st}/\underline{R}^{r}$-Ec $\underline{m}^{st}$ x $\underline{r}^{g}\underline{m}^{st}/\underline{r}^{g}\underline{m}^{st}$, 25 proved to be recombinants between R and the leaf stripe factor (14 $\underline{R}^r$, no stripe: 11 $\underline{R}^{st}$, stripe). This places the factor 1.7 units from R.

29 light-stippled (i.e., $\underline{R}^{st}\underline{m}^{st}$, crossovers between R and $\underline{M}^{st}$) and 709 stippled ($\underline{R}^{st}\underline{M}^{st}$) kernels were produced from the above crosses. When the 29 $\underline{R}^{st}\underline{m}^{st}$ kernels were planted, of the 22 that germinated, 9 carried the leaf striping factor. The factor, therefore, is between R and $\underline{M}^{st}$, and at a distance of about 1 or 2 units distal to R.

R. A. Bray

2. A duplicate R locus.

An R factor originally from Peru and sent us by P. C. Mangelsdorf under the designation Peru 1497, has been found to segregate independently of the known R locus in chromosome 10. Plants heterozygous for both loci give ratios as expected for duplicate factors. By the use of inversions and the wx-9 translocation series, the duplicate factor has been located on the second chromosome, probably near the B locus. The data obtained were as follows:

I. $\underline{r}^{g}/\underline{r}^{g}$; wx/wx ♀ X $\underline{r}^{g}/\underline{r}^{g}$; Wx 'R'/wx T2-9b (2S.18, 9L.22) ♂

Kernel phenotypes from 5 plants

Wx 'R'	Wx r	wx 'R'	wx r
482	138	126	459

Recombination between 'R' and wx on T2-9b : ca. 22%

II. C/c; r^r/r^g; b Gl_2 'R'/B gl_2 Inv.2a (2S.7, 2L.8) ⊗

Seedling phenotypes from colored kernels of five plants

Non-glossy	Glossy
468	13

Recombination between 'R' and gl_2 in Inv.2a : ca. 33%

III. C/C; r^g/r^g; b/b ♀ X C/c; r^r/r^g; b Gl_2'R'/B gl_2 Inv.2a ♂

Seedling phenotypes from colorless kernels of five plants

Red B	Red b	Green
441	3(1)	3(2)

(1) these plants were classified as 'doubtful B'

(2) these could be contaminants

Recombination between 'R' and B in Inv.2a is a maximum of 3% from these data, and could be less.

'R'-Peru 1497, in plants that are r^g for the locus on chromosome 10, conditions colored aleurone, green anthers and seedlings, and is complementary to the aleurone factor C. There is no mottling of the aleurone when the factor is present in a single dose. There is no apparent reduction in the level of pigment after it has been in the same genome with a paramutagenic R gene. It is non-paramutagenic, in that R genes known to be paramutable remain apparently unmodified after they have been together with this duplicate factor for one generation.

Derek Styles

III. STOCKS AVAILABLE AND WANTED

A. Wanted:

W. D. Bell, Department of Botany, Pennsylvania State University

Selections providing mineral-deficiency symptoms.

E. H. Coe, Jr., Curtis Hall, University of Missouri

B chromosomes in colored-seeded, purple plant, multiple dominant (e.g., Su, etc.)

D. V. Glover, Department of Agronomy, Purdue University

Stocks of the following A-B chromosome translocations: B-2, B-5, B-6 and B-8.

Endosperm mutants affecting carbohydrate biosynthesis in maize and any mutants which are allelic to amylose extender, ae.

J. Kohashi, Botanical Museum, Harvard University

Stocks of identical or nearly identical genetic background and carrying $\underline{a_1}\ \underline{A_2}$, $\underline{A_1}\ \underline{a_2}$, and $\underline{A_1}\ \underline{A_2}$ for biochemical studies on anthocyanin development.

C. C. Wernham, Department of Agricultural Plant Pathology, Pennsylvania State University

Inbreds, varieties, or hybrids—not later than 900-1000 maturity--that have unusually long leaves.

Inbreds that have unusually wide leaves—in excess of 5 inches, not later than 900-1000 maturity.

B. Available:

C. R. Burnham, Department of Agronomy and Plant Genetics, University of Minnesota

The following set of interchanges is available for use in chromosome identification tests: 1-2a, 2-4d, 3-7c, 5-7c, 8-9a, and 8-10b. These are segregating 1 heterozygous: 1 homozygous interchange.

H. Garrison Wilkes, Botanical Museum, Harvard University

Continuing field studies on teosinte begun last year (see Wilkes, MNL 37, 1963), seed collections are available for distribution from the following locations in Mexico and Central America.

Mexico

Generalized habitat: 800-1900 m., limestone country rock, seasonally dry, growing season June through October, flowering September-October, mature seed in November.

	Altitude in meters
Guerrero	
Chilpancingo & area	800-1400 m.
Teloloapan - Arcelia (80 km.)	800-1900 m.
Ixcateopan & area	1200-1950 m.
Taxco	1750 m.
Edo. De Mexico	
Chalco	2250 m.
Los Reyes	2180 m.
Amecameca	2350-2500 m.
Michoacan	
Huetamo & drainage of Rio Balsas	400-800 m.
Tiquicheo	800 m.
Tzitzio	1000-1650 m.
Churintzio	1800 m.
Zacapu & area	1750-1950 m.
Cuitzeo	1880 m.
Copandaro de Galeana	1850 m.
Ciudad Hidalgo	2080 m.
Guanajuato	
Moroleon - Uriangato	1750-1900 m.
Manuel Doblado	1750 m.

Central America - Guatemala and Honduras

Generalized habitat: Teosinte from Jutiapa, Jalapa, Chiquimula, and Choluteca is essentially similar to teosinte in Mexico in habitat (but not morphology). In Huehuetenango the habitat is seasonally wet 8 months of the year in contrast to Mexico which is seasonally dry 8 months of the year. Huehuetenango teosinte is two months later than Mexican teosinte forming tassels in November and mature seed in January.

Guatemala

Depto. Jutiapa	
El Progreso	1200 m.
Jutiapa & area	1200 m.
Laguna Retana & area	1050-1250 m.
Agua Blanca	1000 m.
Santa Catarina Mita	800 m.
Depto. Jalapa	
San Luis Jilotepeque & area	950 m.
San Manuel Chaparron & area	950 m.

Guatemala (Continued)	
Depto. Chiquimula	
Ipala & area	900 m.
Depto. Huehuetenango	
San Antonio Huista & area	1200-1650 m.
San Ana Huista	900-1200 m.
Buxup	950-1050 m.
Jacaltenango	1700 m.
Chitsba & area	1200-1550 m.
Tablon	1000 m.
Honduras	
Depto. Choluteca	
San Antonio de Padua	400 m.

IV. REPORT ON MAIZE COOPERATIVE

In 1962, seed increases were made of 165 older, mostly permanently-lettered reciprocal translocations. These stocks are now available for distribution and are listed in this report. In most cases individual translocations can be supplied either in homozygous or heterozygous condition. Most are also available with linked gene markers. This series includes consecutively-numbered translocations from 1-2b through 4-9c.

The remaining lettered translocations (from 4-9c through the 9-10's) were grown the past summer. These have not yet been fully catalogued and pedigreed for distribution.

Increases were made of our entire collection of stocks in Chromosomes 1 through 5, and of selected stocks in Chromosomes 9 and 10. Additional increases were made of exotics and popcorns, endosperm (starch) mutants, glossy leaf traits, A-B translocations, multiple gene testers, and wx-marked translocations.

Most of the unplaced genes which have appeared on our stock list were crossed last summer with wx-translocations and A-B translocations in an effort to place these genes to chromosome and determine their map positions. Progenies from these crosses are being grown in the current Florida generation and will also be grown next summer.

Dr. R. J. Lambert joined our staff last summer and has been assisting in all phases of the Maize Cooperative work.

During 1963, 1,416 seed samples were supplied in response to 92 requests. Of the total, 997 samples were distributed within the U.S. (81 requests) and 419 samples were sent to foreign countries (11 requests).

The following listing of 165 reciprocal translocation stocks is a supplement to stock lists in the 1962 and 1963 Maize News Letters. Requests for seed or for copies of stock lists should be sent to E. B. Patterson, S-116 Turner Hall, Agronomy Department, University of Illinois, Urbana, Illinois.

RECIPROCAL TRANSLOCATIONS

The interchange positions for these translocations are listed in the following publication: Longley, A. E. Breakage Points for Four Corn Translocation Series and Other Corn Chromosome Aberrations. U.S. Dept. of Agr., Agr. Res. Serv. ARS 34-16, 40 pp., 1961.

Translocation	Temporary Symbol	Translocation	Temporary Symbol
1-2b		1-10a	
c		b	Conn R-41
d	17	c	A-50
e	B-75	d	A-84
1-3a		e	B-98
c		f	C-36
d		g	C-47
e	A-33	2-3b	
h	C-15	c	
i	C-43	d	
j	F-10	e	
k	G-3	f	A-61
1-4a		g	F-35
b	Conn R-29	h	K-7
c	A-57	2-4a	
f	C-46	b	
g	C-49	c	
h	X-22-61	d	
	K-40	e	Conn R-42
1-5a		f	A-29
b		g	C-31
c		j	K-10
e	A-90	k	X-1-1
f	D-5	l	X-2-64
g	I-24	m	X-47-41
h	X-1-37	2-5a	
i	X-23-2	b	
1-6a		c	Conn R-50
c		d	A-74
d	Conn R-28	e	B-69
e	A-80	f	K-3
f	B-92	g	X-14-122
g	F-30	2-6a	
h	X-41-13	b	
1-7a		c	
b		d	
c		e	
d		f	84-2
e	42		78
f	A-69	2-7b	
g	B-49	c	
h	B-94	d	B-108
i	I-17	e	C-44
j	X-55-16	f	F-29
	A-37	2-8b	A-1
1-8a	Conn R-20	d	C-24
b	B-42	e	C-40
1-9a		f	C-57
b		g	G-2
c		h	X-42-32
d	I-9		84

Translocation	Temporary Symbol	Translocation	Temporary Symbol
2-9a		3-9a	
b		b	
c	C-61	c	
d	H-7	d	A-41
2-10a		e	A-94
b	F-2	f	B-103
	I-3	g	F-24
3-4	A-21	h	X-23-158
3-5a		3-10a	
b		b	
c		c	
e	A-101	4-5a	
g	X-4-108	b	
h	X-7-38	c	
	B-104	d	
3-6a		e	Conn R-18
b		f	Conn R-30
c	Conn R-34	g	Conn R-32
d	A-53	i	B-74
3-7a		j	X-6-77
b		k	X-19-5
c		4-6a	
d	C-75	b	
e	F-25	c	
3-8a		d	Conn R-43
b		e	X-57-31
c	Burnham	4-7a	
e	A-22	4-8a	
f	A-104	b	X-17-108
g	B-37	4-9a	
h	X-23-26	b	
		c	bp

E. B. Patterson

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