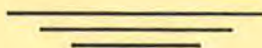


MAIZE GENETICS COOPERATION

NEWS LETTER

42



April 15, 1968

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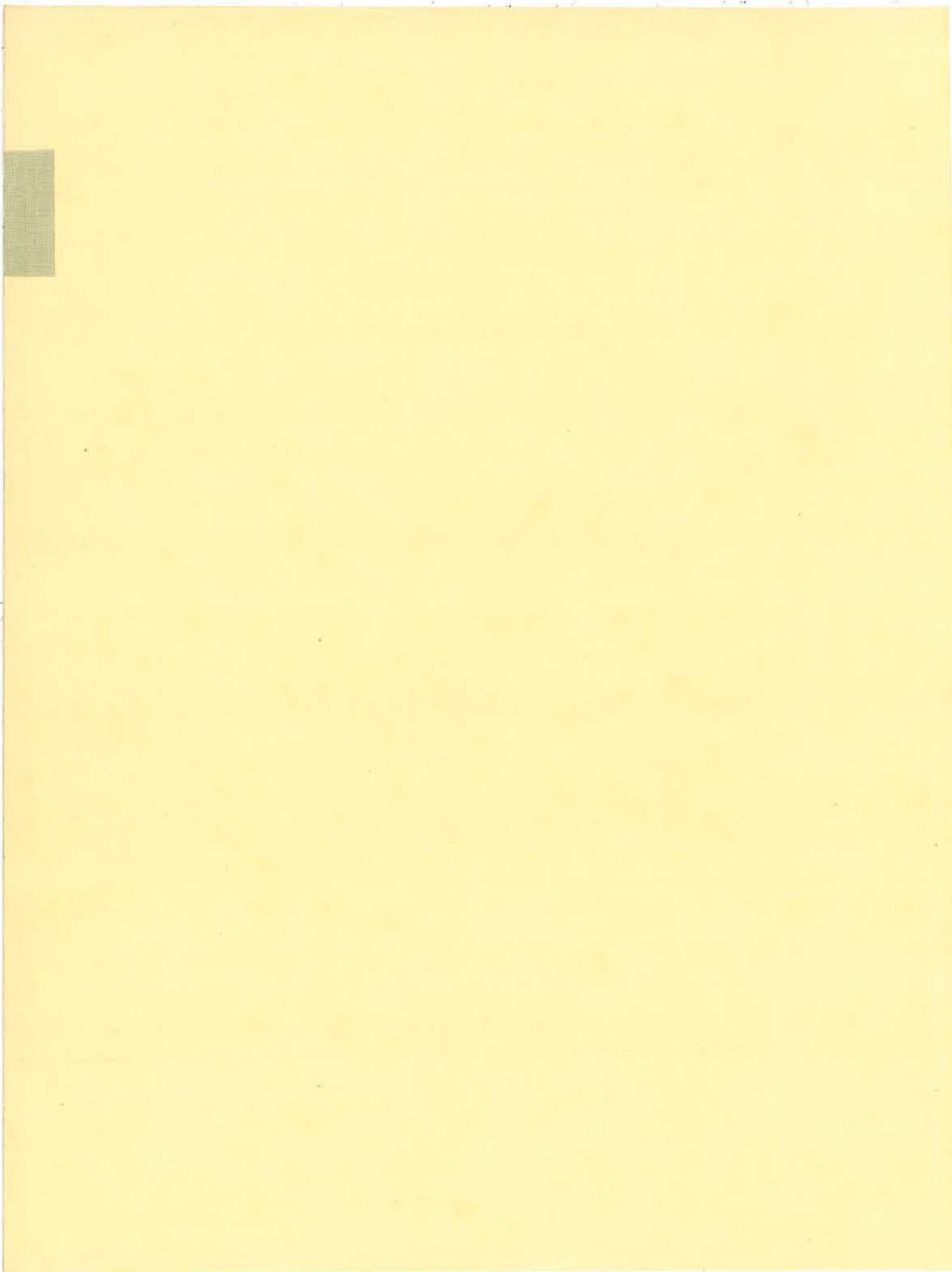


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I. FOREWORD

Once again it is a pleasure to acknowledge the dedicated and efficient services of Miss Ellen Dempsey in the editing, supervising, and assembly of the Maize Genetics News Letter. This is an arduous and responsible task and all of us who derive benefit from these News Letters are in her debt for a job extremely well done. Recognition of the voluntary assistance of Wayne Carlson, Prem Chourey, Achille Ghidoni, William Laughner, Reid Palmer and Edward Ward in proof reading is gratefully acknowledged. We are indebted to Dr. Achille Ghidoni for preparing the bibliography.

Volumes 1-29 and Volume 33 have been placed on microfilm. Copies can be obtained from this laboratory for \$8.50. Checks should be made out to M. M. Rhoades.

The cost of publishing this year's News Letter has been met from a grant by the National Science Foundation to the Maize Genetics Stock Center. We are truly appreciative of this financial help.

Our attention has been called to the following errata in the Maize Genetics News Letter 41:

Page 2: Line 23 $\frac{A + C}{C + D}$ should be $\frac{A + G}{C + T}$

Line 27 $\frac{A + C}{C + D}$ should be $\frac{A + G}{C + U}$

Page 78: Fourth row below backcross and fourth row below self in the first column.

$P < 0.05$ should be $P > 0.05$

Page 134: Line 22 of text.

Type 2a should be Type 1a.

M. M. Rhoades

II. REPORTS FROM COOPERATORS

ANDHRA UNIVERSITY
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Department of Botany

1. Chromosomal instability in individual plants of *Coix aquatica*.

During a cytological study of the chromosomal variants in a mixed population of *Coix aquatica* (n=5) reported last year (MNL 41:5-6, 1967), two plants (8-2 and 9-11) showed variation in chromosome number and behavior within the same individual. The observations on these are summarized in the table below.

Plant No.	Spike No.	Chromosome No.	Nucleolar bivalents	Meiotic behavior
<u>8-2</u>	1	n=6 and 2n=12	one	6ii; A few cells had an association up to 4 chromosomes (not involving nucleolar bivalent).
	2 to 4	n=5 and 2n=10	one, sometimes two	5ii; A few cells had a ring or chain of 4 chromosomes involving one of the nucleolar bivalents.
	5	n=5 and 2n=10	one	5ii; Usually 1-4 univalents per cell were found. Occasionally a chain of 3 chromosomes (not involving nucleolar bivalent) is found. In one cell a fragment and the resulting heteromorphic bivalent were present. 1-3 laggards were recorded at AI.
<u>9-11</u>	1 to 6	2n=11	one	5ii + li; Univalent often found attached to the nucleolus but when separate it formed a small nucleolus of its own. It often showed fold back pairing which persisted till diakinesis and MI giving at these stages the appearance of a small ring bivalent.
	7 to 11	n=5 and 2n=10	one	5ii

The manner in which the material was collected was such that it is not possible to know whether this variable situation exists between different branches of the same plant or between different spikes in the same branch.

The extra bivalent, occurring in plant no. 8-2, resembles the other chromosome pairs in the complement in staining intensity and length at diakinesis. In fact, it could not be identified from the rest at this stage. The presence of an extra chromosome pair and the formation of higher associations involving up to 4 chromosomes in a proportion of cells are suggestive of a tetrasomic condition. Since the higher associations comprised chromosomes that are not equal in size, and further, such associations were also observed even in spikes showing a chromosome number of $n=5$, a tetrasomic nature of pairing for the higher associations is overruled. Therefore, it is believed that the occurrence of the higher associations is due to chromosomal interchanges and that the extra chromosome pair is not involved in them. The occurrence of one or two nucleolar bivalents may be explained on the assumption, based on widespread existence of chromosomal interchanges in the karyotype of this species, that small portions of the nucleolus organizing region of the nucleolar chromosome were translocated to one of the non-nucleolar chromosomes. If the region involved is adequate enough for the function, two nucleolar bivalents may be found consistently. If not, one or sometimes two bivalents may be found associated with the nucleolus. If there is no such translocation, only one nucleolar bivalent may be found. The fragment observed in one of the cells of this plant could be of localized nature and of spontaneous occurrence. The differences observed in the formation of higher associations may be taken as related to the occurrence of chiasmata at appropriate places.

In plant no. 9-11, the extra univalent occurring in 6 of the 11 spikes studied did not pair with other chromosomes in the complement. It is as long and stains as much as other chromosomes and in this it resembles the extra bivalent in plant 8-2. Whether this univalent represents a single chromosome of the extra bivalent in 8-2 remains to be determined. The fact that the extra chromosome pair in 8-2 and the extra univalent in 9-11 did not show pairing affinities with other chromosomes in the complement and further, that they occur in some portions of the plant and are absent in the rest leads to the suggestion that these may be B-type chromosomes.

J. Venkateswarlu
Panuganti N. Rao
Raju S. K. Chaganti

2. Further cases of spontaneous chromosomal variation in job's tears (*Coix lachryma-jobi*).

A case of spontaneous chromosomal interchange in *C. lachryma-jobi* ($n=10$) was reported earlier (MNL 39:184-185, 1965). Further studies on this species have revealed two plants showing two other types of spontaneous chromosomal variations, viz. a) trisomy and b) desynapsis.

(a) A plant with a chromosome number of $2n=21$ was located. This showed at diakinesis and metaphase I nine bivalents and one trivalent. The trivalent is attached to the nucleolus. Since the third chromosome in the trivalent

was always found to be smaller than the other two chromosomes, it appears that the third one does not represent an entire homologous chromosome but only a large centric fragment of it. The plant is propagated vegetatively and cytological examination of the material from the suckers also showed the same trisomic condition. Seed set was good and from selfed seed of this plant, three plants were raised and checked cytologically. All the plants showed $2n=20$ and regular bivalent formation.

(b) Cytological material collected in midsummer (May 1967) from a plant bearing all sterile seeds showed desynapsis. Univalents varied from 12 to 20 per cell at diakinesis and metaphase I. Other abnormalities associated with univalent formation, such as laggards at anaphase I and II and micronuclei, were common.

When the original culms of this plant were nearing their end by about July 1967, new suckers started coming up. Cytological examination of the material fixed in the rainy season (September 1967) from these suckers revealed regular meiosis with 10 bivalents at diakinesis and metaphase I. Seed setting was fairly good. It is therefore believed that the desynaptic behavior and the consequent seed sterility in the original culms were the result of the effect of high summer temperature.

J. Venkateswarlu
Panuganti N. Rao

3. Cytological studies in the progeny of tetraploid plants of job's tears.

Three tetraploid plants ($4n=40$) obtained by colchicine treatment in 1966 began flowering in September 1966 but seed set was poor during October 1966 to January-February 1967 (MNL 41:7, 1967). The vegetative suckers from these plants produced a good number of black spherical seeds in March-April 1967. From a sample of the seed collected from each of the three open pollinated tetraploids a large progeny was raised in June 1967. One hundred twenty-four out of 170 seeds sown have germinated (72.94%). Germination started in 9 or 10 days and continued till 45-60 days after sowing. These were transferred to the field in 4-5 weeks after germination. White chalky seeds produced on the same plants are usually sterile but a large number of these were also sown separately. One of them germinated and produced a healthy plant.

Pollen mother cells in 70 plants of the progeny were examined at diakinesis, metaphase I, and anaphase I for chromosome numbers. Even though tetraploids and diploids were growing side by side no triploids were obtained in the progeny. The table below shows the frequency and percentage of plants with different chromosome numbers met with in the progeny.

	Chromosome numbers in the Progeny				Total
	21	39	41	40	
Frequency of plants	1	5	8	56	70
Percentage	1.43	7.14	11.43	80.00	100

Only 20% of the plants studied showed any variation in chromosome number. The 21 chromosome plant (trisomic) might have originated by parthenogenetic development of an unfertilized egg carrying 21 chromosomes (due to irregular distribution of chromosomes at anaphase). The plants with 39 and 41 chromosomes were probably resultants of mating between a normal gamete ($n=20$) and a gamete with one chromosome added ($n=21$) or removed ($n=19$) owing to irregular distribution at anaphase. The plants with 40 chromosomes could result by the union of normal gametes or of gametes having 19 and 21 chromosomes. It is not known, however, whether the male gamete carrying the unbalanced number functions normally with other pollen, but on the female side such gametes seem to function. All the plants with $4n$ number in the progeny showed multivalent formation (up to quadrivalents). In the 41 chromosome plants, as expected, a pentavalent was observed in a proportion of cells. In the 39 chromosome plants, in all the cells, either a trivalent or an univalent was clearly seen. The trisomic plant showed 9 bivalents and 1 trivalent.

Seed setting is generally good in all the progeny but some plants produced abundant seed. Seed size and shape are varied, sometimes even within the plant. The color is usually black but in some it is diluted to brown.

J. Venkateswarlu
Panuganti N. Rao

4. Meiosis in *Sclerachne punctata* R. Br.

The genus *Sclerachne*, with only one species (*S. punctata*) and restricted in distribution to Java, Madoera, and Timor (Henrard, 1931), is an Oriental relative of maize. Cytological studies on this species were limited to observations made by Mangelsdorf and Reeves (1939) and Larsen (1963) on somatic chromosomes ($2n=20$). Some observations on meiosis were made now and reported here. Seeds were kindly provided by Professor Paul Weatherwax. The chromosomes at pachytene appear uniformly stained. Formation of 10 bivalents at diakinesis and metaphase I and a 10:10 distribution of chromosomes at anaphase I were observed. Two bivalents were usually found near the nucleolus. Among the 10 bivalents, 2 are large, 2 slightly smaller than the large ones and 6 are small. All the bivalents do not always orient on the metaphase plate, but are often found to occur in 6 or 7 groups due, perhaps, to secondary associations; 3 or 4 groups of two bivalents each and

2 or 4 groups of one bivalent each were found. The second division is also quite regular but in two cells, out of several studied at anaphase II, chromosome bridges in one cell and a laggard in the other were observed. Pollen fertility and seed setting are good.

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1. Absence of a detectable change in Ds at the A_1 locus following mutagenic treatments.

In last year's MNL we reported our observations in regard to the stability of Ds at the A_1 locus. We now present additional data regarding the absence of a detectable change in Ds at the A_1 locus following certain "mutagenic" treatments viz. Ultraviolet radiation, gamma-rays and Mitomycin C.

It is known that UV irradiation of pollen produces discrete changes at the genic level. It was assumed that a "change" in Ds, without affecting the A_1 locus, would restore the function of A_1 . No such change was detected as Table 1 shows. That the treatment was in general mutagenically effective is shown by the fact that a very large number of cases of sh₂ were obtained, although most of these must be losses of Sh₂ following the generation of breakage-fusion-bridge cycles.

Similarly no change was detected for Ds following gamma irradiation of pollen or plants. Gamma radiation in general does not produce discrete changes and practically all the changes must be due to marker loss. However, the B-F-B cycles are correlated with the "recreation" of Dt-like elements but in the present case no Ac-like elements were generated.

Mitomycin (MC) was used since it is a known agent for the induction of lysogenic bacteria. If Ds were like a prophage, then conceivably it could be induced by MC treatment. MC was apparently very mildly mutagenic. Its ability to "induce" Ds, if it is an inducible prophage, remains in doubt. No colored kernels were obtained (Table 1).

Chandra Mouli
N. K. Notani

2. Induced mutation of I and C : a comparison.

The complex inter-relationships of expression and dominance among I, C and c are not readily interpretable in terms of the structure and function of the locus or loci involved. By themselves III, CCC and ccc genotypes respectively condition colorless, colored and colorless aleurone. I and c are resolved only when present together with C, the former being dominant and the latter recessive to C. Further, because both I and C are mapped very close together, it is generally considered that I and C or c are either components of a compound locus or form an allelic series of a

Table 1
 Types of kernels obtained from treatment with different mutagenic agents of $\underline{A_1 Ds Sh_2}$, no Ac stocks
 crossed to $\underline{a_1^S sh_2}$, no Ac tester

Treatment	Total kernels	Phenotype of the kernels				Colored shrunken or non-shrunken	Total change for $\underline{sh_2}$ %
		Colorless non-shrunken	Colorless				
			$\frac{1}{4}$ shrunken %	$\frac{1}{2}$ shrunken %	Full shrunken %		
Control	1799	1791	6(0.35)	2(0.11)	0(0.00)	0(0.00)	8(0.46)
UV irradiation of pollen	2197	2066	64(2.91)	45(2.04)	22(1.01)	0(0.00)	131(5.96)
Seeds treated with Mitomycin C	2543	2517	10(0.39)	12(0.47)	4(0.16)	0(0.00)	26(1.02)
Seeds treated with Mitomycin C, pollen irradiated with UV	2268	2124	53(2.33)	63(2.77)	28(1.25)	0(0.00)	144(6.35)
Plants exposed to chronic gamma radiation	1822	1774	24(1.31)	14(0.76)	10(0.56)	0(0.00)	48(2.63)
Plants exposed to chronic gamma radiation, pollen treated with UV	2647	2455	47(1.77)	101(3.77)	44(1.66)	0(0.00)	192(7.20)

Table 2

Changed kernel types obtained from the cross $\frac{I\ Sh\ Bz\ Wx}{I\ Sh\ Bz\ Wx}$ (treated) X $\frac{C\ sh\ bz\ wx}{C\ sh\ bz\ wx}$ and their subsequent breeding behavior

Treatment	Number of seeds treated	Colored			Shrunken			Waxy			Remarks
		Obtained	Failed to propagate	Nonconcordant	Obtained	Failed to propagate	Nonconcordant	Obtained	Failed to propagate	Nonconcordant	
A.											
Control	250	0	-	-	0	-	-	0	-	-	
γ -ray irradiated	500	5	0	4	0	-	-	1	1	-	
Diethyl-sulfate	202	5	1	3	0	-	-	0	-	-	One case: All 4 markers lost
Ethyl-methane-sulfonate	350	3	1	2	3	0	0	4	2	0	
EMS + γ -ray irradiated	146	5	0	4	0	-	-	0	-	-	
Total	1198	18	2	13	3	0	0	5	3	0	
True mutations		3			3			2			
B. EMS											
$\frac{I\ Sh\ Bz\ Wx^*}{I\ Sh\ Bz\ Wx}$ X $\frac{C\ sh\ bz\ wx}{C\ sh\ bz\ wx}$	150	0	-	-	0	-	-	0	-	-	
$\frac{C\ Sh\ Bz\ Wx^*}{C\ Sh\ Bz\ Wx}$ X $\frac{c\ sh\ Bz\ wx}{c\ sh\ Bz\ wx}$	180	6	1	0	-	-	-	-	-	-	∞

*Treated

Table 3

Transmission of linked factors on the short arm of chromosome 9 in crosses heterozygous for a gametophyte factor

Cross	Progeny kernels							
	<u>I Sh (Bz) Wx</u>	<u>C sh bz wx</u>	<u>I sh (bz) wx</u>	<u>C Sh Bz Wx</u>	<u>C sh Bz Wx</u>	<u>I Sh (Bz/bz)wx</u>	<u>C sh bz Wx</u>	
<u>C sh bz wx</u> X <u>C sh bz wx</u> <u>I Sh Bz Wx</u> <u>I Sh Bz Wx</u>	3	196	0	6	5	0	20	
<u>C sh bz wx</u> X <u>C sh bz wx</u> <u>I Sh Bz Wx</u> <u>I Sh Bz Wx</u>	1	133	0	3	4	0	14	
<u>C sh bz wx</u> X <u>C sh bz wx</u> <u>I Sh Bz Wx</u> <u>C sh bz wx</u>	54	61	1	0	1	6	9	
<u>C sh bz wx</u> X <u>C sh bz wx</u> <u>I Sh Bz Wx</u> <u>C sh bz wx</u>	92	117	1	4	0	12	10	
<u>C sh bz wx</u> X <u>C sh bz wx</u> <u>I Sh Bz Wx</u> <u>C sh bz wx</u>	110	106	1	0	1	8	15	
<u>C sh bz wx</u> X <u>C sh bz wx</u> <u>I Sh Bz Wx</u> <u>C sh bz wx</u>	90	86	0	1	0	16	11	

bifunctional locus. The latter model was proposed by Coe (1964) because he did not obtain the crossovers expected on the basis of the compound locus model. While this may be the case, the dominance behavior of (the mutation) I appears to be somewhat similar to the so-called super-repressed mutations of the regulator gene (R^S type) in E. coli which when heterozygous (R^S/R^+) are unable to synthesize the enzyme (B-galactosidase). This is a very striking result from the genetic point of view since an R^S mutation corresponds to a dominant loss of function (Jacob & Monod in Cytodifferentiation and Macromolecular Synthesis, Academic Press, 1963).

The data to be presented here were collected with a view to study similarities and differences between I and C in regard to their direction of mutation, mutation rates, and any other information which would have a bearing on the above models. Our observations are summarized in Table 2. The following points are noteworthy:

- (1) There is a very high proportion of non-concordant changes of I. In contrast no such class was observed from C, Sh and Wx mutations.
- (2) The direction of mutation of I is only to i (phenotypically indistinguishable from c) never to C. These findings are similar to those of Coe (1962) for the direction of spontaneous mutation of I.
- (3) The mutation rates of I and C are apparently dissimilar but the data are insufficient on this point.

Chandra Mouli
N. K. Notani

3. A new gametophyte factor on chromosome 9.

Drastically reduced pollen transmission for the factors located on the short arm of chromosome 9 was observed following, apparently, a spontaneous mutation of a Ga factor to ga. I was transmitted to the extent of about 1%, Sh about 3%, Bz about 4%, and Wx about 10%. No reduction in transmission of these factors was noted in the reciprocal cross (Table 3). Assuming 100% non-transmission for the pollen carrying ga and assuming the transmitted gametes as due to crossovers, the locus of ga is placed very close to I and distal to it. Crossing-over between I and Wx is about half the usual value. Its relationship with ga, if any, is not clear at the moment.

Chandra Mouli
N. K. Notani

4. High rate of induced change for anther color in maize.

During the course of a study designed to reveal the type of sectorial mutations induced by ionizing radiations and chemical mutagens, homozygous ACR^T (original stock kindly supplied by Prof. R. A. Brink) seeds were irradiated with Co^{60} gamma-rays and ethylmethane sulfonate (EMS). A large number of plants arising from the treated seeds were observed to have green anthers (Table 4). EMS was found to be particularly effective in inducing this change with as many as 103 out of 280 plants showing some

Table 4

Effect of treatment with ethyl methane sulfonate or gamma-rays on anther color of plants grown from A C R^r and A B P1 C R^r homozygous seeds

II

Treatment	Genotype	No. plants scored	No. plants showing green anthers	Extent of green anther sector (No. of Plants)									
				3 branches affected		2 branches affected		1 branch affected		Spike-lets affected or more			1 anther affected
	Homozygous for			Entirely	Partly	Entirely	Partly	Entirely	Partly	4	2	1	
Control	<u>A B P1 R^r</u>	185	1	-	-	-	-	-	-	-	-	-	1
	<u>A C R^r</u>	226	1	-	-	-	-	-	-	-	-	-	1
Seeds soaked 24 hrs. & irradiated 2000r, gamma rays	<u>A B P1 R^r</u>	178	12	-	-	-	1	-	-	-	-	6	5
	<u>A C R^r</u>	401	15	-	1	1	2	-	4	1	-	2	4
Treated with 0.01M EMS 24 hrs.	<u>A B P1 R^r</u>	123	26	-	-	-	-	-	-	-	-	20	6
	<u>A C R^r</u>	280	103	1	4	2	5	4	41	2	13	11	20

change. Forty-four of the changed plants were examined for pollen fertility. In general the pollen fertility was higher in red anthers than in the green ones. Similar changes were observed with the $\underline{A} \underline{B} \underline{P1} \underline{R}^r$ homozygous stock but the incidence was much lower and the sectors very much smaller.

These observations are not readily explained in conventional terms. Since the colored anther color phenotype is dominant over the green anther phenotype, a simultaneous mutation or deletion of the \underline{P} component of \underline{R}^r locus would have to occur in both chromosomes to manifest this change in the first generation. This is highly improbable from what we know of the mutation rates of certain gene loci. The determination of the basis of this change must await further work.

Chandra Mouli
N. K. Notani

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1. Male-sterility as affected by seed storage.

Seed of Llera III, a derivative of the Tuxpeno race of maize from Mexico, was produced by sib pollination during the 1963 winter at Hyderabad. During summer 1965 a yield trial was planted at Indian Agricultural Research Institute, New Delhi, in which Llera III was one of the entries. While taking notes on various characters it was observed that Llera III had 2.9 per cent male-sterile plants. The anthers of the male-sterile plants were shriveled and there was no pollen formation in any of the anthers. When a random sample from the same seed lot of Llera III, which was increased at Hyderabad during the 1963 winter, was grown at the Birla Institute of Scientific Research, Rupar, during the 1967 summer, 12.5 per cent male-sterile plants were observed--an increase of 9.6 per cent over what was observed in 1965 summer.

Two possibilities seem to have given rise to an increased percentage of male-sterile plants during the 1967 summer, when the source of seed for both the years of study happened to be the same. Either there has been inadequate sampling during the 1965 study or else the seed storage for another two years has resulted in an increase of male-sterile plants in the population. Such a storage effect has been noted in mutation studies.

B. K. Bhat
M. C. Pande

UNIVERSITY OF BOLOGNA
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1. Analysis of variation of an autodiploid strain of maize by means of diallel cross analysis.

In genetic studies, especially for quantitative characters, it is sometimes very important to have available strains with an homogeneous genotype;

therefore the possibility to produce autodiploid strains, that are strains resulting from the duplication of a haploid genome, was regarded as a possibility to provide better tools for genetic studies. On the other hand, Sprague et al. (1960) have shown that in autodiploid strains there is a very high rate of spontaneous mutagenesis, thus pointing to the problem of maintenance of homogeneity of these strains over a number of generations.

It seemed of interest to us to investigate the relative importance of spontaneous mutagenesis in changing the homogeneity of an autodiploid strain, and also the possible relations between the occurrence of spontaneous mutation and the establishment of overdominance phenomena.

Plants derived from a single grain of an autodiploid ear of maize, stock HD 73, 1375/II, kindly supplied by Prof. Sprague, were reproduced by selfing through 4 generations. The F_4 plants were classified according to the pedigree in five groups obtained from five F_2 plants.

In 1965 using five parents, one from each line and generation of selfing, we realized all possible crosses within generations, obtaining three series of crosses, classified according to the generation of selfing from which the parents were taken.

The F_1 plants, together with their parents were sown in 1966 following a randomized block experimental scheme, replicated four times. The height of the plant was measured and the data analyzed following the method of Hayman (1954).

Table 1 summarizes the estimates of the components of variance, obtained from the analysis, together with their standard errors.

Table 1
Estimates of components of variance for the height of the plant from the diallel analysis

	Parents from F_2	Parents from F_3	Parents from F_4
D	5.54 \pm 0.75	5.72 \pm 1.80	4.53 \pm 0.60
F	6.17 \pm 1.88	0.01 \pm 4.49	1.36 \pm 1.49
H_1	0.55 \pm 2.03	-3.27 \pm 4.86	-1.88 \pm 1.41
H_2	-23.76 \pm 1.84	-82.92 \pm 4.41	-34.47 \pm 1.46
h^2	-2.03 \pm 1.34	-5.01 \pm 3.20	0.04 \pm 1.60
$H_2/4H_1$	11.0	6.3	4.9
h^2/H_2	0.86	0.64	0.00

The results obtained show that the additive portion of variation is highly significant in all the three series of crosses considered, whereas the estimates of H_1 are not statistically different from zero. These results suggest that the determination of the height of the plant in our material is completely additive.

Table 1 shows also that the estimates of the H_2 component are statistically different from zero, whereas the estimates of h^2 are not significant. As a consequence the estimator, $H_2/4H_1$, which is expected to give a measure of asymmetry, is very high, while the estimator, h^2/H_2 , indicates that a very small number of groups of genes are involved in the manifestation of the variability observed.

Considering all the results obtained we should draw the conclusion that between plants derived from a single grain of an autodiploid strain there are differences which are genetically determined in an additive way. The high values of the asymmetry estimator suggest that over all loci there is a disproportion between the number of alleles of different types (+ and -). This supports the hypothesis that the autodiploid strain was originally very homogeneous and that the additive genetic variability observed is not probably due to residual heterogeneity, but mostly to spontaneous mutations. This view is also supported by the low value of the estimates of the number of groups of genes involved in the manifestation of the variability present in the considered population.

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1. Further studies on teosinte chromosomes.

- a. Huixta Teosinte. Microsporocytes of five F_1 hybrids of Huixta teosinte and maize were cytologically investigated. The teosinte seeds employed in this study are different from those studied previously (Cytologia, Ting, 1958). At pachytene, seven chromosome knobs, all terminal, were observed. Except for those on the long arms of chromosomes 4 and 8, all of them were homozygous. This is the first report that only terminal knobs were present in this teosinte. However, in a few cases asynapsis involving the distal one-third of the short arm of chromosome 4 was identified.

The knobs on the short arms of chromosomes 1, 2, and 6 were small. On the long arm of chromosome 4 and the short arm of chromosome 7, the knobs were large in size. Medium-sized knobs were present on the long arm of chromosome 9.

Despite the occasional occurrence of terminal asynapsis in the short arm of chromosome 4, no definite inversion in this chromosome was identified at pachytene. However, at anaphase I of the microsporocyte divisions, chromatid bridges and fragments were frequently observed in certain hybrids. It is likely that some short inversions were present in this teosinte. Perhaps the lengths of these inversions were so short that the force of homologous pairing could not overcome that of non-homologous pairing. Hence, only rod-shaped configurations were formed at pachytene.

- b. Perennial teosinte: Microsporocytes of four perennial teosinte plants were studied. These plants belong to the progeny of a selfed plant obtained through the courtesy of Dr. D. L. Shaver of Cornnuts Inc., California. It was found that the pachytene chromosomes of these plants were virtually knobless. There were terminal large chromomeres on the short arms of chromosomes 8 and 9. Most of the chromosomes formed regular bivalents at pachytene. Very few multivalents were demonstrated. No deficiencies, inversions or translocations were observed. Apparently these plants are different in terms of chromosome characteristics from those studied earlier by the author (Chromosomes of Maize-teosinte Hybrids, 1964).

Y. C. Ting
Mary E. Dougall

2. Further studies on the B-chromosomes of maize.

In the last year, 79 plants of the selfed progenies of two inbred maize strains, carrying six B-chromosomes each, were examined. For the inbred 67-14, the number of B's per plant among a subtotal of 18 plants was found to vary from three to 12, with the largest number of plants in the six B class. As for the other inbred 67-17, among a subtotal of 61 plants studied the number of B's per plant was found to range from two to 12, with the largest number of plants in the class with five B's.

For a study of the effect of B-chromosomes on the seedset of maize, three additional inbreds were used. They were 66-14, with 15 B's, and 66-15 and 66-16, each with 12 B's. Selfings of three plants in each of these strains were attempted in the summer of 1966. It was later observed that seedsets of all of these plants were very poor, averaging less than five per cent. However, seedsets of sib plants having zero to five B's per plant were close to normal. Hence, the poor seedsets of these plants were attributed to their possessing a large number of B-chromosomes. However, before a definite conclusion can be drawn, a study on a large number of plants together with a statistical analysis should be carried out. Furthermore, the plants with many B's were also

found to be later in flowering, and slower in growth than their sibs.

For another study on the differential DNA synthesis in eu- and heterochromatin of maize, kernels with varying numbers of B-chromosomes were grown. At the early seedling stage, when length of their primary roots averaged about two inches, they were fed with H^3 -thymidine in Hoagland's solution. In preparing autoradiographs a standard dipping technique was followed. Data gathered up to the present indicate that the time of DNA synthesis in eu- and heterochromatin (B-chromosomes) differs. The euchromatin of maize, or A-chromosomes, started DNA synthesis before the heterochromatin. The investigation, it is hoped, may also lead to a detailed analysis of the mitotic cycle of maize.

Y. C. Ting
Richard Phillips

3. Extra chromosome element.

At the first meiotic prophase of the microsporocytes of a maize plant 67-44-2, an extra chromosome element was consistently observed. It was stained as well as the regular chromosomes either with propionic carmine or with Schiff's reagent. At pachytene, it always formed a circular configuration and its length on the average measured about 20 μ . Its location was not confined to a certain part of the cell.

As the division advanced to diakinesis, no evidence of shortening of this element was obtained. At metaphase I, it fragmented into two elements. No centromeres were identified. Apparently due to their lack of regular movement at anaphase I, both of these elements were always found in only one part of the spindle. However, at telophase I, they were no longer identifiable in most of the cases. Among a total of approximately 500 cells examined, these elements were definitely observed in only about two per cent of the cells.

A few years ago a similar element was seen in one of the teosinte derivatives. That element was somewhat shorter than the one reported in the present communication. But its meiotic behavior appeared to be the same. Selfings and crosses with this plant, 67-44-2, were attempted last summer in order to know more about the significance of this element.

Y. C. Ting

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1. Genetic recombination among spontaneous and ethyl methanesulfonate-induced waxy mutants in maize.

Ethyl methanesulfonate (EMS) has been reported to produce "point mutations"⁴ and "single locus mutations"¹ in maize. Since intracistron

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

recombination has been demonstrated at the waxy (wx) locus in maize³ an opportunity is provided to use this locus to distinguish among genetic alterations, e.g. the occurrence of intracistron recombination among mutants is evidence for "point mutations" as opposed to extensive deletions. Intracistron recombination can be studied in pollen of wx plants because of the differential staining reactions of Wx and wx pollen grains and is facilitated because large populations of pollen grains can be observed.

Results are reported for two EMS-induced wx mutants and for three spontaneous wx mutants. Treatments were made on seeds with the genotype C^I Sh Bz Wx. The mutant designated wx^{BL-A} was obtained by treating kernels with 0.025 M EMS solution for 3 days at 3° C. The mutant designated wx^{BL-B} was obtained from 0.1 M EMS treatment for 2 hours at 25°C. These wx mutants, detected by crossing the treated material to a recessive tester C sh bz wx^C, were produced by Amano and Smith¹ and further details on methods of EMS treatment and detection of wx mutants were reported there.

Preparatory to an analysis of the EMS-induced wx mutants it is essential to establish the induced mutant site in the homoallelic condition. In order to do this, plants arising from M₁ generation wx kernels (C^I Sh Bz wx^{induced}/C sh bz wx^C) were self-pollinated. Portions of tassels from these M₁ plants were collected for a pollen analysis.² The material was segregating for the distal marker genes, and only those wx kernels with the dominant distal markers C^I Sh Bz, were selected for further study. Second generation plants arising from these kernels were self-pollinated and those plants that did not recombine (give Wx pollen) at the wx locus and that were also C^I Sh Bz were apparently homoallelic for the induced site as well as homozygous for the distal marker genes. Plants meeting these criteria were included in this study and were used in the final analysis. Third generation plants (C^I Sh Bz wx^{induced}) were crossed in all combinations (diallel) with the spontaneous wx mutants, wx^C, wx⁹⁰, and wx^{H21}. These hybrids were planted and portions of the tassels were collected to use in the assay of pollen. A single plant was used in the first (M₁) and second generations to represent each mutant. However, to make the diallel crosses several plants were used and to obtain the diallel data 3-5 plants were used.

The frequency of Wx pollen grains $\times 10^{-5}$ that represent reversion and recombination between the wx^C tester site and the EMS-induced wx site in the M₁ generation was 41.39 ± 5.17 for wx^{BL-A} and 29.28 ± 4.36 for wx^{BL-B}; 155×10^3 and 154×10^3 pollen grains respectively were used to obtain these estimates. Out of necessity the M₁ data and diallel data (Tables 1 and 2) were taken in separate growing seasons.

Table 1
Average number of Wx pollen grains due to reversion from homoallelic ethyl methanesulfonate induced and spontaneous wx mutants

Alleles	Estimated no. microspores $\times 10^3$	\bar{X} no. <u>Wx</u> $\times 10^{-5} \pm s\bar{x}$
<u>wx</u> ^C	133	2.25 \pm 1.30
<u>wx</u> ⁹⁰	342	0.58 \pm 0.41
<u>wx</u> ^{H21}	166	0.60 \pm 0.60
<u>wx</u> ^{BL-A}	266	0.0 \pm 0.0
<u>wx</u> ^{BL-B}	308	1.29 \pm 0.65

Table 2
Average number of Wx pollen grains from intercrosses among homoallelic ethyl methanesulfonate induced and spontaneous wx mutants

Alleles	Estimated no. microspores $\times 10^3$	\bar{X} no. <u>Wx</u> $\times 10^{-5} \pm s\bar{x}$
<u>wx</u> ^C x <u>wx</u> ^{BL-B}	178	0.0 \pm 0.0
<u>wx</u> ^C x <u>wx</u> ^{BL-A}	217	5.99 \pm 1.66
<u>wx</u> ^C x <u>wx</u> ^{H21}	245	50.68 \pm 4.56
<u>wx</u> ^C x <u>wx</u> ⁹⁰	319	86.11 \pm 5.19
<u>wx</u> ^{BL-B} x <u>wx</u> ^{BL-A}	142	7.73 \pm 2.33
<u>wx</u> ^{BL-B} x <u>wx</u> ^{H21}	211	3.79 \pm 1.34
<u>wx</u> ^{BL-B} x <u>wx</u> ⁹⁰	188	0.0 \pm 0.0
<u>wx</u> ^{BL-A} x <u>wx</u> ^{H21}	184	0.54 \pm 0.17
<u>wx</u> ^{BL-A} x <u>wx</u> ⁹⁰	168	12.48 \pm 2.72
<u>wx</u> ^{H21} x <u>wx</u> ⁹⁰	349	30.63 \pm 2.96

If the attempt to obtain homoallelic induced sites fails and if instead homoallelic wx sites from the tester stock were in actuality analyzed, the so-called induced site will recombine like the wx site. The more

important evidence to indicate that the wx mutants are homoallelic for the EMS-induced site is that the EMS-induced mutant wx^{BL-A} recombines with wx^C (Table 2), but wx^{BL-B} does not. However, wx^{BL-B}, unlike wx^C, does not recombine with wx⁹⁰; also wx^{BL-B} and wx^{BL-A} recombine with each other.

The M₁ results show a higher recombination rate than the diallel results and lead to nonadditivity. In fact wx^{BL-B} recombines with wx^C in the M₁ generation but does not recombine in the diallel (Table 2). Nonadditivity has been previously reported at the wx locus in maize.³ More important than obtaining additivity of the data is the fact that recombination occurs among both the spontaneous and induced mutants in the diallel.

The frequency of intracistron recombination of various EMS-induced wx mutants in the M₁ generation led to the conclusion that EMS induces independent mutations at sites within the wx locus in maize. Also the occurrence of recombination in the M₁ generation between mutant and tester sites indicates that "point mutations" (gene mutations) have been induced by this mutagen.² The occurrence of recombination between EMS-induced and spontaneous wx mutants crossed in all combinations confirms the earlier report² and is further indication that "point mutations," or at least minor deletions, have been induced by this mutagen.

The author is grateful to Gary McGovern for assistance in performing this research.

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Robert W. Briggs

2. Relative response of maize to X-rays vs. neutrons over a wide range of doses.

A problem of continuing interest in radiobiology is to determine why radiations which give different patterns of energy distribution in exposed tissues produce different degrees of response for equal amounts of total energy absorbed. A commonly used measure of this difference is the relative biological effectiveness (RBE), computed as the ratio of doses for two radiations of different quality required to produce the same effect. RBE values characteristically change with dose levels of X-rays (X) vs. neutrons (N); that is, no single ratio of X/N for equal effects holds throughout a range of absorbed doses.

Maize plants, grown from Yg₂/Yg₂ seeds that had received various absorbed doses of fission neutrons or of 250 kVp X-rays, were scored for radiation damage on the basis of 9 criteria (Table 1). The responses ranged from those caused by a sublethal genetic effect (yg₂ leaf sectors), to eventual gamete lethality (pollen sterility and reduced seed set), to growth retardation due to somatic cell death (reduction in plant height, survival and emergence), to complete cessation of cell division ("reversal" of emergence

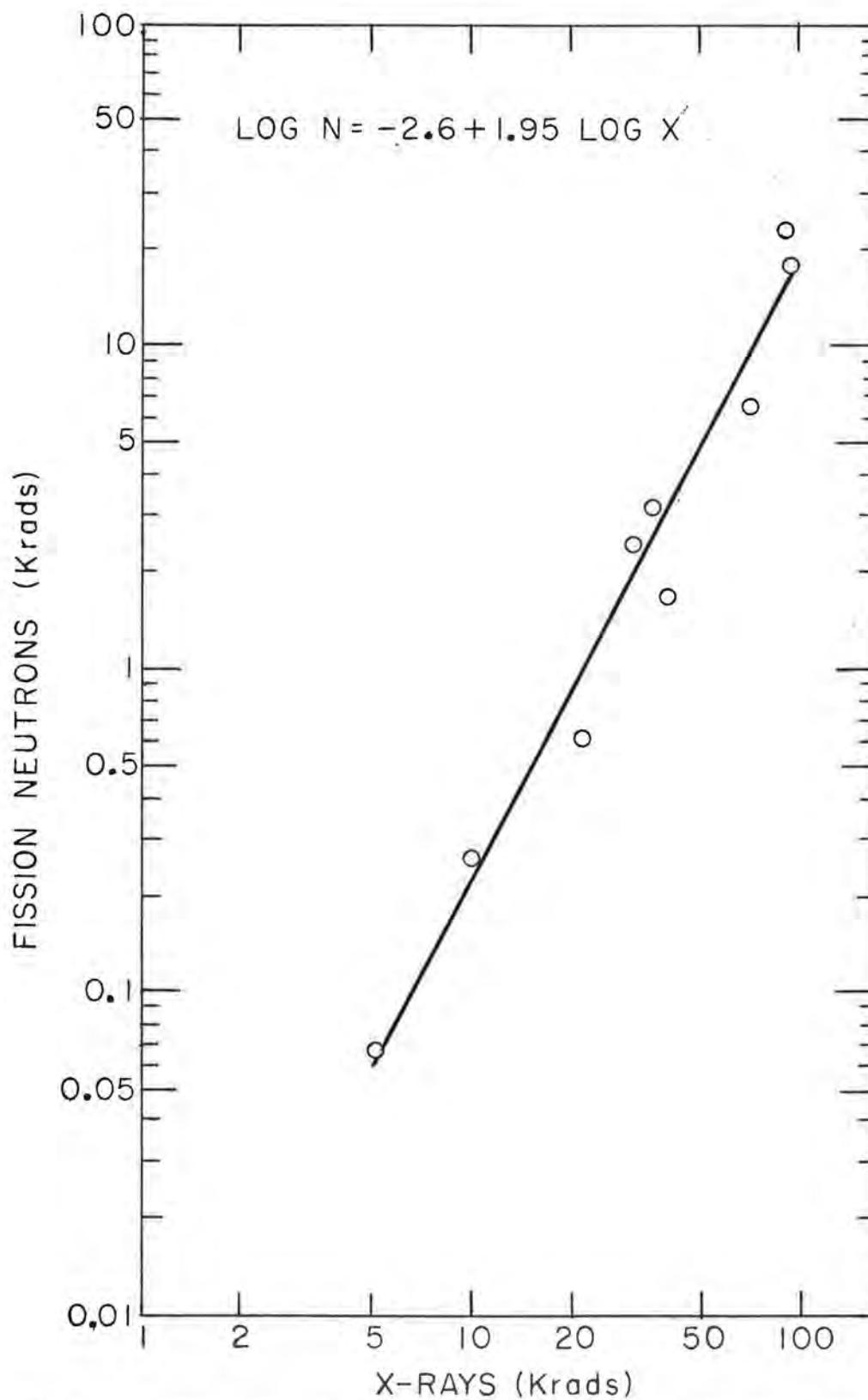


Fig. 1. Logarithmic plot of 9 criteria of equal effect (see Table 1) in maize plants grown from seeds which had received a wide range of absorbed doses of fission neutrons or of 250 kVp x rays.

and plant height). The steadily decreasing RBE with increasing doses results from the proportionally larger increments of neutron dose (N), relative to X-ray dose (X), that are required to give the equal effects measured.

Table 1
Criteria of response, doses, and RBE values for maize irradiated with fission neutrons and 250 kVp X-rays

Criteria	Neutrons (K rads)	X-rays (K rads)	RBE
1) 1 yg_2 sector per leaf 5	0.068	5.15	75.7
2) 2 yg_2 sectors per leaf 5	0.27	9.91	36.6
3) 50% seed set	0.63	21.06	33.4
4) 50% pollen fertility	1.73	37.50	21.7
5) 50% plant height	2.51	29.76	11.9
6) 50% survival	3.31	34.01	10.3
7) 50% emergence	6.77	66.70	9.9
8) Emergence reversal	18.65	93.10	5.0
9) Plant height reversal	24.04	84.96	3.5

Figure 1 shows a logarithmic plot of X vs. N for each of the 9 criteria. These points can be fitted to a straight line, for which the least square equation is:

$$\log N = -2.6 + 1.95 \log X$$

This simple power function, which spans 4 orders of magnitude of neutron doses, is amenable to the following straightforward explanation: (1) the responses measured have a common cause which is chromosome breakage and genetic loss; (2) increasing somatic growth inhibition and gamete lethality are attributable directly to quantitatively more genetic damage; (3) neutron-induced damage increases linearly with dose and X-ray-induced damage with the approximate square (slope = 1.95) of the dose; (4) this relationship remains uncomplicated from irradiated seed through the development of the maize plant.

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1. Transpositions of Dt_1

A search was made for transpositions of the controlling element Dt_1 in a homozygous $a_1 Dt_1$ stock that had been maintained for several generations by self-pollinating or sib-crossing. The mutation frequency was uniformly high in this stock. Because the frequency of aleurone mutations is exponentially related to Dt dosage, a Dt_1 transposition would result in a greatly increased number of dots if the egg or sperm nucleus contained both the transposed Dt and the Dt_1 remaining on chromosome 9. In the triploid endosperm the normal Dt_1 dosage of three could be increased to four if transposition took place in the pollen parent or to five if it occurred in the egg parent.

To this end 1255 $a_1 a_1 Dt_1 Dt_1$ plants were self-pollinated and the progeny ears examined. Several kernels were selected which had significantly higher mutation rates of the a_1 gene than did the remainder of the kernels on their respective ears. Plants were grown from these exceptional kernels and crosses made to test the hypothesis that a transposition of Dt_1 had occurred.

Testcrosses of plants descended from six of these kernels produced ears bearing kernels in the ratio of 3 dotted: 1 dotless, indicating the presence of two independently segregating Dt 's. There is 7% recombination between Dt_1 and Yg_2 (yellow green seedling) in the short arm of chromosome 9. Table I summarizes data from crosses of the type $Dt_1^T dt Yg_2 Yg_2, a_1 a_1$ X $dt dt Yg_2 Yg_2, a_1 a_1$ involving two independent transpositions, Dt_1^{TA} and Dt_1^{TB} . The absence of linkage between Yg_2 and either Dt_1^{TA} or Dt_1^{TB} verifies the independent location of these Dt_1^T 's and Dt_1 of chromosome 9. Furthermore Dt_1^{TA} shows 39% recombination with Y (Yellow endosperm) on the long arm of chromosome 6. Sib ears of those included in the table, which had Dt_1 at its standard location, gave 6.0% recombination between Yg_2 and Dt_1 .

The significantly greater recovery of dt kernels from Dt_1^{TB} testcrosses ($X^2_{Total} = 10.818, P = .001; X^2_{Heterogeneity} = 4.993, P = .7-.6, D. F. = 7$) indicated that a change in stability was associated with its different location since Dt_1 at the standard location showed normal Mendelian inheritance ($X^2 = .003, P > .9, D. F. = 15$). This altered behavior is a "change in state" and may be due to a high transposition rate, "changes in state" of Dt_1^{TB} activity, and/or Dt_1^{TB} losses. That a higher transposition rate may have been responsible was suggested by the finding of ears involving Dt_1^{TB} , segregating two Dt_1^T 's as well as Dt_1 . Such ears were common among testcrosses of $Dt_1^{TC}, Dt_1^{TD},$ and Dt_1^{TF} . One ear segregated four independent Dt 's.

Earle Doerschug

Table 1
 Analysis of \underline{Dt}_1^{TA} and \underline{Dt}_1^{TB} . Testcross data of $\underline{Dt} \underline{dt} \underline{Yg}_2 \underline{yg}_2 \underline{Y} \underline{y}$ plants.

$\underline{Dt} \underline{dt}, \underline{Yg}_2 \underline{yg}_2, \underline{Y} \underline{y} \overset{\circ}{\sigma} X$	$\underline{Dt} \underline{Yg}$	$\underline{Dt} \underline{yg}$	$\underline{dt} \underline{Yg}$	$\underline{dt} \underline{yg}$	<u>Total</u>	<u>Dt Y</u>	<u>Dt y</u>	<u>dt Y</u>	<u>dt y</u>	<u>Total</u>
\underline{Dt}_1^{TA}	1328	1352	1341	1313	*5334	1744	1094	1080	1711	5629
\underline{Dt}_1^{TB}	695	633	749	754	*2831	648	707	710	816	2881
\underline{Dt}_1 (sib ears of those possessing \underline{Dt}_1^{TB})	2067	123	135	2051	*4376					

* X^2 Total \underline{Dt}_1^{TA} : $\underline{dt} = 0.127$, $P = .8-.7$; X^2 Heterogeneity = 7.242, $P = .9-.8$, D.F. = 12

* X^2 Total \underline{Dt}_1^{TB} : $\underline{dt} = 10.818$, $P = .001$; X^2 Heterogeneity = 4.993, $P = .7-.6$, D.F. = 7

* X^2 Total \underline{Dt}_1 : $\underline{dt} = .003$; $P > .9$; X^2 Heterogeneity = 12.077, $P = .7-.6$, D.F. = 15

2. Stability of Dt_1 .

In the same population of self-pollinated ears from which Dt_1^T 's were derived, 9% of the ears possessed dotless kernels present either in a very low percentage or accounting for 1/4 of the total. Tests of dotless kernels of different origin are shown in Table 2. Dotless kernel No. 1 proved to have an altered state of Dt_1 activity showing a greatly reduced frequency of aleurone mutations such that frequently no dots were found on an individual kernel. Crosses of plants from dotless kernels Nos. 2-8 with $dt\ dt$ plants carrying the standard a_1 allele or the highly mutable a_1^m-1 found by Nuffer (Nuffer, M. G. 1961. Genetics 46: 625-640) produced only dotless kernels; when crossed with $a_1^s\ Dt_1$ (the $a_1^s\ Dt_1$ stock has dotless aleurones since a_1^s does not mutate in the presence of Dt), all of the dotless stocks gave F_1 kernels with aleurone mutations with the exception of family No. 6 which produced ears with all dotless kernels, ears segregating for dotted and dotless, and ears with only dotted kernels. Thus, a dottedable a_1 allele was present in the dotless stocks and the loss of mutability was attributed to loss of Dt_1 activity. Additional crosses involving family No. 6 substantiated the finding that both a mutable and a stable a_1 allele were present. It is possible that the loss of Dt_1 activity was coincident with a mutation of a_1 to a_1^s . It is not known whether the families 2-8 have an inactive Dt_1 allele or whether they experienced an actual physical loss of Dt_1 . Numerous sib crosses of the dotless stocks (Table 2) as well as self-pollinations and crosses with $a_1^m-1\ dt$ have produced no reactivation of Dt_1 . The chromosomes 9 of these families, when observed at pachynema, appeared to be normal and had the small terminal knob characteristic of Dt_1 stocks. Several losses of Dt_1 and one mutation of a_1 to a_1^s accounted for the occurrence of dotless kernels on otherwise $a_1\ a_1\ Dt_1\ Dt_1$ ears. The apparent discrepancy between this result and those of Rhoades (Rhoades, M. 1941. Cold Spring Harbor Symp. Quant. Biol. 9: 138-144) and Peterson (Peterson, P. 1953. Maize Gen. Coop. News Letter 27: 61), who attributed the dotless kernels to mutations of a_1 to a_1^s , is probably the result of sampling errors inherent in small populations. It is also possible that the stocks used contained different states of the a_1 and/or Dt_1 alleles, where changes to the inactive a_1^s and losses of Dt_1 occurred at different rates.

A number of crosses of the type $a_1\ a_1\ Dt_1\ Dt_1^{\circ} \times a_1\ a_1\ dt\ dt^{\sigma}$ were made. Four ears in a total of about 40 had dotless kernels which were randomly distributed and not in clusters. The proportion of dotless kernels was high on some ears but never accounted for half the kernels. Dotless kernels were also observed on ears of the reciprocal crosses. Thus, in these plants independent losses of Dt_1 (or loss of Dt_1 activity) must have occurred after divergence of the cell lines forming individual ovules in the female parent. In the pollen parent, however, one cannot distinguish between frequent independent losses of Dt_1 and a single event involving a sector of the tassel. The rate of Dt_1 losses cannot be accurately determined since the majority of the ears showed no losses of Dt_1 whatsoever, whereas a few ears had several dotless kernels. A similar distribution was observed with the self-pollinated ears.

It is possible that dotless kernels are the reciprocal product of Dt_1 transpositions; i.e., after a transposition has occurred in a homozygous

Table 2

Phenotypes of kernels resulting from crosses between dotless types of different origin and tester strains. The dotless types were derived from the selfed progeny of homozygous \underline{Dt}_1 stocks

No.	Dotless families	X $\underline{a}_1^* \underline{sh}_2 \underline{dt}$	X $\underline{a}_1^m -1^* \underline{dt}$	X $\underline{a}_1^s \underline{Dt}$	X sib (No. of crosses)
1	741	a few kernels dotted	a few kernels dotted	all kernels dotted	-----
2	743	----	dotless	dotted	dotless (6)
3	744	dotless	dotless	dotted	dotless (4)
4	745	dotless	dotless	dotted	dotless (3)
5	747	dotless	dotless	dotted	dotless (7)
6	748	dotless	dotless	dotless or dotted+	dotless (2)
7	749	dotless	dotless	dotted	-----
8	1255	dotless	dotless	dotted	dotless (2)

* \underline{a}_1 , the standard allele that responds to \underline{Dt}

\underline{a}_1^m , a highly mutable \underline{a}_1 allele described by Nuffer (1961)

\underline{a}_1^s , an \underline{a}_1 allele that does not respond to \underline{Dt}

+The ears testing individual sib plants were completely dotted, completely dotless, or segregated 1:1.

Dt₁ cell line, ¼ of the gametes derived from the line would contain no Dt.

Earle Doerschug

3. Activation Cycles of Dt₁^{TB}

Kernels sectored for dotted expression were first observed among the back-cross progeny of a Dt₁ Yg₂/dt yg₂, Dt₁^{TB} dt^{TB}, a^{m-1} a^{m-1} plant. Several kernels had no dots at all in the aleurones but were heavily dotted in the scutella. Whereas three-fourths of the kernels were expected to have fully dotted aleurones, the ear contained 172 kernels with uniformly dotted aleurones, 117 completely dotless types, and 96 with dots found in sectors of the aleurone and/or dotted scutella. The aberrant types from the latter class were grown and crossed with tester strains to determine the cause of the altered dotted expression in these kernels.

Self-pollinations of plants grown from sectored kernels produced kernels having basically colorless aleurones, some of which had well-defined dotted sectors; the dotting in the scutella, when it occurred, was uniform (color in the scutellum requires additional genes which were not being followed here). A very few kernels were dotted throughout the aleurone. A plant arising from a kernel with reduced Dt activity was selected for intensive study and used as pollen parent in a variety of crosses. All the kernels on an ear from a cross between this plant and an a₁^s a₁^s Dt₁ Dt₁ stock (colorless aleurone) were uniformly and highly dotted. Since a₁^s does not respond to Dt, the only source of a mutable a₁ allele was the plant being tested; thus, the presence of two normally mutable a^{m-1} genes in the pollen parent was established.

The activity of Dt was studied by crossing the test plant to an a₁^s a₁^s dt dt (colorless aleurone) female parent. The resultant ear showed that Dt was inactive in most of the aleurones but occasionally became active, producing sectors of a₁ mutability. The same behavior was observed in a similar cross to an a^{m-1} a^{m-1} dt dt plant.

An independent modifier of Dt activity could not have caused the sectoring since the Dt from the a₁^s Dt₁ stock was not affected. A modifier linked to Dt whose influence is restricted to genes in the same homologue might be postulated, but it seems more likely that induction of Dt activity and inactivity was autonomous.

Since the tested plant was homozygous for the highly mutable a^{m-1}, sectors of Dt reactivation were clearly defined. The reactivation sectors on self-pollinated ears were larger than on outcrossed ears, where only one Dt was present in the triploid endosperm. It is possible that Dt reactivation in these kernels was dose-dependent; i.e., the inactive Dt's acted synergistically to reactivate other inactive alleles. According to this explanation, the combined action of the Dt's caused earlier reactivation realized by larger sectors of Dt activity.

One ear from the cross a₁^s a₁^s dt dt X a^{m-1} a^{m-1} dt Dt^{in-ac} ("Dotted, inactive-active") segregated 1:1 for kernels with dotted and colorless scutella (Table 3, No. 1). Kernels in the two classes were scored for

Table 3

Tissue dependence of Dt^{in-ac} phase reversals. Analysis of the crosses $a_1^s a_1^s dt dt$ (1191) ♀ or $a^m-1 a^m-1 dt dt$ (1183) ♀ X $a^m-1 a^m-1 Dt^{in-ac} dt$ (1179-1181) ♂. Both parents of Nos. 2-5 were probably heterozygous for a scutellar color factor

No.	Cross	Number of kernels			
		Dotted scutella; dotless endosperms	Dotted scutella; sectors of mutability in endosperm	Dotless scutella; dotless endosperms	Dotless scutella; sectors of mutabil- ity in endosperm
1	1191 X 1181-1	129	22	154	0
2	1183-8 X 1181-1	84	35	185	1
3	1183-10 X 1180-1	99	13	177	0
4	1183-3 X 1180-3	114	13	195	0
5	1191 X 1179-1	128	48	256	3

aleurone sectors showing Dt activity; sectors of Dt^{in-ac} reactivation were found only on kernels with dotted scutella. The 3 : 5 ratios for kernels with dotted: dotless scutella on the four remaining ears of Table 3 (Nos. 2-5) were attributed to the segregation of a scutellar color factor in both parents. Nevertheless, sectors of Dt^{in-ac} reactivations were found mainly on kernels with dotted scutella. The dotless scutella of the four exceptional kernels (last column, Nos. 2 and 5) were not unexpected since a scutellar factor was presumably segregating in addition to Dt^{in-ac}. It was concluded that inactivations of Dt^{in-ac} followed by occasional reactivations were tissue-dependent, occurring in the aleurone but not in the scutellum. Thus, kernels with colorless scutella (with the proper scutellar factor constitution) were assumed to be of dt dt dt constitution.

Preliminary crosses show independent inheritance of yg₂ and Dt^{in-ac} suggesting that Dt^{in-ac} was derived from Dt₁^{TB} rather than Dt₁. No tests of Dt₁^{TB} and Dt^{in-ac} allelism have been made, however.

Reversals of phases of activity were found for the regulatory element Spm (McClintock, B. 1961. Am. Nat. 95: 265-277). Alternations of phase occurred during any period of the life cycle and were interpreted to be autonomously controlled by Spm. The active and inactive phases of En^(crown) or En^(flow), however, were associated with specific areas of the aleurone (Peterson, P., 1966. Genetics 54: 249-266). Dt^{in-ac} has characteristics in common with both En^(crown) or En^(flow) and Spm. Random reversals of phase occurred in a single tissue (the endosperm), but such fluctuations were a property of the endosperm and not of the scutellum where Dt^{in-ac} was fully active.

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1. Survival of Maize Borer (Chilo partellus Swinhoe) Larvae and Varietal Resistance in Maize.

Maize borer is the most devastating insect pest of maize in West Pakistan. It is very active from March to July after which it starts to hibernate. Sporadic attack, however, continues till the end of November but damage done from August onwards is not very severe. It is due to the ravages of this pest that the growing period of maize in West Pakistan has been relegated to the fag end of the long summer season and the farmers are forced to plant short duration varieties because the crop planted earlier in the season is completely destroyed by this pest. A late sown crop often does not mature in time for planting wheat that is supposed to follow maize in the same field. Consequently, early maturing varieties are planted that in general give low yields. Recently high yielding composites containing Central and South American germ-plasm have been introduced in some parts of West Pakistan. These varieties are late maturing, therefore have to be planted early in the season with the result that very intensive plant protection measures are required to save them from borer attack. Obviously in a developing country like Pakistan,

it is not feasible to provide adequate plant protection facilities to all the farmers in the villages; therefore use of late maturing varieties will remain restricted. It was therefore considered necessary that efforts for the development of high yielding varieties that are resistant to maize borer attack should be intensified.

Controlled experiments were conducted on feeding of larvae (Chilo partellus Swinhoe) on the leaves of the following varieties both in the spring as well as in the normal crop season. These varieties were selected on the basis of differential survival of plants under natural infestation in the field. Percentage survival of plants recorded in the field during the previous season for each of the selected varieties as compared to double cross hybrid 59 is given.

Varieties.	Percentage survival of plants under natural infestation in the field.
Antigua Gr I	60.9
OH45	60.2
Antigua Gr II	47.2
Caribbean (Composite)	46.3
Renala (West Pakistan)	44.0
J-1 (Composite)	40.3
Double Cross Hybrid No. 59	24.2

Larvae of the same brood were collected from the field immediately on hatching and released on the leaves of different varieties kept in specimen tubes. Seventy such tubes were kept in each case. Fresh succulent portions of the leaves were put in the tubes after every twenty-four hours. The open end of the tube was covered by muslin cloth held in position by a rubber band. Only one larva was reared in each tube. The number of larvae that survived and pupated was recorded. The size of larvae was also measured and recorded on every alternate day with a view to ascertaining the differential growth rate of the larvae feeding on the leaves of different varieties. This experiment was conducted during the month of July on the normal season crop. Results obtained from these preliminary experiments are given below:

Survival and mortality of Chilo larvae fed on the leaves of different varieties

Variety.	Percentage of larvae survived and pupated.
<u>Spring crop.</u>	
Double Cross Hybrid No. 59	100.0
J-1 (Composite)	80.5
Caribbean (Composite)	79.3
Renala (West Pakistan)	70.4
Antigua Gr. II	69.5
OH45	49.5
Antigua Gr. I	49.5
<u>Normal season.</u>	
Double Cross Hybrid No. 59	100.0
J-1 (Composite)	79.5
Caribbean (Composite)	80.7
Renala (West Pakistan)	69.6
Antigua Gr. II	70.5
OH45	49.5
Antigua Gr. I	51.5

The results given in the above table show that observations made in the normal season corroborate those recorded in the spring. Double cross hybrid 59 was the most susceptible variety while OH45 and Antigua Gr I were the most resistant types of the varieties under study. Caribbean and J-1 composites also appear to contain some resistance against the pest. These results also confirm the original observations in the field.

Differential survival of larvae when fed on the leaves of various varieties appears to indicate some sort of genetic resistance in maize against Chilo partellus Swinhoe. One hundred per cent survival of larvae when fed on the leaves of double cross hybrid No. 59 shows that the conditions in the laboratory for the growth and development of the

larvae were quite congenial. The 50% mortality of larvae fed on Antigua Gr I and OH45, against 100% survival in the case of variety No. 59 under the same laboratory conditions, justifies the conclusion that there is definite genetic resistance in maize against Chilo partellus Swinhoe. It is difficult to say anything about the exact nature of the resistance at this stage.

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1. Heritability of repressed R expression noted in $R^6 R^6$ aleurone cells.

In Vol. 41 (under Washington University) we reported a high level of repression for R when introduced into aleurone tissue of an $R^6 R^6$ (paramutated for six generations with R^{st}). The lightest kernels ($R^6 R^6$) were selected for planting in 1967 to test the heritability of the observed repression of R. Table 1 shows that the selected lightest phenotypes observed the previous year scored the same as unselected kernels in the testcrosses in 1967 (for testcross methods see our previous reports). Following the mating $R^6 R^6 \times RR$, it can be concluded that the degree of repression of R in the presence of two R^6 chromosomes in the aleurone cannot be used to select for lighter phenotypic expression in the sporophyte. Several points may be noted:

(1) Under the conditions of this experiment, specific levels of pigment expression in the endosperm phenotype will not identify a specific level of pigmentation for the testcross of the sporophyte included in the seed.

(2) The repression effect of R^6 , when the lightest mottle kernels are selected, is a "preview" of the general level of secondary paramutation from R^6 to be observed when the $R^6 R$ "heterozygotes" are planted out and testcrossed the following year.

(3) The degree of "immediate" paramutation (repression) in the $R^6 R^6$ was greater than that in the $R^{st} R^{st} R$ (Vol. 41) though in the following generation the effect of the standard R^{st} on R was greater than that of R^6 on R.

(4) The degree of paramutation on R from R^6 is of the order of that encountered with a weakly paramutagenic R^{st} allele.

Table 1
Comparison of \underline{R} Expressions from Testcrosses of $\underline{R}^6\underline{R}$
"Heterozygotes"

Unselected	Selected	Selected	Selected	Standard
17.30	15.06	19.44	15.10	20.94
17.36	14.04	15.18	16.64	20.90
15.55	16.88	16.16	17.64	20.88
15.82	16.37	19.42	18.50	20.66
16.16	16.36	16.84	18.54	20.94
16.06	15.26	17.96	17.00	20.96
Pooled \bar{X} 16.38	15.66	17.50	17.23	20.88

Three families of selected light kernels are compared with standard \underline{R} and unselected kernels, all of which were planted out and testcrossed to Inbred W23. Kernel selections for planting were based on an $\underline{R}^6\underline{R}^6$ x \underline{RR} (standard) mating where pigment repression was observed in certain $\underline{R}^6\underline{R}^6$ kernels.

Bernard C. Mikula
Richard Sherman
Robert Locy

2. Tassel induction and alteration of \underline{R}^1 expression by short-term LD treatments.

In Vol. 41 MGCNL we reported that the degree of pigmentation from \underline{R}^1 (paramutated \underline{R}) in testcrosses could be related to the environmental treatments which \underline{RR}^{st} plants had been given during the 3rd and 4th weeks of seedling development. Plants which received LL (constant light) conditions had lower aleurone pigmentation scores than those plants which received LD (12 hr. light: 12 hr. dark) during the 3rd and 4th weeks. It was believed that tassel initiation took place during this interval and that the effect of environment upon \underline{R}^1 expression might in some way be correlated with the tassel induction period. A preliminary report follows on the relationship of these two points.

Light was supplied by six 200W daylight-type fluorescent bulbs approximately 1 m above the seedlings which were grown in 4" pots at 22°C in constant light. At given ages during the 3rd and 4th weeks of development (Table 2) plants were exposed to various numbers of 24 hr. LD periods then returned to LL (constant light) conditions. At the end of 30 days all plants were transferred to field conditions and testcrossed in the summer to assay the effect of the LD treatments on \underline{R}^1 expression. It was of interest to know: (1) how soon plants were susceptible to

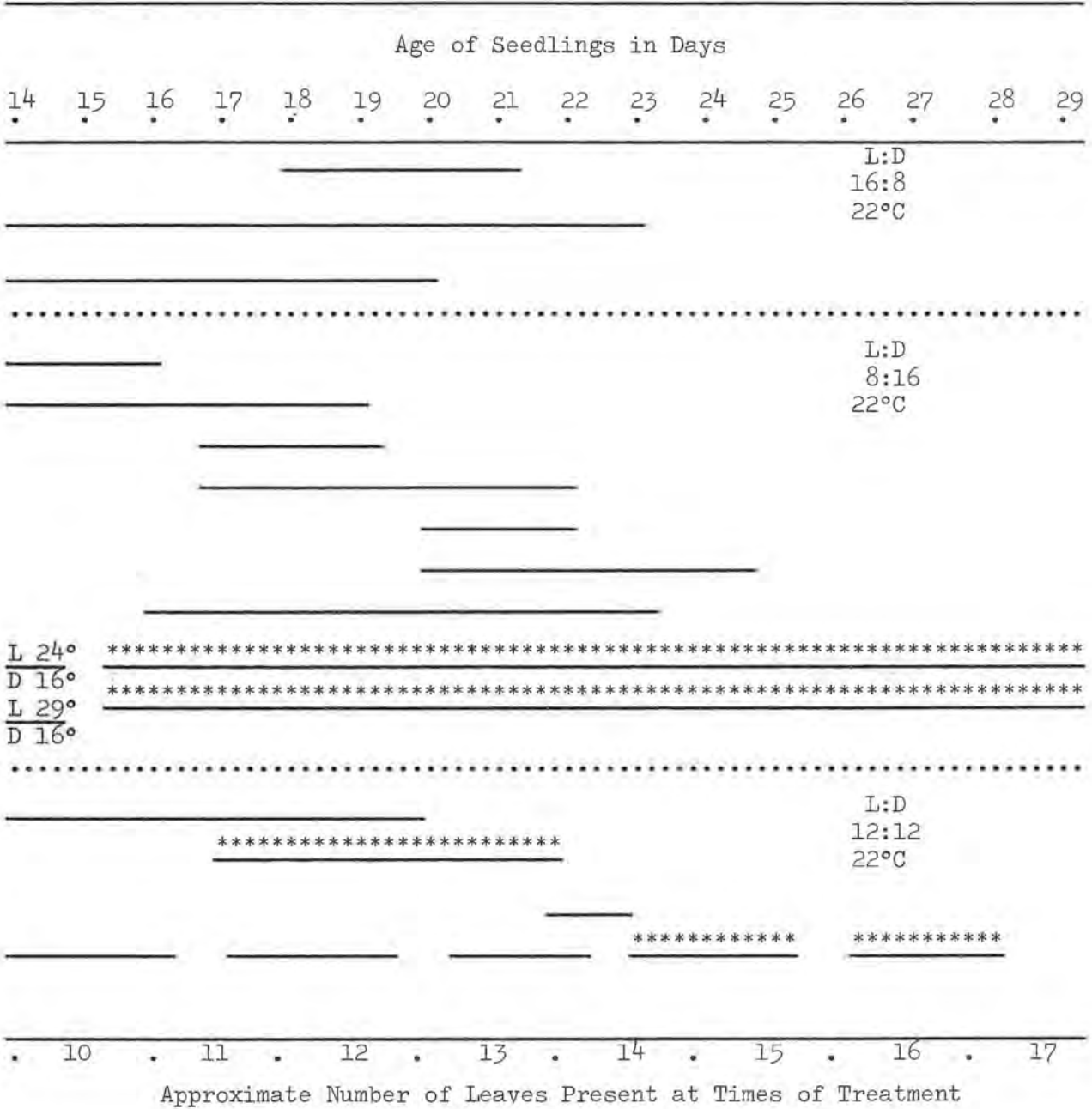
tassel induction, (2) what daylength conditions were necessary--given our light intensities and temperature regimes, (3) how few cycles of an LD period were sufficient for tassel induction.

Table 2 shows that tassel induction for the W22 inbred line could be brought about in 12:12 LD conditions when the plants were 23 days old, if the LD cycles were continued for three 24-hr cycles. Three 24-hr. cycles did not bring about tassel induction earlier than the 23-25 days of seedling age. Six days, however, did bring about tassel formation when the LD periods began on the 17th day and continued through the 22nd day of seedling life. 16:8 and 8:16 LD cycles were not successful for tassel induction at the given temperatures and light intensities. Tassels formed in 8:16 LD periods when day and night temperatures were varied from 24° day to 16° night or 29° day to 16° night--this temperature regime was continued over a 14 day period. There are, of course, many combinations of variables which have not been tried. Effort was made to find the shortest and earliest time during which the seedling was susceptible to tassel induction, given the limited growth chamber space and growing conditions available at the time.

Since three LD cycles can induce tassel formation, can these same LD cycles affect R^1 expression? The effect of short tassel inductive periods on R^1 pigment expression from the RR^{st} treated plants is given in Table 3. As reported previously, plants given only LL conditions score lightest upon testcrossing. Plants given LD conditions score darker than those given constant light conditions for the first 30 days of seedling development. It appears from our preliminary results that three 24-hr. LD cycles can condition a darker R^1 expression compared to R^1 expressions from plants given only LL conditions during this same period. Seven days of LD cycles also conditioned a darker R^1 expression. Field-grown plants in 1967 gave consistently darker R^1 testcross scores than had been noted in previous years; these scores are in the range of those plants which had the LD cycles.

Table 2

Light (L): Dark (D) Treatments of Maize Seedlings for Tassel Induction



*** represents LD treatments which resulted in tassels; ___ represents LD treatments which failed to produce tassels. The length of the line above represents the length of the treatment and the age of the plant during the LD conditions.

Table 3
Effect of Short-term LD Cycles on R^1 Expression

LL	Age of Plant in Days and No. of Cycles of LD				Field Grown
	17-23 7	23-25 3	23-26 4	23-29 7	
12.84	14.50	16.14	14.38	16.30	15.91
11.88	13.82	15.60	14.71	16.50	16.13
12.18	12.76	16.26	16.46	12.73	14.24
10.65	12.88	12.96	12.66	14.44	16.05
12.72	14.26	11.24	13.82	15.25	15.22
9.14				16.16	17.98
12.26				14.26	15.91
11.52					14.57
<u>11.65</u>	<u>13.64</u>	<u>14.44</u>	<u>14.41</u>	<u>15.09</u>	<u>15.75</u> Pooled \bar{X}

Testcross results of RR^{st} plants given LD cycles during the 3rd and 4th weeks of seedling development. Plants were kept under constant light except for the periods of LD treatment indicated.

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1. Modified phyllotaxis in maize. Dispersion, spirodistichy, decussation, and similar alterations in other parts of maize plants. Multiple germination in distichous, spirodistichous and decussate plants.

In an inbred line of maize, inherited decussate phyllotaxis was observed in association with brachysm and other modifications in height. Some other abnormalities were found, such as alterations in the position of grains in the ear, increased numbers of plumules, plurality of embryos and multiplicity of plants after germination.

The decussation is present both in leaves and ears, and it may involve higher physiological efficiency and higher yield than in normal distichous plants having the same number of nodes. The decussate plants have the trend to subtend a high number of ears.

There is also a tendency to a helical disposition of several organs or parts of plants. The gyre of the helix may be turned to the right or to the left side.

The position of the grains in the spike may be alternate or opposed and in pairs coupled on the same peduncle, in all of the possible positions. The grains may weld together, giving rise to grains with two embryos or two endosperms. This abnormality has also been detected in normal distichous plants. The alterations in the ear are often symmetrical. An odd number of rows has been repeatedly found.

After germination of the seeds, two, three and more seedlings emerge, all coming from the same grain. The multiple germination can be predicted after inspection of the seed, owing to the presence of apparent swellings, corresponding to the preformed coleoptiles. Embryos carrying these anomalies have also been detected in seeds from normal distichous plants.

Welding, fasciation and abnormalities of this order, have been observed in leaves, stems and inflorescences. The decussate plants may give rise indistinctly to distichous, spirodistichous and decussate plants.

A program of studies is now developing in relation to these abnormalities.

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1. Peroxidase isozymes in maize; designation of locus Px₁.

Eighteen peroxidase isozymes were reported previously in several lines of maize (Hamill, Maize News Letter 41: 62, 1967). Using the same techniques of starch gel electrophoresis, 6 additional isozymes have been found, bringing the total to 24 (13 moving cathodally and 11 anodally). Twelve of these isozymes migrated to positions comparable to isozymes observed in commercial preparations of horseradish peroxidase, making this a useful reference. Plants within inbred lines of dent and sweet corn normally displayed identical isozyme patterns for a given tissue, while plants within open-pollinated varieties and diverse tropical races exhibited much variation.

Peroxidase isozyme patterns have been studied in some detail for 8 seedling tissues and 13 mature tissues. Isozyme complements varied greatly among tissues, with certain tissues showing a rather characteristic pattern. Two isozyme bands were found to be unique to mature tissues. No tissue studied had all the 24 isozymes and no tissue completely lacked peroxidase isozymes. Within tissues of any one line of maize the number of isozymes

has been found to range from as few as 4 (anther, prior to anthesis) to as many as 16 (mature leaves).

Ontogenetic studies have been initiated for leaf blades, leaf sheaths, and internodes. These tissues were studied at anthesis from the base to the top of the plant. In the leaf tissues, there was an increase in the number of peroxidase isozymes with increasing maturity; the basal leaves had approximately twice as many isozymes as the upper leaves. In contrast, all internodes had essentially the same pattern at this time. At stages prior to anthesis, this sequential change in leaf peroxidases was even more dramatic, and a similar change was also noted in internodes. Differences in peroxidase isozyme patterns appear to reflect differences in rates of tissue enlargement and development.

Genetic analyses were completed for two of the cathodal isozymes which were designated C10 and C20. Seedling roots of inbred lines commonly exhibited either C10 or C20; both isozymes were never observed together in an inbred line. The F_1 from C10 x C20 always showed both isozymes and hybrid bands were never seen. Chi square analysis showed that segregations in the F_2 and backcross populations fit 1:2:1 (32 C10 : 62 C10, C20 : 42 C20) and 2^2 1:1 (48 C10 : 55 C10, C20 and 39 C20 : 32 C10, C20) ratios respectively. Since plants lacking both bands were never observed in these F_2 and backcross progenies, and no inbreds have yet been found with both isozymes, it was concluded that the two isozymes are conditioned by co-dominant alleles at one locus. The locus has been designated Px_1 , with alleles Px_1^1 and Px_1^2 .

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1. Genetic marker stocks adapted to the tropics.

To an increasing extent maize genetic marker stocks appear to be of interest for teaching and research activity in tropical countries. At most tropical agricultural universities and schools, maize can be planted for genetic research problems or class demonstrations any month of the year.

Corn Belt maize is generally ill adapted to the tropics, principally due to its daylength sensitivity and disease susceptibility. It is difficult enough to recover seed from genetic marker stocks under the comparatively congenial conditions in Hawaii (20° N. latitude). However, the added burden from near-epiphytotic conditions of blights and rusts in much of the tropics

makes perpetuation of these stocks laborious if not impossible, especially in the short-day months of the traditional academic year.

A series of conversions of genetic marker stocks was therefore started in Hawaii, and is presently being conducted under the aegis of the National Corn and Sorghum Program of Thailand (in cooperation with The Rockefeller Foundation).

Inbreds Amarillo Theobromina 21(B)-6#-1S-7# and Peru 330-2#-2S-6# (# = sib; S = self), derived from stocks of the Coordinated Maize Improvement Scheme in India and the corresponding single cross were chosen for the initial conversion. Both lines are vigorous, dark-yellow flints with excellent disease resistance and high general combining ability. Both appear to be widely adapted in the tropics. Initial conversions include the T cytoplasm and the following genes: \underline{a}_1 , \underline{a}_2 , \underline{ae} , \underline{an}_1 , \underline{B} , \underline{br}_1 , \underline{br}_2 , \underline{bt}_1 , \underline{bz}_1 , \underline{Cg} , \underline{d}_1 , \underline{d}_2 , \underline{d}_3 , \underline{de}_{16} , \underline{Dt}_1 , \underline{et} , \underline{fl}_1 , \underline{fl}_2 , \underline{lg}_1 , \underline{ms}_1 , \underline{ms}_8 , \underline{na} , \underline{o}_1 , \underline{o}_2 , \underline{PvV} , \underline{Prr} , \underline{Pl} , \underline{pr} , \underline{py} , \underline{r} , \underline{sh}_2 , \underline{su}_1 , \underline{Ts}_5 , \underline{wx} , and \underline{y} .

Small samples of seedstocks representing early generations of these conversions are available from the authors.

Approximately 50 other genes are being introduced in a second series of conversions to the flinty inbreds. An additional series of genetic stocks, representing about 40 alleles affecting isozyme polymorphisms, is also in preparation. These are being developed in sweet corn inbreds derived from the variety Hawaiian Sugar (widely adapted in the tropics, but susceptible to blight (*H. turcicum*) and rust). Cytological aberrations have not yet been introduced into the program.

Conversions are being made on a cycle of 3-4 generations/year. The scope and rate of progress of these conversions will depend to some extent on funds for, and interest in, the program. Contributions of tropically-adapted genetic and cytogenetic marker stocks would be appreciated.

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1. Cytoplasmic influence on the inheritance of oil content.

Reciprocal crosses between Illinois High Oil (IHO) and eight lines were made in the summer of 1967 to estimate the differential influence of the maternal and pollen parents on oil content. The results obtained using wide-line nuclear magnetic resonance (NMR) are listed in Table 1.

Table 1
Mean Oil Content in Parental and Reciprocal F_1 Seed

Parent 1	Oil Content in Per Cent						Parent 2
	P_1 Selfed	$P_1 \times P_2$	$P_2 \times P_1$	P_2 Selfed			
IHO (1)	11.27 *	6.78 *	1.93 *	.53		ILO	
IHO	12.44 *	10.28 *	6.51 *	4.32		WF9	
IHO	13.79 *	10.27 *	7.90 *	4.64		B37	
IHO	13.49 *	10.32 *	6.40 *	3.70		H49	
IHO	12.63 *	9.22 *	5.06 *	3.62		Oh7A	
IHO	12.60 *	9.79 *	6.72 *	4.51		B14	
IHO	14.35 *	12.20 *	7.47 *	4.83		M14	
IHO	14.34 *	10.43 *	6.92 *	3.61		Oh43	
Average	13.11 *	9.91 *	6.11 *	3.72			

*Significant difference between all pairs of means listed in each of the two columns, P_1 Selfed vs. $P_1 \times P_2$; $P_1 \times P_2$ vs. $P_2 \times P_1$; and $P_2 \times P_1$ vs. P_2 Selfed, at the 5% probability level.

(1) IHO = Illinois High Oil, ILO = Illinois Low Oil

As the F_1 differences are large, this material should give the maximum potential for separating the influence of the female sporophyte from that of the cytoplasm contributed by the female parent, both postulated causes of the observed maternal differences. To test for cytoplasmic effects, F_1 reciprocals were selfed in separate replicated experiments yielding seed born on genetically identical plants with different cytoplasm. The

results are presented in Table 2. Significant differences were found in four of the IHO-line combinations; however, combined analysis for all experiments did not show significance as the IHO did not have a consistent effect.

Table 2

Number of Reciprocals, Total Number of Subsamples and Per Cent Oil Means for F_2 Seed Representing Both Cytoplasm Sources From Initial IHO By Line Crosses

Line Crossed Reciprocally With IHO	Number of Separate Reciprocals	Total Number of Subsamples	Per Cent Oil	
			IHO Cytoplasm	Line Cytoplasm
ILO	5	473	4.71*	4.81
WF9	2	284	9.24*	9.38
B37	2	284	8.81*	8.70
H49	2	241	8.69	8.75
Oh7A	2	272	7.52	7.60
B14	2	196	9.01	8.97
M14	2	239	9.23	9.17
Oh43	1	164	8.33*	8.29
		Average	8.19	8.21

*Significant difference between means at the 5% probability level.

It can be concluded from this data that a cytoplasmic effect on oil content exists. However as the magnitude of the F_2 differences is small and sometimes reversed from that expected based on the F_1 , the physiological influence of the maternal parent must be the primary factor causing the observed differences in the F_1 .

Douglas L. Garwood
Robert J. Lambert

2. Lipid and protein characteristics of a Peruvian archaeological specimen.

A well-preserved cache of corn ears 800 to 1,000 years old was uncovered during a road-building operation in the La Rinconada area near LaMolina. According to Alexander Grobman, the sample is representative of the ancestor of the Chilcano and Huachano complexes of early, drought resistant coastal floury corns.

NMR scans demonstrated that liquid oils were not present. However, petroleum ether and carbon tetrachloride extractions removed fats. In addition, fatty acid and amino acid analyses were made by GLC and automatic amino acid analyzer. Kjehldahl nitrogen determination also was made.

Fatty Acid	%	
	Peruvian Sample	Ill. Hi. Oil
Myristic C ₁₄	1.54	Trace
Palmitic C ₁₆	31.28	11.5
Palmitoleic C _{16:1}	1.92	0.4
Stearic C ₁₈	4.78	1.8
Oleic C _{18:1}	51.67	33.0
Linoleic C _{18:2}	8.78	50.0
Linolenic C _{18:3}	0.0	0.2
Total oil (Gravimetric)	1.7	15
Protein	7.8	15

If one assumes that the Peruvian sample was originally similar to modern corns in fat content and quality, it is apparent that the disappearance of linoleic acid over time was more pronounced than for other fatty acids.

Traces of short chains and other breakdown products were also observed.

Amino acid analyses are incomplete, but short column analysis suggests that lysine level was not different from ordinary corns.

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Ian de la Roche
Dennis Elmore
Floyd Collins
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3. Lysine content of Peruvian flourey varieties.

Nineteen Peruvian highland and selva flourey varieties were selected from the germ-plasm bank maintained by the Programa Cooperativo de Investigaciones en Maize at LaMolina. Varieties were selected on the basis of phenotypic

similarity to opaque-2 or floury-2. Lysine content was determined by short column method on an automatic analyzer. All varieties were found to be similar to ordinary corns. (List and analytical data available on request).

D. E. Alexander
C. D. Elmore

4. Inheritance of palmitic acid.

A study of the level of palmitic acid was made during the summer of 1967. K6 (11.28% palmitic acid) and H51 (16.01% palmitic acid) inbreds were used to produce F_1 , F_2 , BC to K6 and BC to H51. All analyses were run on single kernels by G.L.C., 456 analyses in all.

The results of analysis of BC to K6, BC to H51 and F_2 were tested against a single gene hypothesis by Chi square. This was rejected as probability approached zero.

The data, when plotted in histograms, appeared to approach a normal distribution; portioning of variance by Mather's formulae yielded an additive genetic variance 2.4 times as large as the dominance genetic variance. Heritability was .83, suggesting that C16 is under direct gene control.

The F_3 generation of this cross is now being analyzed so that an estimate of the number of genes controlling palmitic acid can be made. Other populations are also being prepared for analysis to provide supporting evidence for a genetic model.

R. J. Swieringa
D. E. Alexander

5. Location of fl_2 .

(a) Preliminary crosses which included T 4-9g (4S.27; 9L.27) and T 4-9 5657 (4L.33; 9S.25) confirmed the location of fl_2 on Chromosome 4.

$$\frac{Wx}{wx} \frac{T 4-9g}{+} \frac{+}{+} \frac{fl_2}{+} \quad X \quad wx \quad \rightarrow \quad 149 \frac{Wx}{Fl} \frac{Fl}{1304} = 11.42 \times 2 = \text{estimated } 22.8\% \frac{wx-fl_2}{\text{recombination}}$$

$$\frac{Wx}{wx} \frac{T 4-9 5657}{+} \frac{+}{+} \frac{fl_2}{+} \quad X \quad wx \quad \rightarrow \quad 32 \frac{Wx}{Fl} \frac{Fl}{371} = 8.6 \times 2 = \text{estimated } 17.2\% \frac{wx-fl_2}{\text{recombination}}$$

Ears from the above crosses were scored for percentage of non-waxy, non-floury kernels among the total ($fl_2 \frac{fl_2}{Fl_2}$ endosperms are usually floury in phenotype). Since these represent only one of the two recombinant types, the percentages thus obtained were doubled to arrive at an estimate of total $wx-fl_2$ recombination.

(b) fl₂ - su₁ recombination

$$\frac{fl_2 +}{+ su_1} \times su_1$$

Parental		Recombinant		Total	%
fl ₂ +	+ su ₁	fl ₂ su ₁	+ +		Recombination
159	116	12	8	295	6.8

Kernels of the above cross were separated for the sugary trait at planting time. Resulting plants were pollinated by a homozygous fl₂ source, and the harvested ears were classified for presence or absence of floury kernels.

(c) la - fl₂ - su₁ recombination

$$\frac{+ fl_2 +}{la + su_1} \times la su_1$$

		Families	
(P)	+ fl ₂ +	<u>67-(19-23)</u>	<u>la - fl₂</u> = 6/246 = 2.4%
(1)	+ + ² su ₁	219	
(2)	+ fl ₂ su ₁	5	<u>fl₂ - su₁</u> = 22/246 = 8.9%
(1,2)	+ + ² +	21	
		1	<u>la - su₁</u> = 26/246 = 10.6%
	Total	<u>246</u>	Order: <u>la - fl₂ - su₁</u>

Kernels of the above cross were separated for the sugary trait at planting time. Non-lazy plants were pollinated by a homozygous fl₂ source and harvested ears were classified for presence or absence of floury kernels.

(d) Ga₁ - fl₂ - su₁ recombination

$$\frac{Ga_1 su_1}{su_1} \times \frac{ga_1 fl_2 Su_1}{Ga_1 + su_1}$$

(26% Su) (25% Su)

Region		<u>67-14</u>	<u>%</u>	<u>67-16</u>	<u>%</u>
1	<u>Ga fl Su</u>	25	65.8	31	68.9
2	<u>Ga + Su</u>	<u>13</u>	<u>34.2</u>	<u>14</u>	<u>31.1</u>
1 + 2		38	100.0	45	100.0

Family 67-14 was planted from an ear with 26% non-sugary kernels (59Su: 168 su); family 67-16 was planted from an ear with 25% non-sugary kernels (59Su: 177 su). On the assumption that only Ga_1 -carrying pollen functioned in these crosses, the percentage of non-sugary kernels measures $Ga_1 - su_1$ recombination. It is known, however, from previous work that in similar crosses, ga_1 -carrying pollen may function with a frequency of perhaps 2 to 5%.

Both families above were planted with non-sugary kernels. All plants were pollinated by a homozygous fl_2 source, and ears were classified for presence or absence of floury kernels.

In each of the above crosses, about one-third of the assumed $Ga_1 - su_1$ recombination occurred in the $fl_2 - su_1$ segment. This yields an estimated value of about 8% recombination between fl_2 and su_1 , which is in good agreement with the data in (b) and (c), above. In the absence of other information, these results could be interpreted as indicating either the gene order $Ga_1 - fl_2 - su_1$ or the order $Ga_1 - su_1 - fl_2$; in the latter instance, $su_1 - fl_2$ recombination would be about 30-35%. From the other data above, however, it is clear that the first gene order is correct.

Combined data indicate that $fl_2 - su_1$ recombination is about 8%. On the current linkage map of Chromosome 4, fl_2 might therefore be assigned tentatively to position 63. Incidentally, the $la - su_1$ recombination value of 10.6% in (c), above, is in good agreement with the current tentative assignment of la to position 60 on the genetic map:

Ga ₁	st Ts ₅	la	fl ₂	sp ₁	su ₁
35	(55) 56	(60)	(63)	66	71

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1. Linkage relations of Ht.

In previous reports in the Maize News Letter (1963, 1965), data were presented which indicated that the dominant gene, Ht (chlorotic-lesion resistance to Helminthosporium turcicum), is located in the central region of the long arm of Chromosome 2. Additional data have now been accumulated on its position relative to w_3 , and on some linkage relations in stocks heterozygous or homozygous for Inversion 2a (2S.7; 2L.8).

(a) Position of Ht relative to w_3 in normal stocks

Progeny from the crosses indicated below were classified for Ht, and all plants were self-pollinated. The harvested ears were classified for Ch and seedling tested for segregation of v_4 and/or w_3 .

(1)

$$\text{Standard (+ + + +) } \times \frac{+ w_3 + \text{Ch}}{v_4 + \text{Ht} +}$$

Region		66-(8353-8356)
P	+ w + Ch	22
	v + Ht +	13
1	+ + Ht +	3
	v w + Ch	6
2	+ w Ht +	1
	v + + Ch	1
3	+ w + +	12
	v + Ht Ch	5
1,2	+ + + Ch	1
	v w Ht +	0
1,3	+ + Ht Ch	5
	v w + +	2
	Total	71

$$\frac{v_4}{v_4} - \frac{w_3}{w_3} = 17/71 = 24\%$$

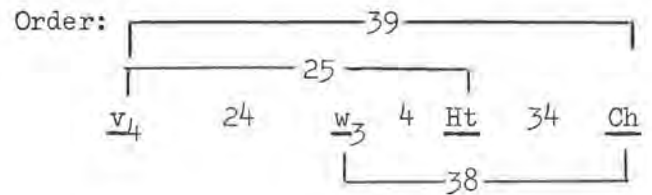
$$\frac{v_4}{v_4} - \frac{\text{Ht}}{\text{Ht}} = 18/71 = 25\%$$

$$\frac{v_4}{v_4} - \frac{\text{Ch}}{\text{Ch}} = 28/71 = 39\%$$

$$\frac{w_3}{w_3} - \frac{\text{Ht}}{\text{Ht}} = 3/71 = 4\%$$

$$\frac{w_3}{w_3} - \frac{\text{Ch}}{\text{Ch}} = 27/71 = 38\%$$

$$\frac{\text{Ht}}{\text{Ht}} - \frac{\text{Ch}}{\text{Ch}} = 24/71 = 34\%$$



(2)

$$\text{Standard (+ + +) } \times \frac{+ \text{Ht} +}{w_3 + \text{Ch}}$$

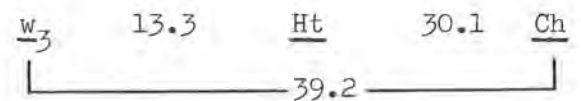
Region		65-(6171-6178)
P	+ Ht +	39
	w + Ch	45
1	+ + Ch	6
	w Ht +	10
2	+ Ht Ch	24
	w + +	16
1,2	+ + +	2
	w Ht Ch	1
	Total	143

$$\frac{w_3}{w_3} - \frac{\text{Ht}}{\text{Ht}} = 19/143 = 13.3\%$$

$$\frac{\text{Ht}}{\text{Ht}} - \frac{\text{Ch}}{\text{Ch}} = 43/143 = 30.1\%$$

$$\frac{w_3}{w_3} - \frac{\text{Ch}}{\text{Ch}} = 56/143 = 39.2\%$$

Order:



Combined data from these and other linkage tests involving markers in the long arm of Chromosome 2 are as follows:

$$\begin{array}{ll} \underline{v}_4 - \underline{w}_3 = 134/486 = 27.6 & \underline{w}_3 - \underline{Ht} = 22/214 = 10.3 \\ \underline{v}_4 - \underline{Ht} = 129/506 = 25.5 & \underline{w}_3 - \underline{Ch} = 108/297 = 36.4 \\ \underline{v}_4 - \underline{Ch} = 233/502 = 46.4 & \underline{Ht} - \underline{Ch} = 191/562 = 34.0 \end{array}$$

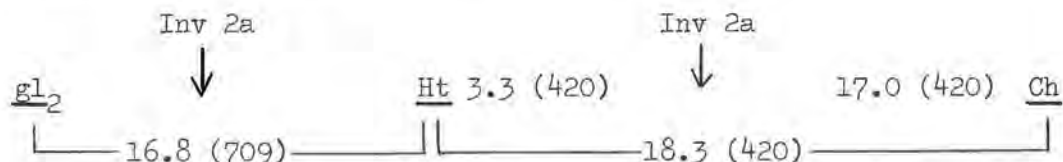
The two progenies in which \underline{w}_3 and \underline{Ht} were classified simultaneously (the crosses tabulated above) involve small numbers but agree in the location of \underline{Ht} to the right of \underline{w}_3 . On the basis of combined data, the gene order and distances are as follows:

$$\underline{v}_4 \quad 27.6 \quad (486) \quad \underline{w}_3 \quad 10.3 \quad (214) \quad \underline{Ht} \quad 34.0 \quad (562) \quad \underline{Ch}$$

(b) Linkage relations of Ht in plants heterozygous for Inversion 2a

Region	+ Inv 2a Ch			X	+ + +
	Ht	+	+		
	(2961-3010)				
P	+	Inv	Ch	177	
	Ht	+	+	162	$\underline{Ht} - \text{Inv} = 14/420 = 3.3\%$
1	+	+	+	10	$\underline{Ht} - \underline{Ch} = 77/420 = 18.3\%$
	Ht	Inv	Ch	0	$\text{Inv} - \underline{Ch} = 71/420 = 17.0\%$
2	+	Inv	+	34	
	Ht	+	Ch	33	Order: $\underline{Ht} - \text{Inv} - \underline{Ch}$
1,2	+	+	Ch	0	
	Ht	Inv	+	4	
	Total			420	

The data above, together with \underline{gl}_2 - \underline{Ht} recombination reported in the 1963 MNL, may be summarized as follows:



The indicated \underline{Ht} -Inv 2a recombination is presumably a measure of the frequency of 2-strand double crossovers within the inversion loop in which one crossover is to the left and one to the right of the \underline{Ht} locus. Crossing over within the inversion is expected to yield two types of duplicate-deficient

The breakpoints of Inversion 2a are thus between gl_2 and B in the short arm and between Ht and Ch in the long arm. There is an indication that lg_1-gl_2 recombination may be increased in stocks homozygous for Inversion 2a.

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2. Mapping studies of Rp_3 .

In the 1964 MNL (p. 66), Hooker and Russell reported that a dominant gene for resistance to Puccinia sorghi present in line 178 showed linkage with T 3-9c (3L.09; 9L.12). This gene later proved to be allelic to Rp_3 . In the data they reported, in plants heterozygous for T 3-9c, wx and Rp_3 showed 11.7% recombination (32/274).

Further efforts to determine the map position of Rp_3 yielded the following information:

(a) In greenhouse classifications: $d_1 - Rp_3 = 51/288 = 17.7\%$ recombination

(b) In field classifications (255 plants): d_1 23.1 Lg_3 7.1 Rp_3
└──────────27.1──────────┘

(c) In greenhouse classifications (244 plants):

Rp_3 3.3 gl_6 25.8 lg_2
└──────────28.3──────────┘

(d) Progeny of the following cross were scored in the field in 1967:

+++ X $\frac{+ + Rp_3}{Lg_3 Rg +}$

	P	+	+	Rp			
		Lg	Rg	+	202		
				+	233		$Lg_3 - Rg = 17/456 = 4.1\%$
	1	+	Rg	+	7		$Lg_3 - Rp_3 = 21/456 = 5.0\%$
		Lg	+	Rp	10		$Rg - Rp_3 = 4/456 = 0.9\%$
	2	+	+	+	4		Order: $Lg_3 - Rg - Rp_3$
		Lg	Rg	Rp	0		
					456		
			Total				

At right above are indicated the recombination values based on the data as recorded. However, the four wild-type plants tabulated as region 2 recombinants in the table may represent contaminants, since no contamination marker was present in the male parent and hence their origin could not be verified. The occurrence of $Rg Rp_3$ progeny would have established the

$$(b) \quad \frac{+ \quad + \quad +}{+ \quad \underline{Ga_1} \quad \underline{su_1}} \times \frac{\underline{Rp_4} \quad + \quad +}{+ \quad \underline{Ga_1} \quad \underline{su_1}} \quad \text{Ear ratio:} \quad \begin{array}{l} \underline{Su} \quad 121 - 58 = 63 \\ \underline{su} \quad 58 + 58 = 116 \end{array}$$

$$\text{Estimated } \underline{Ga_1} - \underline{su_1} \text{ recombination} \\ = 63/179 = 35.2\%*$$

			<u>Observed</u>		<u>Estimated</u>	
P	+	Ga su	41	+ 41 =	82	Recombination:
1	Rp	Ga su	4	+ 4 =	8	$\underline{Rp_4} - \underline{Ga_1} = 12/160 = 7.5\%$
2	+	Ga Su	107	- 41 =	66	$\underline{Ga_1} - \underline{su_1} = 70/160 = 43.8\%*$
1,2	Rp	Ga Su	8	- 4 =	4	$\underline{Rp_4} - \underline{su_1} = 74/160 = 46.3\%*$
			<u>160</u>		<u>160</u>	

Combined data from (a) and (b), above:

$$\underline{Rp_4} - \underline{Ga_1} = 23/278 = 8.3\%$$

$$\text{(Ear ratio)} \quad \underline{Ga_1} - \underline{su_1} = 112/309 = 36.2\%*$$

$$\text{(Plants)} \quad \underline{Ga_1} - \underline{su_1} = 117/278 = 42.1\%*$$

$$\underline{Rp_4} - \underline{su_1} = 120/278 = 43.2\%*$$

*These values are based on estimated gametic frequencies of alleles at the su₁ locus.

All calculations above have assumed 100 per cent functioning of Ga₁-carrying pollen. If there was some functioning of ga₁ pollen in production of these progenies, estimates of both Rp₄ - Ga₁ and Ga₁ - su₁ recombination are too high; the Rp₄ - su₁ recombination values, however, would not be altered. The sequence of Rp₄ with respect to Ga₁ is not clearly established by these data, but it appears more probable that Rp₄ is distal to Ga₁. Rp₄ is probably not more than about 10 units from Ga₁ and may be considerably closer. Accurate mapping of Rp₄ would require testing of progeny for constitution at the Ga₁ locus, and preferably should employ a closer proximal marker (e.g., fl₂) in place of su₁. The de₁ locus, at the left end of the map, would also provide useful marking, but apparently stocks of this trait have been lost.

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1. On the use of the TB-3a translocation to localize the lethal effects of the interaction of the mutant etched allele and M^{et} .

In M.G.C.N.L. 40:39-42, 1966, I reported on a system of zygotic lethality involving the interaction of the recessive etched allele of chromosome 3 and the previously unreported modifier, M^{et} . Individuals homozygous for the modifier, $M^{et}M^{et}$, and heterozygous for the etched locus produce no etched kernels as a result of selfing or testcrossing by standard etched testers. The elimination of etched individuals was demonstrated to be postzygotic in nature by genetic and histological tests. At that time we had in hand at least circumstantial evidence that the elimination of etched individuals was based on the existence of two or more "doses" of the modifier, M^{et} , in endosperm tissues and was totally independent of the modifier genotype of the embryo.

In order to verify that this is the actual situation, we turned to the use of the TB-3a translocation. Previous studies have indicated that the dominant allele of the etched locus (Et) overcomes the lethality conditioned by the interaction of et and the modifier M^{et} . It was reasoned that a TB-3a stock, lacking the mutant etched allele but homozygous for the modifier, could be established and used to vary the "doses" of et in the endosperm and embryo as a result of post-meiotic non-disjunction of the B-centromere (see Table 1.) Such a test should allow us to localize the lethal effect of this system of genic interaction in either the endosperm or embryo.

TB-3a tester stocks of the appropriate modifier genotype have been established and some crossing was done this past summer. The analysis of the data from these crosses is incomplete as of this writing because further field testing is necessary.

Delano K. Cox

2. On the nature of the interaction of M^{et} and the mutant etched allele.

During the course of the establishment of the TB-3a stocks discussed above, new information on the nature of the interaction of et and M^{et} has become available.

Crosses designed to introduce the modifier (M^{et}) into the TB-3a background are expected to produce kernels which develop into either of three types of plants with respect to their chromosome three constitution: (1) normal $3/3$, (2) hypoploid $3/3^B$, and (3) hyperploid $3/3^B/B^3/B^3$.

Table 1
Expected genotypes (chromosomal and genic)
from the cross:

$3(a \underline{Et}) / 3(a \underline{et}) ; \underline{M}^{et} \underline{M}^{et} \text{♀} \times 3(\underline{A} \underline{Et}) / 3^B / B^3(\underline{A} \underline{Et}) ; \underline{M}^{et} \underline{M}^{et} \text{♂}$

All individuals are $\underline{M}^{et} \underline{M}^{et}$

Normal disjunction:

Seedling	Embryo Genotype	Endosperm Genotype	Kernel Phenotype
<u>A</u> Norm. green	$3(a \underline{et}) / 3(\underline{A} \underline{Et})$	$3(a \underline{et}) / 3(a \underline{et}) / 3(\underline{A} \underline{Et})$	<u>A</u>
<u>A</u> Norm. green	$3(a \underline{Et}) / 3(\underline{A} \underline{Et})$	$3(a \underline{Et}) / 3(a \underline{Et}) / 3(\underline{A} \underline{Et})$	<u>A</u>
<u>A</u> TB-3a green balanced	$3(a \underline{et}) / 3^B / B^3(\underline{A} \underline{Et})$	$3(a \underline{et}) / 3(a \underline{et}) / 3^B / B^3(\underline{A} \underline{Et})$	<u>A</u>
<u>A</u> TB-3a green balanced	$3(a \underline{Et}) / 3^B / B^3(\underline{A} \underline{Et})$	$3(a \underline{Et}) / 3(a \underline{Et}) / 3^B / B^3(\underline{A} \underline{Et})$	<u>A</u>

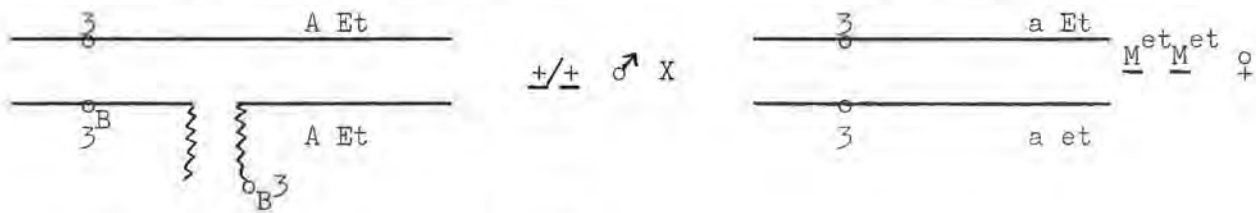
Nondisjunction:

<u>A</u> Hyper. green	$3(a \underline{et}) / 3^B / B^3(\underline{A} \underline{Et}) / B^3(\underline{A} \underline{Et})$	$3(a \underline{et}) / 3(a \underline{et}) / 3^B$	<u>a et</u> (Type I)*
<u>A</u> Hyper. green	$3(a \underline{Et}) / 3^B / B^3(\underline{A} \underline{Et}) / B^3(\underline{A} \underline{Et})$	$3(a \underline{Et}) / 3(a \underline{Et}) / 3^B$	<u>a Et</u> (Type II)*
<u>a</u> Hypo. vires.	$3(a \underline{et}) / 3^B$	$3(a \underline{et}) / 3(a \underline{et}) / 3^B / B^3(\underline{A} \underline{Et}) / B^3(\underline{A} \underline{Et})$	<u>A</u> (Type III)*
<u>a</u> Hypo. green	$3(a \underline{Et}) / 3^B$	$3(a \underline{Et}) / 3(a \underline{Et}) / 3^B / B^3(\underline{A} \underline{Et}) / B^3(\underline{A} \underline{Et})$	<u>A</u> (Type IV)*

*Localization of lethal effect will be accomplished by comparing frequencies of Types I, II, III, and IV.

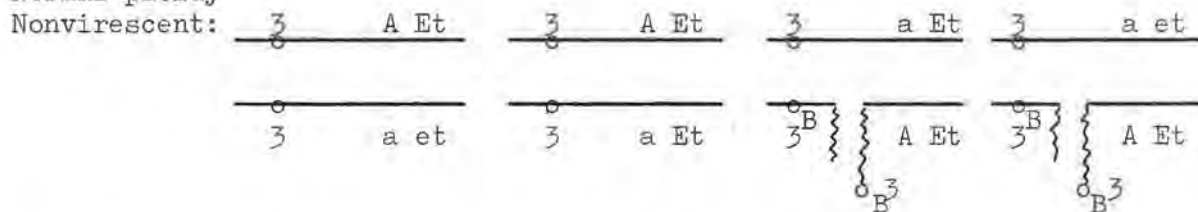
Balanced TB-3a
(pollen 15-20% aborted)

Normal 3/3
(pollen normal)

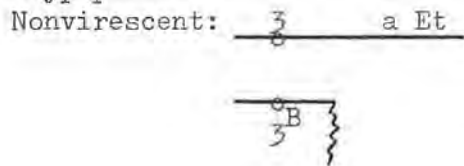


PROGENY: All individuals are $\frac{+}{M^{et}}$

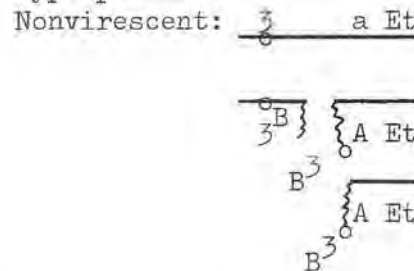
Normal ploidy



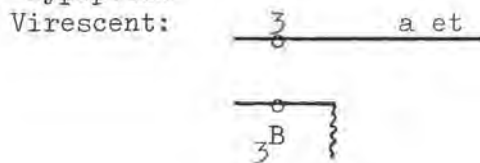
*Hypoploid



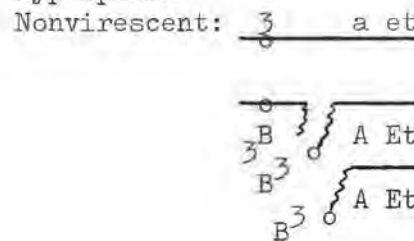
Hyperploid



*Hypoploid



Hyperploid



The mutant etched allele is pleiotropic and expresses itself as an "etching" of the endosperm of homozygous kernels as well as producing a virescence in the seedling stage. As is well known, chromosome three hypoploids are easily recognized by their short stature, pointed leaves, and 50% pollen sterility. Such a cross as the one just described is expected to produce equal numbers of nonvirescent hypoploids ($\frac{3(a Et)}{3^B}$) and virescent hypoploids ($\frac{3(a et)}{3^B}$). The results of field scoring of seedlings for two summers appear in Table 2.

Table 2
 Results of seedling tests of progeny of the cross:
 $3(\underline{a} \underline{Et})/3(\underline{a} \underline{et}); \underline{Met} \underline{Met}^{\text{et}} \text{♀} \times 3(\underline{A} \underline{Et})/3^{\text{BB}}(\underline{A} \underline{Et}); +/+ \text{♂}$. All individuals
 are $\underline{Met}^{\text{et}}/+$.

	Normal Green <u>Aa</u>	Hypoploid Green <u>aa</u>	Hypoploid Virescent <u>aa</u>
<u>1966 (U. of Ill.):</u>			
No. of seedlings	1025	220	107
Frequency (%)	75.8	16.2	8.0
<u>1967 (Ill. St. Univ.):</u>			
No. of seedlings	479	125	56
Frequency (%)	72.5	19.0	8.5

It is obvious from the data that the two types of hypoploids are not present in equal frequencies as was the expectation. The degree of virescence associated with the deficient class (aa virescent) was extreme, bordering on albinism even though they eventually gained pigment. Further, these plants were only about 20% the size of the aa green hypoploids and formed an unexpected third category of plants with respect to size and rate of maturation. At maturity (though they never were observed to shed pollen), they were quite small resembling more fox tail grass than corn (though corn they were).

It is important to remember that all of these individuals are heterozygous for the modifier, $\underline{Met}^{\text{et}}/+$. We consider this observation to be a reflection of an "enhancement" of the etched phenotype which is caused by the interaction of the mutant etched allele and the modifier $\underline{Met}^{\text{et}}$.

Further evidence of such enhancement effects is available but not previously noted. As a result of conversations with Dr. I. Greenblatt (see also M.G.C.N.L. 36 & 37) I have become interested in the development of the etched kernel phenotype. While screening kernels for possible use in developmental studies, I made the purely subjective observation that etched kernels of the $+/\underline{Met}^{\text{et}}$ background consistently have a more severe "etching" of their endosperm tissues than do etched kernels lacking the modifier. This past year etched kernels that were heterozygous for the modifier were planted in the field. Etched (et/et) kernels lacking the modifier were also planted. The seedlings were strikingly different with respect to their expression of virescence. Those plants that were heterozygous for the modifier displayed a level of virescence that borders on albinism. Those plants lacking the modifier had a "normal" level of

virescence. The severely virescent plants did, however, develop pigment and produced kernels which were used in a histological study.

These observations indicate that there is indeed an enhancement effect associated with the interaction of et and M^{et} . It apparently affects the development and maturation of plastids (chloroplasts and leucoplasts) as first suggested by Greenblatt (M.G.C.N.L. 36 & 37).

If we try to reconstruct this system in terms of enhancement effects and the postzygotic lethality associated with the etched locus (see Cox M.G.C.N.L. 40:39-42), the following picture emerges.

<u>Kernel Genotype</u>	<u>Endosperm Phenotype</u>	<u>Seedling Phenotype</u>
I. <u>et/et</u> ; <u>+</u> <u>+</u>	Moderate to poor etching	Virescence <u>+</u>
II. <u>et/et</u> ; M^{et} <u>+</u>	Severe etching	Extreme virescence
III. <u>et/et</u> ; M^{et} / M^{et}	Postzygotic arrest, no mature kernels	-----

The above scheme suggests that the modifier, M^{et} , which is independent of the linkage group of the mutant etched allele, interacts with etched to upset normal plastid development.

The author would like to thank the laboratory of Dr. J. R. Laughnan and the Department of Agronomy, University of Illinois for the use of their field facilities during the summer of 1966. Our current work is being carried out at the Illinois State University Farm, Normal, Illinois where space has been set aside, largely through the efforts of Dr. D. F. Weber, for the establishment of maize genetics research plots.

Delano K. Cox

3. The effect of abnormal chromosome 10 on recombination in Tp9/N9 plants.

Rhoades (1958, M.G.C.N.L. 32:66-70 and unpublished) has intensively studied a segment transposed from the long arm of chromosome 3 (3L) to the short arm of chromosome 9 (9S). Recombination values were determined in bivalents consisting of one normal chromosome 9 (N9) and one carrying the transposed segment from chromosome 3 (previously designated as Dp9 but now designated as Tp9 by Rhoades). Recombination along the entire length of 9S was strongly decreased in plants heterozygous for the transposition. A corresponding decrease in the precision of chromosome pairing in 9S at pachynema has also been demonstrated by Rhoades and Dempsey (unpublished). They found that the buckle induced by the transposition is not located at a constant position in 9S, but it could be found at essentially any position in 9S. Frequently the buckle is not even seen since it was retracted into the chromosome. These genetic and cytological results have been confirmed by the author. (The same transposition was used by the author in a previous study reported in this newsletter (Weber, M.G.C.N.L. 41:204-206).

The present study is designed to determine the effect of abnormal chromosome 10 on this system. Recombination in sister plants of the following constitutions was analyzed:

Table 1

Female parent	Male parent	Total	<u>C-Sh</u> recombinants	<u>Sh-Wx</u> recombinants	% <u>C-Sh</u> recombinants	% <u>Sh-Wx</u> recombinants	# plants
<u>c sh Tp wx</u> <u>k10</u> <u>C Sh N Wx</u> <u>k10</u>	<u>c sh N wx</u> <u>k10</u>	1137	42	10	3.7	0.9	3 (3 ears)
<u>c sh Tp wx</u> <u>K10</u> <u>C Sh N Wx</u> <u>k10</u>	<u>c sh N wx</u> <u>k10</u>	1753	84	30	4.8	1.7	5 (5 ears)
<u>c sh N wx</u> <u>k10</u>	<u>c sh Tp wx</u> <u>k10</u> <u>C Sh N Wx</u> <u>k10</u>	1411	70	54	5.0	3.8	2 (8 ears)
<u>c sh N wx</u> <u>k10</u>	<u>c sh Tp wx</u> <u>K10</u> <u>C Sh N Wx</u> <u>k10</u>	1587	80	43	5.0	2.7	2 (7 ears)

Table 2

Female parent	Male parent	Total	Recombinants	% Recombination	# Plants
<u>Yg c Tp</u> <u>k10</u> yg C N k10	<u>yg c N</u> <u>k10</u>	535	29	5.4	1 (2 ears)
<u>Yg c Tp</u> <u>K10</u> yg C N k10	<u>yg c N</u> <u>k10</u>	1530	322	21.0	4 (6 ears)
<u>Yg c Bz Tp</u> <u>k10</u> yg C bz N k10	<u>yg c N</u> <u>k10</u>	367	48	13.1	1 (1 ear)
<u>Yg c Bz Tp</u> <u>K10</u> yg C bz N k10	<u>yg bz N</u> <u>k10</u>	244	30	12.3	1 (1 ear)
<u>Yg c Bz Tp</u> <u>K10</u> yg C bz N k10	<u>yg c N</u> <u>k10</u>	3287	796	24.2	9 (11 ears)
	<u>yg bz N</u> <u>k10</u>	256	66	25.8	1 (1 ear)

$$\begin{array}{c} \underline{c \ sh \ Tp \ wx} \\ C \ Sh \ N \ Wx \end{array} ; \underline{KlO} \quad \text{and} \quad \begin{array}{c} \underline{c \ sh \ Tp \ wx} \\ C \ Sh \ N \ Wx \end{array} ; \underline{klo}$$

Tp = the point of insertion of the transposed segment in a chromosome carrying the transposed segment

N = the point of insertion of the transposed segment in a chromosome not carrying the transposed segment

KlO = abnormal chromosome 10

klo = normal chromosome 10

The cytological constitutions of all plants in this study were determined by analysis of microsporocytes. Both types were crossed by c sh N wx testers. The results are presented in Table 1.

To examine recombination between Yg and C or Bz in this system, the following cross was made:

$$\begin{array}{c} \underline{Yg \ c \ sh \ Bz \ Tp \ wx} \\ Yg \ C \ Sh \ Bz \ N \ Wx \end{array} \underline{KlO} \quad X \quad \underline{yg \ C \ sh \ bz \ N \ wx} \underline{klo/klo}$$

(from the above cross)

This cross produces four plant types, two of which were used in further tests. These are:

$$\begin{array}{c} \underline{Yg \ c \ sh \ Bz \ Tp \ wx} \\ yg \ C \ sh \ bz \ N \ wx \end{array} \underline{KlO} \quad \text{and} \quad \begin{array}{c} \underline{Yg \ c \ sh \ Bz \ Tp \ wx} \\ yg \ C \ sh \ bz \ N \ wx \end{array} \underline{klo}$$

These were crossed by yg c or yg bz plants. The results are given in Table 2.

From these data it can be seen that recombination is essentially unaffected in the C-Sh-Wx region by the presence of one abnormal chromosome 10. However, abnormal chromosome 10 greatly increased recombination in the Yg-C region.

A cytological examination of these plants is being undertaken to determine if there is a corresponding alteration of chromosome pairing in 9S at pachynema.

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1. Cytoplasmic effects on quantitative characters in maize:

Reciprocal F_1 and backcrosses were studied between SP2 and Llera III, Yellow Tuxpan, Eto Amarillo, San Andres Tuxtla and Colorado, and Colorado and Pira Blanco, Pollo Segregaciones and Pollo Amarillo. Quantitative characters such as yield, days to 75 per cent silking, plant height, ear height and no. of ears per plant were studied in all the reciprocal crosses with a view to find out whether the cytoplasm showed any effect on these characters. Significant cytoplasmic effects were obtained with four of the five characters studied. The character, number of ears per plant, did not show any cytoplasmic effect.

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2. Instability of cytoplasmic effects in different environments.

Reciprocal F_1 crosses were studied between Indian, Mexican, and Colombian varieties of maize. Data were recorded during 1964 and 1965 growing seasons on quantitative characters such as yield, maturity, plant height and ear height. The reciprocal crosses studied and the characters showing significant reciprocal differences in the two years are given in the table.

Pedigree	1964	1965
SP2 X Yellow Tuxpan	-	Days to silk
Yellow Tuxpan X SP2		
SP2 X Eto Amarillo	-	Plant height
Eto Amarillo X SP2		Ear height
SP2 X San Andres Tuxtla	-	Days to silk
San Andres Tuxtla X SP2		
Pira Blanco X Colorado	Days to silk	Plant height
Colorado X Pira Blanco		Ear height
KT ⁴¹ X San Andres Tuxtla	Plant height	Yield
San Andres Tuxtla X KT ⁴¹		
Basi X Eto Amarillo	-	-
Eto Amarillo X Basi		

It will be noted from the table that cytoplasmic effects on a particular character in one year were not repeated in the other year of study. Reciprocal crosses of SP2 with Yellow Tuxpan, Eto Amarillo and San Andres Tuxtla did not give cytoplasmic effects on any of the four characters studied during 1964, whereas significant effects were obtained on days to silk, plant height and ear height in the 1965 study. Reciprocal crosses between Pira Blanco and Colorado gave a significant cytoplasmic effect on days to silk in 1964, whereas no cytoplasmic effect was obtained for this character in 1965; instead plant height and ear height were significantly affected. KT41 X San Andres Tuxtla and its reciprocal revealed a significant cytoplasmic effect on plant height in 1964 and on yield in 1965. Reciprocal crosses of Basi and Eto Amarillo did not show significant cytoplasmic effects on any of the four characters studied.

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3. Threshold concentration of plasmon sensitive polygenes in the expression of quantitative characters.

During the course of studies of cytoplasmic effects on quantitative characters such as yield, maturity, plant height, ear height and number of ears per plant, it was observed that, if a character was under the dual control of nuclear genes and cytoplasm, cytoplasmic effects were expressed only up to a certain threshold concentration of the plasmon sensitive polygenes. Once the concentration of these genes crossed the threshold limit, they alone controlled the character and no cytoplasmic effects would be expressed.

This concept, to the knowledge of the authors, is a new one and has a direct bearing on the expression of cytoplasmic effects on quantitative characters.

If a cytoplasmic effect on a certain quantitative character is expressed in the F_1 and disappears in backcross generations, it might be due to the increased number of genes of one parent introduced by backcrossing which now exceed the threshold limit and thus nullify the cytoplasmic effect. Such a phenomenon was seen to exist in nine of the eighteen cases showing significant cytoplasmic effects on yield, days to 75 per cent silking, plant height and ear height.

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4. Male sterility caused by nucleus-cytoplasm interaction.

Llera III, a derivative of the Tuxpeno race of maize from Mexico, was reciprocally crossed with SP2, a primitive variety of maize from Sikkim (MNL 38:70), and it was observed that male sterility was produced only when Llera III contributed the cytoplasm. The data are presented in the table.

Pedigree	Per cent male sterile plants
Llera III	2.9
SP2	0.0
(Llera III X SP2) X Llera III	4.2
(SP2 X Llera III) X Llera III	0.0
Llera III X SP2	12.2
SP2 X Llera III	0.0
(Llera III X SP2) X Sp2	100.0
(SP2 X Llera III) X SP2	0.0

On increasing the dosage of SP2 in Llera III cytoplasm, the percentage of male sterile plants also increased. When 25, 50, and 75 per cent of the nuclear component of SP2 was present in Llera III cytoplasm the percentages of male sterile plants were 4.2, 12.2, and 100.0, respectively. The reciprocals, however, were male fertile. It may be mentioned that Llera III itself had 2.9 per cent male sterile plants. Since it is not known what type of male sterility is present in Llera III, no clear cut explanation could be given. It is presumed that with increase of the SP2 nuclear component in Llera III cytoplasm a strong nucleus-cytoplasm interaction took place which resulted in male sterility. The interaction when 75 per cent of the nuclear component of SP2 was present in Llera III cytoplasm was strong enough to produce 100 per cent male sterile plants. The interaction, however, did not occur between SP2 cytoplasm and the Llera III nuclear component. Studies are in progress to find out what type of male sterility is present in Llera III.

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1. The effect of abnormal chromosome 10, and a possible effect of B chromosomes, on crossing over in chromosome 5.

Rhoades (J. Am. Soc. Agron. 33:603) found crossing over in chromosome 5 to be higher in male than in female sporocytes and it has been reported that abnormal chromosome 10 increases crossing over in the proximal region of chromosome 3 (Rhoades & Dempsey, MNL 31:77 and Genetics 53:989) and in chromosome 9 (Kikudome, Genetics 44:815).

To determine the effect of abnormal chromosome 10 (K10) on crossing over in the proximal region of chromosome 5, a plant which was homozygous for a₂bt₁pr and for normal chromosome 10 (k10) was crossed as a female to one which was homozygous for A₂Bt₁Pr and heterozygous for K10. Sporocytes were taken from the progeny and cells in diakinesis were examined for the presence of K10. While doing this, it was noticed that some plants had from 2 to 4 B chromosomes and some had none. The plants were crossed, both as males and as females, to a chromosome 5 tester and crossover values were calculated. Since the a₂ kernels lacked aleurone color and could not be classified with respect to Pr and pr, crossing over in the Bt₁-Pr region was calculated from the A₂ classes only.

The results are as follows:

Sporocytes	# Plants	Used as	# Progeny	% C.O.		Increase in σ^2	
				<u>A₂-Bt₁</u>	<u>Bt₁-Pr</u>	<u>A₂-Bt₁</u>	<u>Bt₁-Pr</u>
k10k10; 0 B's	4	♂	1560	4.8	20.0	+ 9.9	+ 7.9
	4	♀	1372	14.7	27.9		
k10k10; 2-4 B's	8	♂	2910	7.1	22.3	+12.8	+13.5
	8	♀	1983	19.9	35.8		
Total k10k10	12	♂	4470	6.3	21.5	+11.5	+11.0
	12	♀	3355	17.8	32.5		
K10k10; 0 B's	4	♂	1497	16.8	35.7	+ 7.2	+ 0.1
	4	♀	791	24.0	35.8		
K10k10; 2-4 B's	9	♂	3005	17.9	35.2	+10.4	+ 5.9
	9	♀	2399	28.3	41.1		
Total K10k10	13	♂	4502	17.6	35.4	+ 9.7	+ 4.3
	13	♀	3190	27.3	39.7		

The crossover values for male flowers are higher than those for female flowers, except for the Bt_1-Pr region of K10k10 plants lacking B chromosomes. K10 increases crossing over in both the A_2-Bt_1 and Bt_1-Pr regions. A similar effect for the A_2-Bt_1 region has also been found by Robertson (this MNL, p. 89). This increase is greater in the females than in the males, resulting in smaller differences in crossing over between the sexes, particularly in the longer Bt_1-Pr region.

Hanson (MNL 35:61, MNL 36:34 and Ph.D. thesis) has reported that B chromosomes increase crossing over in chromosomes 3 and 9. From the present study, although the numbers of plants in classes without B chromosomes were small and tests of significance were not done, it would seem that B chromosomes may cause an increase in crossing over in chromosome 5 and that the effect, in contrast to that of K10, is greater in males than in females.

Further work, which should make definite conclusions possible as to the effects of B chromosomes, will be done during the coming summer.

Paul Nel

2. The influence of the female parent on preferential fertilization by B^9 -containing sperm.

The B chromosome of maize possesses an accumulation mechanism, whereby non-disjunction of the B chromosome at the second pollen mitosis, followed by preferential fertilization of the egg by the B-containing sperm, results in an increase in B chromosome number (Roman). Preferential fertilization of the egg has been found to occur at approximately the same rate in different genetic backgrounds (Catcheside) and is a constant feature of B chromosome inheritance.

In 1966, plants carrying one of Roman's A-B translocations, TB-9b, were crossed as male parents onto two different inbred lines, a $c\ sh\ wx$ tester and a $c\ sh\ wx\ gl_{15}$ tester. The constitution of the TB-9b plants was $9C\ Sh\ B^9\ C\ Sh$. The Wx locus is very close to the translocation breakpoint of TB-9b, and crossing-over of the Wx allele onto the normal chromosome 9 occurs less than 0.5% of the time (Robertson). It may be assumed, therefore, that when Wx kernels are selected from the progeny of a $c\ sh\ wx \times 9C\ Sh\ B^9\ C\ Sh$ cross, the vast majority of the individuals each contain the $9^B\ Wx$ chromosome. It is also known, from the work of Robertson, that crossing-over of the $c\ sh$ markers from the normal chromosome 9 onto the B^9 chromosome is a very rare event (0.26%). As a result, classification of C and Sh is an accurate method for determining the presence or absence of the B^9 chromosome in the endosperm. In the crosses that were made onto the $c\ sh\ wx$ tester and the $c\ sh\ wx\ gl_{15}$ tester, the Wx seeds were selected and classified for C and Sh . The results are given below:

$c\ sh\ wx$ X 678-5 (TB-9b)
$c\ sh\ Wx = 422$ (62.5%)
$C\ Sh\ Wx = 252$
$C\ sh\ Wx = 1$

$c\ sh\ wx$ X 808-1 (TB-9b)
$c\ sh\ Wx = 238$ (58.4%)
$C\ Sh\ Wx = 170$

$c\ sh\ wx\ gl_{15}$ X 678-5
$c\ sh\ Wx = 224$ (38.5%)
$C\ Sh\ Wx = 356$
$C\ sh\ Wx = 1$

$c\ sh\ wx\ gl_{15}$ X 808-1
$c\ sh\ Wx = 131$ (43.5%)
$C\ Sh\ Wx = 170$

According to Roman's method of classification, the c sh Wx seeds represent cases of nondisjunction of the B⁹ chromosome, in which the embryo received two B⁹ chromosomes. The C Sh Wx seeds represent either cases of nondisjunction of the B⁹ in which the endosperm received two B⁹'s, or cases of proper division of the B⁹ in which the endosperm and embryo each received one B⁹ chromosome. Growing the plants from the C Sh Wx seeds and backcrossing to a c sh wx tester classifies the plants for the presence (C Sh) or absence (c sh) of the B⁹ chromosome.

In the data given above, a difference exists between the percentage of c sh Wx seeds found in crosses with the c sh wx tester and that found in crosses with the c sh wx gl₁₅ tester. The genetic background of the female parents apparently had an effect on the behavior of the B⁹ chromosome. The difference in c sh Wx seeds could be accounted for by a change in the rate of nondisjunction of the B⁹ chromosome, or a change in the degree of preferential fertilization of the egg by the B⁹-containing sperm. It is difficult to see how the genetic background of the female parent could influence the rate of nondisjunction of the B⁹ chromosome, but it is possible that pollen grains in which nondisjunction has occurred are selected for or selected against during fertilization. For this reason, an estimate of the total rate of nondisjunction of the B⁹ chromosome was made for each cross.

In order to calculate the rate of nondisjunction of the B⁹ chromosome, the C Sh Wx class must first be divided into cases of nondisjunction and cases of proper division of the B⁹. C Sh Wx seeds were planted and the progeny classified for the presence or absence of the B⁹ chromosome, by backcrossing to a c sh wx tester. The absence of the B⁹ chromosome in the embryo (backcross seeds all c sh) indicates that nondisjunction has occurred. Among 93 C Sh Wx individuals from the cross c sh wx X 678-5, 88 had resulted from nondisjunction of the B⁹. In the c sh wx gl₁₅ X 678-5 cross, the figure was 86/91. From this data, the overall rate of nondisjunction in the c sh wx X 678-5 cross was found to be 93%, and the rate in the c sh wx gl₁₅ X 678-5 cross 96%. Obviously there was no difference in the rate of nondisjunction between the two crosses. A similar result was found in the crosses of plant 808-1. Among 92 C Sh Wx individuals from the cross c sh wx X 808-1, 57 had resulted from nondisjunction. The rate of nondisjunction in this cross was calculated to be 84%. In the cross c sh wx gl₁₅ X 808-1, nondisjunction was found in 51/75 of the C Sh Wx individuals tested. The rate of nondisjunction for this cross was 82%. Again, no difference in the rate of nondisjunction between the two crosses was found.

Preferential fertilization in the crosses of 678-5 and 808-1 was determined by the per cent of the total amount of nondisjunction that was contributed by c sh Wx seeds. The results for preferential fertilization are given below:

<u>c sh wx</u> X 678-5	Pref. Fert. = 64% (422/660)
<u>c sh wx gl₁₅</u> X 678-5	Pref. Fert. = 40% (224/561)
<u>c sh wx</u> X 808-1	Pref. Fert. = 69% (238/343)
<u>c sh wx gl₁₅</u> X 808-1	Pref. Fert. = 53% (131/247)

From these results it was suspected that the c sh wx gl₁₅ stock was capable of preventing preferential fertilization of the egg by the sperm containing the B⁹ chromosome.

This conclusion was supported by results found in 1967. TB-9b plants were again crossed as male parents onto the c sh wx gl₁₅ stock and onto several other tester lines. In each cross a single pollen shedding from a TB-9b plant was used for pollination of the c sh wx gl₁₅ stock and one other tester line. While the progeny have not been grown up for exact calculation of preferential fertilization, it is obvious from the kernel phenotypes that preferential fertilization has been greatly decreased in crosses involving the c sh wx gl₁₅ stock. Classification of the Wx seeds in these crosses is shown below:

Female parent	TB-9b Male parent	Percentage of <u>c sh Wx</u> or <u>bz sh Wx</u> seeds
1. c sh wx	1122-2	65.4% 237/362
c sh wx gl ₁₅	1122-2	27.5% 332/1204
2. c sh wx	1119-1	66.7% 239/358
c sh wx gl ₁₅	1119-1	49.9% 124/249
3. c sh wx/c sh wx gl ₁₅	1016-1	58.0% 351/603
c sh wx gl ₁₅	1016-1	43.0% 142/332
4. c sh wx/c sh wx gl ₁₅	1013-4	64.0% 369/577
c sh wx gl ₁₅	1013-4	46.6% 194/415
5. c sh wx/c sh wx gl ₁₅	1015-2	59.0% 252/428
c sh wx gl ₁₅	1015-2	32.2% 142/441
6. yg sh bz wx	1018-2	57.5% 260/452
c sh wx gl ₁₅	1018-2	40.0% 182/456
7. yg sh bz wx	1020-2	51.0% 211/417
c sh wx gl ₁₅	1020-2	38.0% 159/418
8. yg sh bz wx	1019-1	60.5% 374/619
c sh wx gl ₁₅	1019-1	42.6% 125/300
9. sh bz wx B Pl	1017-3	66.7% 614/925
c sh wx gl ₁₅	1017-3	43.0% 260/660

The above results are consistent with the idea that preferential fertilization never occurs when the c sh wx gl₁₅ stock is used as female parent. However, an exact calculation of preferential fertilization is required to show this. In two of the crosses listed above (1 and 5) the differences in progeny data between the two female parents are so great that it is suspected that preferential fertilization of the polar nuclei by the B⁹

containing sperm has occurred on the c sh wx gl₁₅ ears. The data from crosses 3, 4, and 5, where F₁'s involving the c sh wx gl₁₅ stock are used as female parents, show that the factor responsible for suppression of preferential fertilization acts as a recessive.

The findings with the c sh wx gl₁₅ stock indicate that the ovary of the plants may be structurally or chemically different from that found in most plants. The possibility that the effect is due to abortion of specific seed types has been ruled out by ovule counts. Sectioning of the ears may reveal a difference in the ovary and at the same time give a clue to the exact mechanism of preferential fertilization.

Wayne Carlson

3. Concerning the mechanism of preferential fertilization.

Roman described preferential fertilization of the egg by B chromosome-containing sperm in 1948. He considered two possible explanations for preferential fertilization. One was that the presence of B chromosomes in a sperm cell increases the ability of the sperm to fertilize the egg. The other explanation assumes that preferential fertilization by a certain sperm cell occurs even in the absence of B chromosomes, but, when B chromosomes are present, they enter the sperm cell which has the advantage in fertilizing the egg. The latter idea is favored by Catcheside.

The explanations for preferential fertilization can be distinguished from each other by observing the nondisjunction of two B chromosomes in the same pollen grain. For example, if B chromosomes must enter a specific sperm cell in order to effect preferential fertilization, then two B chromosomes, undergoing nondisjunction simultaneously, should both migrate to the same pole. On the other hand, if preferential fertilization depends on some advantage conferred on sperm cells by the presence of B chromosomes, one expects two B chromosomes to migrate at random with respect to each other at the second pollen mitosis.

The nondisjunction of two B chromosomes, and their distribution with respect to each other, was followed genetically by combining two of Roman's A-B translocations, TB-9b and TB-4a. Cases of simultaneous nondisjunction of the B⁴ Su and the B⁹ C YG chromosomes were examined. Crosses of the following type were made: c YG/+ +, su/+ + X YC YG 9B 9C YG; 4Su 4B B⁴ Su B⁴ Su ♂. Among the progeny, Su kernels were not used, since nondisjunction of the B⁴ Su chromosome was not assured in this case. The su kernels were classified for C vs. c in the endosperm, and for YG vs. yg in the embryo. The YG c su and YG C su individuals that were found represent cases of simultaneous nondisjunction of the B⁴ and B⁹ chromosomes. The nondisjunction, in the case of the YG c su individuals, was followed by inclusion of the B⁴ and B⁹ chromosomes in the same sperm cell. In the case of the YG C su individuals, nondisjunction was followed by inclusion of the B⁴ and B⁹ chromosomes in different sperm cells. The yg C su class should occur rarely if B chromosomes are preferentially included in a specific sperm cell. The results were: YG c su = 461 YG C su = 310. The yg C su class is certainly not rare. The data are consistent with the idea that preferential fertilization

results from a competitive advantage that is conferred on the sperm cell by the B chromosome. However, the possibility that inclusion of B chromosomes in a specific sperm cell is the cause of preferential fertilization has not been entirely ruled out. Until now, this theory has been interpreted to mean that B chromosomes are almost always included in a specific sperm cell which has a certain advantage in fertilizing the egg. The "mistakes" in preferential fertilization (fertilization of the polar nuclei by the B-containing sperm) would be "mistakes" of the specific sperm cell. One can imagine, however, a situation in which a specific sperm cell fertilizes the egg 100% of the time, and "mistakes" are caused by migration of the B chromosome to the "wrong" pole at the second pollen mitosis. This theory would allow for a considerable number of yg C su individuals in the data, while still depending on the inclusion of B chromosomes in a specific sperm cell for preferential fertilization.

For this reason, preferential fertilization by B⁹-containing sperm was tested in the presence and absence of extra B chromosomes. If inclusion of the B⁹ in a specific sperm cell is all that is required for preferential fertilization, the presence of extra B chromosomes at the second pollen mitosis should have no effect upon it. However, if preferential fertilization depends on a selective advantage conferred on the sperm cell by the B⁹ chromosome, extra B's should eliminate the advantage by their presence in both sperm cells. Black Mexican plants, with and without B chromosomes, were crossed as male parents to TB-9b plants. Among the progeny, five TB-9b plants, lacking extra B chromosomes, were selected and crossed as male parents onto a yg sh bz wx stock. Five other TB-9b plants, with 6-8 extra B chromosomes, were also crossed as male parents onto a yg sh bz wx stock. The ratio of bz kernels to yg seedlings was calculated for each cross as a measure of preferential fertilization. It was found that preferential fertilization does not occur in the presence of extra B chromosomes. The data are given below:

Female parent	Male parent (TB-9b with no extra B chromosomes)*	Progeny Data		
		bz	yg	% bz
<u>yg sh bz wx</u>	1028-5	351	170	67.5%
<u>yg sh bz wx</u>	1029-1	516	310	62.5%
<u>yg sh bz wx</u>	1030-3	283	126	69.1%
<u>yg sh bz wx</u>	1156-2	337	190	64.2%
<u>yg sh bz wx</u>	1157-2	505	253	66.6%
		1992	1049	65.5%

Female parent	Male parent (TB-9b with 6-8 extra B chromosomes)*	Progeny Data		
		bz	yg	% bz
yg sh bz wx	886-2	207	212	49.4%
yg sh bz wx	886-4	223	220	50.2%
yg sh bz wx	887-2	257	251	50.8%
yg sh bz wx	887-5	344	296	53.7%
yg sh bz wx	1031-3	231	240	49.0%
		1262	1219	51.0%

*The male parent in each cross was heterozygous for TB-9b: $\frac{9^{Bz} Yg}{B^{9Bz} Yg}$

The data support the hypothesis that preferential fertilization of the egg depends on a selective advantage conferred on sperm cells by the B chromosome. The data also point out that preferential fertilization is a self-limiting mechanism for B chromosome accumulation. This is probably an important factor in determining B chromosome numbers in a natural population.

Wayne Carlson

4. Male and female transmission of B^4 in the presence of chromosome 4.

The chromosome B^4 carrying the dominant Su has been followed for a number of generations in su₁ backgrounds (see MNL 1966, 1967). Reciprocal crosses of such hyperploid genotypes were made to detect the amount of transmission of the hyperploid type through the pollen and through the egg. Self pollinations of hyperploids of the same genotype were also made, and the results are given in Table 1.

In reciprocal crosses a maximum theoretical transmission would be 50% (no loss of B^4). However, the recovery of 28.7% Su kernels when the hyperploid was the egg parent indicates the rate of loss (42.6%) which has presumably taken place during meiosis. A considerably smaller percentage of Su kernels is recovered when the hyperploid was used as the pollen parent. This indicates that the second major factor responsible for the loss of the hyperploid type is gametophyte competition: the hyperploid type is unable in some cases to successfully compete with the normal type for fertilization. Secondary factors affecting the recovery of the B^4 may be loss of the B^4 during embryo sac development or during microspore divisions.

When the hyperploid is used as the male parent, an additional 25.4% of loss is found. This establishes that even through the male most of the loss takes place during meiosis. Previous cytological observations of

microsporocytes indicated that loss of a single B^4 takes place mainly during the first meiotic division, after its failure to reach the equatorial plane. This results sometimes in laggards. However, in a number of cases (25-30%) the single B^4 divides at anaphase I, usually showing a delayed division with respect to the migration of the other chromosomes. If the migration of these divided B^4 's is successful, they will almost invariably be lost in the second meiotic division. Transmission of B^4 's is believed to occur mainly when the single B^4 passes undivided to one pole during the first meiotic division.

Sectors in the endosperm were found more often when the hyperploid was used as the pollen parent (i.e. one dose of B^4 is present in the endosperm, which is su, su, su, + B^4 Su). If loss of the B^4 occurs early in endosperm development, cells which lost the B^4 may multiply faster and the result will be an excess of the sugary fraction as occasionally was found. This is another example of competition.

After selfing hyperploid genotypes, the recovery of the Su type was in a few cases very low, and near the values found for either pollen or female transmission alone. The exceptional ears obtained are interpreted as cases of somatic loss of the B^4 chromosome and were excluded from the data reported in Table 1. Ears with all kernels of the su type were also discarded since they resulted from early loss of the B^4 in the sporophyte, or the plants did not have a B^4 as a result of heterofertilization or non-disjunction.

The results obtained after selfing hyperploid genotypes compare well with the expectation for combined transmission of the B^4 chromosome through the male and through the female.

From Table 1 the relative gamete frequencies of the hyperploid type versus the normal type can be established as 0.16 through the male and 0.29 through the female, assuming no significant abortion of hyperploid zygotes versus normal zygotes. The frequencies of the expected genotypes are given:

	♂	.840 <u>su</u>	.160 <u>su</u> + B^4 <u>Su</u>
♀			
.714 <u>su</u>		.600 (<u>su</u>)	.114 (<u>Su</u>)
.287 <u>su</u> + B^4 <u>Su</u>		.240 (<u>Su</u>)	.046 (<u>Su</u>)

These figures predict a low recovery of the genotype with two B^4 's resulting from the union of two hyperploid gametes when a plant with a single B^4 is self pollinated. The double hyperploid is expected with the frequency

of 4.6% among the progeny, or in one kernel out of 21.8 (average). After counting chromosomes in root tips, eight plants out of a total of 121 had two supernumerary B_4 's (see MNL 1967).

Among the Su kernels, one out of 8.7 (average) is expected to have two B_4 's in the embryo.

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5. Location of the E_4 esterase locus on chromosome 3.

The E_4 esterase gene in maize has five alleles. Four of these alleles (E_4^C , E_4^D , E_4^E and E_4^F) are distinguishable by the relative rates of migration in electrophoresis of the enzyme types which they produce. The fifth (E_4^N) is a null or silent allele. A description of the banding patterns exhibited by the various alleles in electrophoresis is given in Maize News Letter 40: 53-56 (1966).

A series of translocations involving chromosome 9 was used to determine the location of the E_4 locus in the maize genome. These are shown in Table 1. Each of the translocation stocks was homozygous for the waxy gene (wx/wx) which is located approximately eleven crossover units from the centromere on the short arm of chromosome 9. Each of the translocation stocks was crossed with a stock which was normal with respect to chromosome constitution and which was homozygous for non-waxy (Wx/Wx). The stocks carrying translocations T_{1-9c} , T_{6-9b} , T_{7-9a} and T_{8-9d} were crossed with plants which were E_4^F/E_4^F . The stocks carrying translocations T_{3-9c} , T_{4-9g} , T_{5-9a} and T_{9-10b} were crossed with plants which were E_4^N/E_4^N . The stock carrying translocation T_{2-9b} was crossed with plants which were E_4^D/E_4^D . The offspring obtained from this series of crosses were then crossed with stocks which were again normal with respect to chromosome constitution but which were homozygous for waxy (wx/wx). Stocks carrying translocations T_{1-9c} , T_{2-9b} , T_{3-9c} , T_{4-9g} , T_{5-9a} , T_{6-9b} , T_{7-9a} and T_{8-9d} were crossed with plants which were E_4^F/E_4^F . The stock carrying translocation T_{9-10b} was crossed with plants which were E_4^D/E_4^D .

Kernels derived from the series of crosses between the translocation heterozygotes (which were also heterozygous Wx/wx) and the stocks which were normal in chromosome constitution (and also homozygous wx/wx) were then scored for waxy and non-waxy. These kernels were then germinated and root samples from seven day seedlings were run in electrophoresis in order to score for E_4 esterase constitution. The results are shown in Table 2. As can be seen from the data, there was found to be a close linkage between the E_4 locus and the Wx locus when chromosome 9 was involved in a translocation with chromosome 3. No appreciable linkage with Wx was observed when the translocation involved any of the other chromosomes in the maize genome. These results lead to the conclusion that the E_4 gene is located on chromosome 3 rather close to the breakage point (.09 on the long arm of chromosome 3).

Table 2
Results of testcrosses made to determine the location of the E_4 gene in the maize genome

Cross		Results				Total
N/T _{1-9c}	$\frac{Wx/wx}{E_4^F/E_4^D} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	$\frac{D+F}{127} \frac{wx}{133}$	$\frac{F}{142} \frac{Wx}{142}$	$\frac{D+F}{142} \frac{Wx}{142}$	$\frac{F}{142} \frac{wx}{142}$	<u>544</u>
N/T _{3-9c}	$\frac{Wx/wx}{E_4^N/E_4^D} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	170	143	25	11	348
N/T _{5-9a}	$\frac{Wx/wx}{E_4^N/E_4^D} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	6	11	7	10	34
N/T _{6-9b}	$\frac{Wx/wx}{E_4^F/E_4^D} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	38	34	42	33	147
N/T _{7-9a}	$\frac{Wx/wx}{E_4^F/E_4^D} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	57	54	47	49	207
N/T _{8-9d}	$\frac{Wx/wx}{E_4^F/E_4^D} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	3	2	4	3	12
N/T _{2-9b}	$\frac{Wx/wx}{E_4^D/E_4^E} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	$\frac{E+F}{58} \frac{wx}{85}$	$\frac{D+F}{85} \frac{Wx}{65}$	$\frac{E+F}{65} \frac{Wx}{68}$	$\frac{D+F}{68} \frac{wx}{68}$	<u>276</u>
		$\frac{E+F}{29} \frac{wx}{47}$	$\frac{F}{34} \frac{Wx}{50}$	$\frac{E+F}{7} \frac{Wx}{7}$	$\frac{F}{7} \frac{wx}{7}$	<u>160</u>
N/T _{4-9g}	$\frac{Wx/wx}{E_4^N/E_4^E} \times N/N \frac{wx/wx}{E_4^F/E_4^F}$	29	47	34	50	160
N/T _{9-10b}	$\frac{Wx/wx}{E_4^N/E_4^F} \times N/N \frac{wx/wx}{E_4^D/E_4^D}$	$\frac{D+F}{7} \frac{wx}{11}$	$\frac{D}{11} \frac{Wx}{7}$	$\frac{D+F}{7} \frac{Wx}{7}$	$\frac{D}{7} \frac{wx}{7}$	<u>32</u>

Table 1
Translocation stocks used in crosses designed to determine the location of the \underline{E}_4 gene in the maize genome

Translocation	Breakage Points	\underline{E}_4 Esterase Genotype
T ₁ -9c	(1S.48; 9L.22)	$\underline{E}_4^D/\underline{E}_4^D$
T ₂ -9b	(2S.18; 9L.22)	$\underline{E}_4^E/\underline{E}_4^E$
T ₃ -9c	(3L.09; 9L.12)	$\underline{E}_4^D/\underline{E}_4^D$
T ₄ -9g	(4S.27; 9L.27)	$\underline{E}_4^E/\underline{E}_4^E$
T ₅ -9a	(5L.69; 9S.17)	$\underline{E}_4^D/\underline{E}_4^D$
T ₆ -9b	(6L.10; 9S.37)	$\underline{E}_4^D/\underline{E}_4^D$
T ₇ -9a	(7L.63; 9S.07)	$\underline{E}_4^D/\underline{E}_4^D$
T ₈ -9d	(8L.09; 9S.16)	$\underline{E}_4^D/\underline{E}_4^D$
T ₉ -10b	(9S.13; 10S.40)	$\underline{E}_4^F/\underline{E}_4^F$

John W. Harris

6. Association of crossing over and production of unstable a^P alleles.

The a^P -D35 allele arose in two steps from an a_1 exposed to \underline{Dt} : $a_1 \xrightarrow{\underline{Dt}} A:D2 \xrightarrow{\underline{Dt}} a^P$ -D35 (Neuffer). In the absence of \underline{Dt} , the a^P -D35 allele gives a uniformly faint aleurone color; if \underline{Dt} is present, dots of deep color are formed, as well as sectors of intermediate color, on a pale background. A stock of a^P -D35 without \underline{Dt} was obtained from Neuffer and crossed with a T₂-3- a_1 - sh_2 stock also without \underline{Dt} , obtained from Laughnan. F₁ plants of $\underline{N} \underline{a}^P$ -D35 \underline{Sh}_2 $\underline{dt} \underline{dt}$ constitution were testcrossed by \underline{N} - \underline{a} - $\underline{sh} \underline{dt}$

$\underline{T} \underline{a} \underline{sh}$
male parents and the 34 resulting ears were scanned for colored shrunken crossovers. One ear produced two \underline{sh} kernels that were pale colored. With the exception of one $\underline{A} \underline{sh}$ kernel, a possible contaminant, these were the only colored shrunken crossovers detected on the 34 testcrossed ears. Some of the pale \underline{sh} kernels are probably overlooked because of similarity to colorless \underline{sh} . The two pale \underline{sh} kernels mentioned above had fairly deep aleurone color. Both individuals proved to be heterozygous for the translocation; i.e. they arose by a double crossover in the F₁. Self pollinations of the two plants gave ears segregating pale and colorless seeds, all of which were shrunken. Many of the pale seeds had one or two very small dots of color; these are apparent on \underline{sh} seeds only after careful scrutiny, usually with a dissecting microscope. The original two kernels were not closely examined for dots, but the pale \underline{Sh} kernels on the

same ear have been inspected and no dots are present. Furthermore, a second backcross of plants arising from these pale Sh kernels has produced no dotted kernels and no obvious pale sh crossovers on 6 ears.

One of the two exceptional alleles (designated a^{p*}) has been studied further. The $\frac{T a^{p*} sh}{N a sh}$ plant arising from one of the two pale sh kernels was self

pollinated and used as male parent in a cross with $\frac{T a^s Sh}{N a sh}$ female parents.

Both crosses gave pale kernels with infrequent small dots as well as some pale kernels without dots. Backcrosses of resulting $\frac{a^{p*}}{a}$ and $\frac{a^{p*}}{a^s}$ individuals

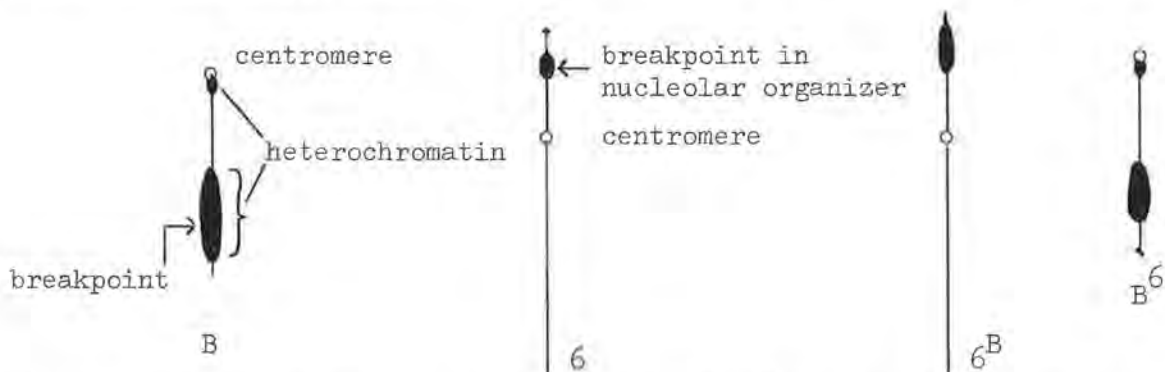
revealed that the dotting was a property of the a^p allele and not due to an independent mutator gene. The pale kernels without dots contain the same a^{p*} allele and appear to be "escapes"; the progeny of plants from such kernels includes dotted pale kernels. There is no evidence of a new Dt factor at the a^p locus since the a allele on the homologous chromosomes in the endosperm is unaffected. Kernels of $\frac{a/a/a^{p*}}$ and $\frac{a^s/a^s/a^{p*}}$ constitution contain the same low number of dots (1-3).

Tests are underway to determine whether unstable a^p alleles will arise in F_1 's of $\frac{a^p-D35/a^s}$ constitution as well as in $\frac{a^p-D35/a}$ plants. If the associated double crossover is a prerequisite for instability, some mutable component of the a allele (or the allele itself) may be included in the mutable a^p allele. If this is the case, no unstable pales should arise from F_1 's involving the a^s allele. Another cross will test whether the stable a^p can be recovered from the unstable a^p following various crossovers.

Ellen Dempsey

7. Cytological location of rgd on chromosome 6.

One of Roman's A-B translocations, TB-6a, was used in an attempt to determine the cytological location of ragged (rgd) on chromosome 6. The breakpoint in chromosome 6 is in the nucleolar organizer region (Roman and Ullstrup, Agron. J., 1951).



Hypoploid plants of the constitution $6^{By} 6^{By} B^6$ (as determined by root tip examination) were transplanted to the field in May of 1967. They were crossed as male parents with plants that were phenotypically Y Rgd. The genotype of the female parent was determined by self-pollinating the

tillers and classifying the progeny for y and rgd. Only crosses of the type Y Rgd/y rgd X $6^{By} 6^{By} B^o$ were further analyzed. Roman's method of mapping genes with A-B translocations was used. Nondisjunction of the B^o chromosome would occasionally give rise to rgd seedlings if the Rgd locus is present on the B^o chromosome. On the other hand, if the Rgd locus is located on the 6^B chromosome, rgd seedlings would not be found. A total of 1846 seedlings were classified and no rgd seedlings were found (Table 1).

Table 1

Ear	Yellow Seed		White Seed	
	+	<u>rgd</u>	+	<u>rgd</u>
1	117	0	105	0
2	110	0	113	0
3	230	0	200	0
4	139	0	166	0
5	111	0	86	0
6	104	0	91	0
7	183	0	191	0
Total	994	0	952	0

From these results it was concluded that the Rgd locus was on the 6^B chromosome.

Several translocation heterozygotes, each with a break in the organizer region of chromosome 6, were crossed as female parents with Rgd/rgd male parents. Following adjacent-1 segregation at meiosis, some of the eggs should contain a deficiency for the terminal portion of $6S$, distal to the break. Eggs of this type, when fertilized by rgd sperms, would give rise to seedlings exhibiting the recessive phenotype. The data are listed below:

Translocation heterozygote	<u>N</u>	<u>rgd</u>
T6-9 ₄₇₇₈ /N	287	0
T4-6 ₄₃₄₁ /N	732	1?
T2-6 ₅₄₁₉ /N	713	0
T6-9a/N	465	0

Only one questionable rgd seedling was observed. It was concluded from these results and those obtained with the TB-6a translocation that the rgd locus is not in the portion of 6S distal to the organizer break. However, the location of rgd in the proximal portion of the organizer was not ruled out by these tests.

Reid G. Palmer
Ellen Dempsey

8. Somatic association of homologues induced by abnormal chromosome 10.

It has been postulated that an association of homologous chromosomes is a general phenomenon found throughout the cells of the organism (Feldman et al, PNAS 56: 1192-1199, 1966), and that the intimate synapsis of homologues in meiosis is the extreme condition. Therefore, an effort was made to determine whether or not homologous maize chromosomes associate at random in mitotic cells of the root tips. Since it is known from the investigations of Rhoades and Dempsey (1966) that abnormal chromosome 10 (K10) induces more intimate pairing of the homologous chromosomes in meiotic cells, root tips of plants with and without K10 were examined.

Plants from isogenic W22 stocks carrying 0, 1, or 2 K10 chromosomes were germinated, the root tips collected, and squashes were prepared according to the Feulgen staining technique. The cells were examined to determine the distances between the homologous chromosomes 6 in all three stocks, between each 6 and each K10 in the stocks with one or two K10's, and between the homologous K10 chromosomes in the stock with two K10's. The 6's could be distinguished from the other chromosomes by the terminal satellites and the K10's by the length, extreme arm ratio and the large terminal knob. Cells which were reasonably flat and circular with all twenty chromosomes visible were selected for counting.

The distances between the chromosomes were measured with an ocular micrometer and, to minimize the differences in cell size due to differential squashing, the distance between the two chromosomes in question was divided by the distance between the two chromosomes which were furthest apart in the cell. This gave a corrected value which will henceforth be referred to as distance between the chromosomes.

In order to determine whether the chromosomes were non-randomly associated, the results of the counts were compared with a theoretical distribution. This theoretical distribution is based on the frequencies with which two points will lie at various distances from each other when randomly

distributed in a circle. It is assumed that the two homologous chromosomes, measured from centomere to centromere, can be considered as two points in a circle. According to the work of Lord (Ann. Math. Statistics 25: 794, 1954) the distance, X, between such a pair of points has the probability density function for a circle of diameter 1:

$$fX(x) = \frac{16x}{\pi} \left[\cos^{-1} x - x(1-x^2)^{1/2} \right]$$

When the distribution is plotted for distances between 0 and 1, a theoretical distribution curve is obtained which has a mean value for X of 0.4527.

Kolmogorov-Smirnov One-Sample or Two-Sample tests of goodness of fit, which take into account both the mean and the shape of the distribution curve, were used to compare the observed measurements with the theoretical distribution.

Table 1 summarizes the data obtained from counts of cells without K10, with one K10 and with two K10's.

Table 1
Mean distance between chromosomes

Class	6-6	6-K10	K10-K10
k10k10	0.439 ⁺ N = 141	-----	-----
K10k10	0.336* N = 74	0.431 ⁺ N = 113	-----
K10K10	0.315* N = 34	0.404 ⁺ N = 92	0.445 ⁺ N = 45

+ = No significant deviation from the theoretical distribution.

* = Deviation from the theoretical distribution is significant above the .01 level.

N = Number of observations.

When the association of the homologous chromosomes 6 was tested in cells from plants not containing K10, the mean distance between the homologues was 0.439. This did not deviate significantly from the expected 0.453 mean of a randomly distributed population. This indicates that during mitotic metaphase in root tip cells the homologous chromosomes are not associated.

Likewise, the mean distance between non-homologous chromosomes, specifically a chromosome 6 and a K10, did not deviate significantly from the value expected for a randomly distributed population. Though this result was anticipated, it is important inasmuch as it strengthens the assumption that

the theoretical distribution actually is a true representation of the distribution of non-associated chromosomes in squashed cells.

When K10 was present in one or two doses, the mean distances between the homologous chromosomes 6 were 0.336 and 0.315, respectively. These means deviated from the random distribution above the .01 level. The values of 0.336 and 0.315 did not differ significantly from each other. The occurrence of a non-random association of the two homologous chromosomes 6 indicates that K10 in some way has initiated or enhanced an attractive force which brings about somatic association of the homologues.

When association between the two K10 chromosomes was investigated no significant deviation from random was observed. This would indicate that the effect of K10 was interchromosomal in nature, affecting only the other homologous chromosomes in the complement. A similar interaction with non-homologous chromosomes has been reported for the effect of K10 on recombination; Rhoades has found that the increase in crossing over induced by K10 in meiotic cells was less in the K10 bivalent than in the other bivalents of the complement.

Since K10 increases the synapsis of meiotic homologues and induces a loose association in mitotic cells, it is possible that both forms of pairing are caused by a single attractive force. This would argue against the hypothesis that both long range and short range pairing forces are operative during meiosis.

Judith Miles

9. The induction of crossing over by B chromosomes.

In the 1960 Maize News Letter I reported that crossing over in the Sh-Wx region was not increased in plants homozygous for a piece of 3L inserted into the short arm of chromosome 9 despite the fact that the length of chromatin separating these flanking markers is approximately twice as great as in a normal chromosome 9. (The chromosome 9 with the inserted segment of 3L was originally designated Dp9 but we have since referred to it as Tp9 since the aberration is more accurately described as a transposition.) In the 1966 Maize News Letter the results of testcrosses of homozygous Tp9 plants heterozygous for the Yg, C, Sh and Wx loci were presented. An unusual feature of the data was the significant increase in recombination for the regions distal to the transposed segment of 3L and the complete, or nearly complete, absence of chiasma interference for double crossovers when one of the regions included the 3L piece. Extensive data from a large number of homozygous Tp9 plants showed no increase in crossing over above the control value for the C-Wx or Sh-Wx regions and the conclusion was reached that crossing over did not occur within the transposed segment of 3L. This conclusion would account for the unchanged recombination in the Sh-Wx region in Tp9 Tp9 and N9 N9 bivalents. Also intelligible are the high coincidence values for those double exchanges where one of the crossover regions is the Sh-Wx interval. The great majority of the exchanges in the Sh-Wx region occur to the right of the inserted piece. Although there is apparently no recombination

in the transposed segment, its presence decreases the interference distance for double exchanges involving the Sh-Wx region and an exchange in this interval does not affect the probability of a second exchange occurring in the adjacent C-Sh region. Interference is normally high for short adjacent regions in the short arm of chromosome 9 and decreases as the regions become longer. The transposed segment of 3L does not undergo crossing over but its physical presence in the Sh-Wx interval has the same effect in reducing interference as that achieved by increasing map distance.

The Df3 chromosome is shorter than the normal 3 by the deleted segment that was inserted into chromosome 9. Since the Lg₂ and A genes lie to the left and right of the deficiency, they are separated by considerably less chromatin and the amount of recombination between these two markers should be reduced in Df3 homozygotes. However, in testcrosses of Tp9 Tp9; G1 Lg Df A/g1 lg Df a plants the percentage of recombination in the physically shorter Lg-A region was as great as in normal chromosome 3 homozygotes. Evidently no exchanges occur in the chromatin of the transposed segment when it is part of chromosome 3 or when placed in the Tp9 chromosome. This segment is not genetically inert since N9 Df3 spores abort and its immunity to genetic exchange, when in the Tp9 chromosome, is lost in the presence of supernumerary B chromosomes as is described below.

Subsequent to the extensive early experiments with Tp9 Tp9 plants where there was no change in C-Wx or Sh-Wx recombination values, segregating families were later encountered in which some individuals had the usual amount of recombination and others had two to three times as much. The genetic basis for high recombination, which has been followed for several generations, proved to be the presence of one or more supernumerary B chromosomes. Sib plants of C Tp wx/ c Tp Wx constitution with B's varying in number from none to three were testcrossed. As is shown in the tabulation below the average amount of recombination between C and Wx in sib plants of family 28064 was 17.7% in 0B, 37.0% in 1B, 40.4% in 2B, and 42.0% in 3B plants.

C-Wx Recombination in Plants with:

<u>0 B</u>	<u>1 B</u>	<u>2 B</u>	<u>3 B</u>
13.3%	40.7%	42.6%	42.5%
15.1	36.0	38.1	43.1
15.0	32.5	44.4	44.6
18.0	40.4	43.7	38.6
20.0		38.4	
21.4		36.4	
21.3			
14.4			
16.6			
Wt. M = 17.7	Wt. M = 37.0	Wt. M = 40.4	Wt. M = 42.0

Given below are C-Wx crossover values from a related family (28062):

<u>0 B</u>	<u>1 B</u>	<u>2 B</u>	<u>3 B</u>
11.9%	24.8%	26.7%	40.2%
23.0	22.4	36.9	40.4
10.9	31.8		
17.2	25.0		
16.6	30.0		
19.6	24.0		
19.3	32.3		
13.0			
14.6			
20.2			
16.1			
<hr/>			
Wt. M = 16.7	Wt. M = 27.4	Wt. M = 30.6	Wt. M = 40.3

The increase in recombination in B-bearing plants compared to OB plants is not as striking as in the first experiment but the enhancement is highly significant and the data from the two sets of crosses are in agreement.

In contrast to the above two families in which the number of B's varied from none to three is the situation in family 28878 where all the plants had high C-Wx crossover values and one or more B chromosomes. These plants were homozygous for the Tp9 chromosome. The data given below likewise show a consistent dosage effect of B chromosomes, but the increase in recombination is less pronounced than that observed in comparisons of OB and 1B sibs in the first two sets of data.

<u>1 B</u>	<u>2 B</u>	<u>3 B</u>
30.1%	35.5%	43.0%
	40.0	42.6
	41.0	40.3
	38.0	
	38.0	
	39.2	
	40.5	
<hr/>		
Wt. M = 30.1	Wt. M = 38.9	Wt. M = 42.0

Although there is variation in the amount of C-Wx recombination within and between the different classes of B chromosomes in the three families, the data were combined to determine the average increase produced by specific numbers of B chromosomes.

0 B	20 ears	M = 16.9	Population total = 5471
1 B	12 ears	M = 30.8	Population total = 3287
2 B	15 ears	M = 38.8	Population total = 4419
3 B	9 ears	M = 41.7	Population total = 2456

Before it was realized that B chromosomes were responsible for the enhanced C-Wx recombination values, testcrosses were obtained from homozygous Tp9 plants heterozygous for Yg, C and Wx. Some individuals had low C-Wx and some had high C-Wx recombination. Although the number of B chromosomes was not ascertained in these plants, they came from a duplicate planting in another season of the kernels giving rise to the populations reported above in the first set of data (family 28064). It is clear that the ears with low C-Wx and those with high C-Wx recombination values were borne on plants with 0B and from 1-3 B chromosomes, respectively. When the kernels were planted and seedlings scored for Yg, the following data were obtained.

Per Cent Recombination

	<u>Yg-C</u>	<u>C-Wx</u>	<u>Total</u>	<u>Coin.</u>
Low <u>C-Wx</u>	28.8	13.0	41.8	0.65
High <u>C-Wx</u>	12.7	37.9	50.6	0.57

The average of 38% C-Wx recombination in those individuals assumed to carry from one to three B's is in good agreement with the recombination frequencies for sib plants with known numbers of B chromosomes. The most striking feature in the 3-point data is the negative correlation between crossover values in the Yg-C and C-Wx regions. Plants with high C-Wx crossing over (M = 38%) have low Yg-C values (M = 13%) and conversely those with low C-Wx recombination (M = 13%) have high Yg-C crossing over (M = 29%). A linear regression coefficient of -1.426 was obtained by plotting C-Wx against Yg-C recombination values. The failure to find individuals with high crossover percentages in both regions is indicative of an upper limit to the amount of recombination in the short arm of chromosome 9.

The plants in family 28878 were heterozygous for Yg, C and Wx. Recombination frequencies obtained from the 3-point testcross data are given below:

No. of B Chromosomes	<u>Yg-C</u>	<u>C-Wx</u>	<u>Population Size</u>	<u>Total Recombination</u>
1	15.6	30.1	379	45.7
2	9.6	35.5	271	
2	13.7	40.0	488	
2	10.4	41.0	424	
2	15.8	38.0	158	
2	16.8	38.0	297	
2	10.1	39.2	337	
2	6.1	40.5	412	
Wt. M =	11.8	38.9	$\Sigma = 2387$	
3	9.7	43.0	330	52.4
3	10.1	42.6	286	
3	11.4	40.3	464	
Wt. M =	10.4	42.0	$\Sigma = 1080$	

These data confirm the conclusions reached above on the negative correlation between crossover percentages in the two regions and on the dosage effect of additional B chromosomes.

M. M. Rhoades

10. A molecular basis for heterosis.

Recent studies on the kinetic properties of alcohol dehydrogenase isozymes in corn scutella have revealed that the enzyme forms specified by the $Adh^{C(m)}$ and Adh^S alleles are strikingly different. For example, the C^m type isozyme found in $Adh^{C(m)}$ homozygotes shows optimal activity at pH 10.5 and a 10 fold reduction in activity at pH 8.0. On the other hand the S type isozyme formed in Adh^S homozygotes is most active around pH 8.0 and is completely inactive at pH 10.5. $Adh^S/Adh^{C(m)}$ heterozygotes which form both the S and C^m type isozymes show high activity at both pH levels as expected (Table 1). The striking difference between the isozymes is quite surprising in view of the fact that they are specified by allelic genes and do not show preferential dimerization.

Table 1
Units activity/gram kernel

	: pH 8.0	: pH 10.5
Adh^S/Adh^S	: 5898	: 0
$Adh^S/Adh^{C(m)}$: 3399	: 3779
$Adh^{C(m)}/Adh^{C(m)}$: 519	: 5702

The alcohol dehydrogenase system can serve as a model for explaining the phenomenon of hybrid vigor although we have no reason to believe that this particular enzyme is implicated in heterosis. We propose that the intracellular milieu such as pH, ionic strength, chemical composition, etc. is not constant and may vary significantly during growth. Furthermore, we propose that enzymes specified by various alleles of the same gene may have different optima for activity. The enzyme specified by one allele may be active in one environment but relatively inactive in a second, while another allelic enzyme may show the reverse relationship. Heterozygotes which contain both alleles would produce enzymes which are active in either environment. This would be expected to result in hybrid vigor since in such heterozygotes the range of intracellular conditions in which high enzyme activity persists is considerably broadened.

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1. Anthocyanin suppressors in the aleurone.

In each of the Mexican varieties, Maiz Chapalote (mc) and Zapalote chico (zc), a suppressor of aleurone anthocyanin has been found. Both are allelic to the standard \underline{C}^I allele that is present in stocks distributed by the Maize Cooperative, and each is given an allelic designation, $\underline{C}^{I(mc)}$ and $\underline{C}^{I(zc)}$. In tests of their color suppressing potency, each is distinguishable.

Using a W-22 color converted stock as female parent and the suppressor stocks as male, the potency of each of the alleles in suppressing color can be graded on a scale where 1 = colorless and 10 = full color.

\underline{C}^I expressed the highest potency with a grade of 4.2; $\underline{C}^{I(mc)} = 6.2$ and $\underline{C}^{I(zc)} = 7.0$.

Results of similar tests utilizing the color suppressor stocks as female parents, though showing a greater overall suppression of color, correlate with the ratings observed when the suppressor alleles are transmitted by the male parents.

In additional tests using other color lines to test the potency of each of the alleles, the three alleles were ranked in a similar manner; $\underline{C}^{I(zc)}$ always shows the least capacity to suppress color.

The expression of these alleles is in some ways similar to the color suppression reported by Mouli and Notani (MGCNL 41).

O. Leleji
 Peter A. Peterson

2. Mutation and En transposition.

En (Enhancer) has been identified at \underline{a}_1^m (pale and purple) an allele at the \underline{a}_1 locus. This unstable allele mutates at a high rate to purple as well as to stable colorless and pale types, which fall into a graded series. A large number of the stable derivatives have been tested and each was found to contain En but at a site apart from the original allele, although on the same chromosome and therefore linked with \underline{a}_1 (MGCNL 39: 102-103). Utilizing 2-point tests, En has been located in both distal and proximal positions with regard to the original site. Thus for this allele, mutation (in this case to $\underline{a}_1^{m(nr)}$ -non-responding to En) appears to be coincident with En transposition.

Peter A. Peterson

3. Amylases in Sh_1 and sh_1 .

The hypothesis that the phenotypic hollowness observed in $sh_1 sh_1 sh_1$ kernels is due to the action of a starch digesting amylase that is not present in the full-kernel $Sh_1 Sh_1 Sh_1$ types was investigated. In developing endosperm of maize kernels that had 0, 1, 2, and 3 doses of the Sh_1 allele, no differences were observed in amylolytic activity. Thus, the Sh_1 protein band observed in disc-gel electrophoresis of endosperm proteins and absent in sh_1 tissue is not associated with amylase activity.

Amylase is active in developing normal and sh_1 endosperm from twelve to thirty-six days after pollination. The level of the amylolytic activity decreases slightly as the kernels mature as seen in the table. Amylase activities were measured by the decrease in iodine blue color of starch.

Amylase Activities of Maize Endosperm

Dose of Sh_1 Gene	0	1	2	3
Genotype	$sh_1 sh_1 sh_1$	$Sh_1 sh_1 sh_1$	$Sh_1 Sh_1 sh_1$	$Sh_1 Sh_1 Sh_1$
Phenotype	<u>shrunken</u>	<u>non-shrunken</u>		
Specific Activity (relative units/mg protein)				
13-day	8.5	8.5	8.5	9.3
18-day	8.3	8.1	7.6	6.8
24-day	6.2	6.6	5.5	6.7
30-day	6.3	6.3	5.0	6.0
36-day	5.0	4.0	6.5	5.3

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1. Genetic and biochemical studies of chlorophyll deficient mutants.

For the past few years we have been accumulating mutants that are defective in the chlorophyll but which might have near normal carotenoid synthesis. Mutants of this type would be expected to have a luteus phenotype, but we have also included pale yellow, yellow-green as well as some near albino types in this study. If the mutant had not been previously located to chromosome we attempted to do this and for most of

Table 1
Phenotype, chromosome location and chlorophyll, carotene, and xanthophyll concentrations for 23 chlorophyll deficient mutants grown at 80°F. and under 2,000 foot candles of light

Mutant	Phenotype	Chromo- some	Chlorophyll mg/gm	Carotene mg/gm	Xanthophyll mg/gm
normal	green	-	1.674	.0980	.1140
$\frac{1}{3}$	almost albino	-	0	.0015	.0055
$\frac{1}{4}$	very slight yellow	10	trace	trace	.0066
$\frac{1}{6}$	yellow-green	9?	-	-	-
$\frac{1}{7}$	pale yellow- green	9	.3220	.0077	.0395
$\frac{1}{10}$	good yellow	6	trace	.0029	.0140
$\frac{1}{1106}$	yellow, leaf tips slightly green	4	.0992	.0019	.0187
$\frac{1}{4106}$	light yellow- green	-	.0781	.0028	.0103
$\frac{1}{4117}$	yellow-green	-	.1824	.0051	.0335
$\frac{1}{4120}$	yellow, leaf tips green	6	.0935	.0021	.0175
$\frac{1}{4920}$	pale yellow, leaf tips green	-	.0486	.0021	.0117
$\frac{1}{4923}$	dark yellow, some green	-	.2667	.0205	.0350
<u>py</u> PI 177593	pale yellow, leaf tips green	4	.1914	.0059	.0165
<u>ye</u> PI 183367	yellow with some green	-	.2380	.0070	.0375
yel nec PI 217486	yellow, necrotic leaf tips	8	.1639	.0128	.0369
$\frac{1}{\text{Blandy \#1}}$	yellow, leaf tips green	-	-	-	-
$\frac{1}{\text{Blandy \#2}}$	yellow	-	-	-	-
$\frac{1}{\text{Blandy \#3}}$	yellow with some green	6	.3115	.0176	.0432
$\frac{1}{\text{Blandy \#4}}$	good yellow	-	.0055	.0240	.0195
$\frac{1}{\text{Brawn \#1}}$	yellow-green	6	.5939	.0222	.0800
yellow dwarf	yellow-dwarf	3	.0140	.0015	.0070
$\frac{w}{1}$	very pale yellow	6	.0180	.0008	.0091
$\frac{w}{\text{IT. \#1}}$	very pale yellow	-	.0140	.0000	.0030
$\frac{w}{8896}$	pale yellow	6	trace	.0006	.0090

them we determined chlorophyll, carotene, and xanthophyll concentrations. For the pigment analysis, the mutants were grown at 80° F. and 2,000 foot candles of light. Table 1 summarizes the results of these studies.

It is obvious from the results reported in Table 1 that most of the mutants make some chlorophyll although they all fall far short of that made by the normals. Five of them, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{10}$, $\frac{1}{\text{Blandy \#4}}$, and $\frac{1}{\text{w8896}}$, are very deficient in chlorophyll formation and come the closest to being true chlorophyll mutants. It is equally obvious that although some of the mutants looked quite yellow none of them approximate the level of carotenoid observed in the normal. The low levels of carotenoids could be due to pigment bleaching in the bright light.

Six of the mutants in Table 1 were grown in the dark and checked for their ability to make protochlorophyllide and to convert it to chlorophyllide and chlorophyll. These tests were run by using whole leaves in a reflectance attachment on a Bausch and Lomb 505 recording spectrophotometer. Protochlorophyllide was indicated by the presence of a peak at 630 m μ in a dark grown leaf. A peak at about 684 m μ after the leaves were exposed to one minute of light indicated that chlorophyllide was formed. After one hour in the dark the peak shifts to approximately 673 m μ , which is thought by some to be the result of phytylation of the chlorophyllide, producing chlorophyll. Table 2 summarizes the results of these studies. Grown under dark conditions $\frac{1}{\text{Blandy \#4}}$ evidently does not make chlorophyll or its precursors and $\frac{1}{3}$ and $\frac{1}{\text{Blandy \#2}}$ make only a trace. The other three mutants make significant amounts of these pigments, although quantitative values cannot be determined from these tests. Mutants which showed an ability to synthesize some chlorophyll in the light (Table 1) also seem to be able to synthesize it in the dark. Mutants with marked chlorophyll deficiency when grown in the light produce little or no chlorophyll precursors in the dark. In this regard they differ from normal plants, the white-albino mutants, and others of the luteus type mutants.

Table 2
Protochlorophyllide, chlorophyllide, and chlorophyll production as measured
in vitro in six "chlorophyll" mutants

Mutant	Protochlorophyllide peak (484 mu)	Chlorophyllide peak (684 mu)*	Chlorophyll**
<u>l</u> ₃	trace	trace	trace
<u>l</u> ₄₉₂₀	trace	+	+
<u>l</u> -Blandy #2	+	+	+
<u>l</u> -Blandy #3	+	+	+
<u>l</u> -Blandy #4	0	0	0
<u>w</u> ₈₈₉₆	trace	trace	trace

*684 mu is an average value. In different samples the peak might vary two or three mu from this value.

**As measured by a shift in the spectrum peak from the 684 mu value to a peak of shorter wave length.

Donald S. Robertson
I. C. Anderson

2. Metabolic block in porphyrin synthesis.

The seedling mutant w₈₈₉₆ forms at most only a trace of protochlorophyll when grown in the dark. It also has a reduced amount of carotenoids in the present genetic background (see accompanying report). This pigment relationship is the opposite of that found in the group of albino mutants which we have been investigating. In these latter mutants the formation of protochlorophyll, chlorophyllide, etc., is normal but there are metabolic blocks in carotenoid biosynthesis.

The enzyme catalase is a porphyrin enzyme so it was of interest to measure the amount of this enzyme and compare the results with the findings obtained with the porphyrin chlorophyll. When dark or dim light grown leaves of w₈₈₉₆ were ground and assayed they were found to contain about one-third the catalase of normal leaves. When the extract was centrifuged there was obtained a chloroplast pellet and a chloroplast free supernatant. No catalase was found in the chloroplast pellet of w₈₈₉₆. All of the catalase of the mutant was in the supernatant. In the normal about two-thirds of the catalase activity was in the chloroplast and one-third in the supernatant fraction.

These results indicate that in the normal plants there are probably two pathways for the biosynthesis of porphyrin compounds, one located in the plastid and the other in the cytoplasm. The plastid system would seem to be responsible for synthesizing prophyrin for both catalase and chlorophyll. The mutant gene w_{8896} evidently interrupts this pathway before the biosynthesis of these two compounds diverge. The w_{8896} locus does not seem to be involved in the cytoplasmic pathway for porphyrin synthesis.

I. C. Anderson
Donald S. Robertson

3. Increased crossing over in chromosome 5 in the presence of abnormal 10.

In the 1966 Maize Cooperation News Letter, (pp. 65-69) data were presented indicating a map distance of 5.05 between a_2 and bt_1 . To determine if crossing over could be increased in this region, abnormal 10(K10) was incorporated in our stocks. Rhoades and Dempsey and Kikudome have reported that the incorporation of abnormal 10 in a stock will increase crossing over in chromosomes other than 10. In the testcross $a_2 a_2 \frac{bt_1}{bt_1}$ x $\frac{A}{a} \frac{+}{bt} \frac{K10}{+}$ there were observed 103 A_2-bt_1 seeds and 113 $a_2 +$ seeds out of a total of 1,125, to give an a_2-bt_1 distance of 19.2. This amounts to approximately a four-fold increase in our previously observed level of crossing over in this region.

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1. Biochemical and breeding aspects of opaque-2.

Experiments have been conducted with the opaque-2 gene in view of breeding problems. Preliminary results lead to the following conclusions:

- (a) The yield of the o_2 plant is lower than its normal counterpart.
- (b) The lower yield is mainly due to a collapse of the kernel, while the o_2 plant seems wholly normal.
- (c) The difference in yield can be evaluated from the difference between normal and o_2 kernels on the same ear provided by a heterozygous plant.

A group of 13 lines has been crossed to an opaque line. The hybrids have been selfed and crossed according to a diallel cross system. The results of selfing the hybrids are reported in the table.

The opaque kernels show a slightly higher total protein content (+ 0.33%). The yield reduction varies from 6.9 to 16.3%. The lysine content ranges from 3.29 to 4.26%. A positive correlation exists for the content in lysine, histidine, arginine, aspartic acid, and glycine.

The total protein content, the weight decrease and the lysine content are not significantly correlated.

Table 1
Some Characters in Opaque-2 Kernels

Background of the selfed hybrid	Total protein content (%)		% Weight reduction between normal and opaque-2 seeds	% Lysine content in $\frac{o_2}{2}$ seeds	% Histidine content in $\frac{o_2}{2}$ seeds	% Arginine content in $\frac{o_2}{2}$ seeds	% Aspartic acid content in $\frac{o_2}{2}$ seeds	% Glycine content in $\frac{o_2}{2}$ seeds
	normal	opaque-2						
W 153 x $\frac{o_2}{2}$	9.30	9.32	12.7	3.61	2.56	5.40	8.66	4.14
W 22 x $\frac{o_2}{2}$	12.66	12.93	12.9	3.76	3.07	6.00	9.52	4.56
R 3b x $\frac{o_2}{2}$	11.03	12.09	10.2	3.29	2.67	5.53	8.46	4.27
W 75 x $\frac{o_2}{2}$	10.10	10.88	13.4	3.87	2.66	5.95	8.36	4.79
M 14A x $\frac{o_2}{2}$	10.06	10.13	7.4	3.45	2.76	5.85	9.79	4.93
W 3c x $\frac{o_2}{2}$	9.46	9.71	16.3	3.84	3.12	6.00	9.28	4.64
W 324 x $\frac{o_2}{2}$	9.53	9.18	7.8	3.93	2.83	5.79	9.49	4.81
W 64A x $\frac{o_2}{2}$	11.48	11.58	13.9	4.11	3.02	6.40	10.46	4.68
W 374R x $\frac{o_2}{2}$	9.18	9.35	15.0	4.26	3.29	6.65	8.90	4.35
Se1 224 x $\frac{o_2}{2}$	10.56	10.48	8.3	3.94	2.79	6.01	8.80	4.31
W 187d x $\frac{o_2}{2}$	9.07	10.60	6.9	4.11	2.83	5.74	9.13	4.57
OH 43 x $\frac{o_2}{2}$	8.00	8.06	11.1	3.54	2.88	5.59	7.95	4.55
A 158 x $\frac{o_2}{2}$	10.32	10.30	9.2	3.81	2.96	6.03	8.04	4.74

The diallel system has provided data on the heritability of the difference between the kernel weight in the normal and opaque-2 phenotypes. A large fraction of the genetical variance is of the additive type. Significant are also the components related to dominance and to maternal effect.

The main conclusions are as follows:

- (a) the lack of phenotypic effect of the o_2 gene on the plant suggests the use of heterozygous plants in selection, which permits the evaluation of weight decrease in o_2 ;
- (b) the selection for a negligible difference between o_2 and normal kernels is expected to be successful on the basis of the statistical significance of the additive component of heritability;
- (c) the variability in lysine content of o_2 kernels justifies the selection for a better expression of the character;
- (d) the simultaneous selection for o_2 kernel weight increase, total protein content, and lysine content appears feasible.

T. Ekpenyong
F. Salamini

2. Further data on an unstable factor affecting anther and aleurone color.

The data obtained recently on the system reported in the 1967 M.N.L. (100-101) permit us to present the following conclusions:

- (1) A factor appears to affect both anther and aleurone tissues; in fact the color pattern in the anther corresponds perfectly with that of the aleurone.
- (2) The segregation data suggest that the instability of the color gene is controlled by an Activator factor. The latter is linked to chromosome 9 markers (sh, wx). When the Activator is absent the phenotype produced by the color gene is pale bronze. Such a gene is not allelic to any of the following factors: A_1 , C_1 , C_2 , R , Bz . The only indication of linkage is with chromosome 1 markers; consequently we suspect that we are dealing with a bz_2 allele.
- (3) A Spm test has been carried out with plants exhibiting the typical anther and aleurone pattern of instability. The wx pollen from these plants on a wx^{m-8} tester produced typical Wx patches in the kernels.
- (4) The activator shows dosage effect. Two or three doses of the factor delay the formation of the colored spots which, in such cases, appear very small.

A. Bianchi
F. Salamini

3. The knob endowment of selected lines of maize.

A number of standard inbred lines have been studied as to knob endowment as appears from the following table:

Table 1
Knob Constitution of American Inbred Lines

Line denomination	Spreading index (1)	Position of each knob (K) or large chromomere (C) (2)									Type of nucleolar organizer (3)	
		1S	2S	2L	3L	4L	5L	6L	7L	8L		9S
B 14	3					K			K		C	1
M 14	3					K	K	Kd	K		C	1
WM 13 R	3					K	K	Cd	K	K	C	2
C 103	2										C	1
38-11	1	K	K?				K?	2c	K			1
W 75	2									K		1
WF 9	1					K	K	Cp	K	K		1
A 158	2					K	K	Cp	K	K		2
OH 07	3			C			K		K		C	2
OH 41	1		K			K	K		K		C	2
W 15	2							Cd			C	2
W 79A	4				K	C	C	Cd	K	K		1
W 9	2	C?			K	K	C		K	K	C	2
W 37A	3	C				K		Cd	K	K		2
W 64A	2			K		K		Cp	K	K		2
B 2	3						C	K	K	K		1
W R 3	4					K	K	2c	K	K	C	1
33-16	4					K						2

- (1) Spreading indexes are those used by Wellwood and Randolph: 0-1-2-3-4; 0 stands for extremely entangled chromosomes, 4 stands for the best spreading, and 1-2-3 for increasing degrees of spreading.
- (2) K stands for reasonably large knob; C indicates a consistently prominent chromomere. In the long arm of chromosome 6 no case has been found of a knob in its median region: the C cases reported refer to the proximal and distal chromomeres.
- (3) The different organizer types were recognized by McClintock (1934) and are distinguishable according to the portion of the elongated organizer which develops the nucleolus. Type 1 refers to the distal portion, type 2 to the median, and type 3 shows the greatest nucleolar organizing activity nearest the proximal end of the organizer.

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1. Linkage relationships for two mutants detected in Italian populations.

Further investigations have been accomplished on linkage relationships of two mutants, described in 1967 MNL, with known genetic markers.

For the ij-type mutant, F_2 segregations (repulsion phase) presented the following data (inclusive of 1966 results):

<u>G1</u> ₁	<u>Ij</u>	<u>gl</u> ₁	<u>Ij</u>	<u>G1</u> ₁	<u>ij</u>	<u>gl</u> ₁	<u>ij</u>
3882		2037		1889		8	

(c.o. 6.5% \pm 1.5 st. error).

The data previously reported about close linkage between a shrunken type (bt) mutant and su₁, have been confirmed by the scoring of ears obtained from backcrossing, to the triple recessive, plants of the constitution Su₁ bt G1₃ / su₁ Bt gl₃, as follows:

<u>Su</u> ₁ <u>Bt</u>	<u>su</u> ₁ <u>Bt</u>	<u>Su</u> ₁ <u>bt</u>	<u>su</u> ₁ <u>bt</u>
113	4124	4157	20

All the seedlings from the su₁ bt kernels had the G1 phenotype, while only 26 plants from Su₁ Bt seeds turned out to be gl, indicating that part of them derived from contamination. Consequently, considering the bt phenotypes only, the su-bt recombination is 0.5% \pm 0.1.

The bt mutant, then, has to be placed on chromosome 4 (probably allelic to bt₂), between su₁ and gl₃ and very close to su₁.

C. Lorenzoni
M. Pozzi

2. Abnormal segregations (significantly different from a 1:3 ratio) of genetic markers in the F_2 of lines derived from Italian populations.

In the analysis of a number of F_2 progenies derived from crossing lines from Italian populations to some F_2 genetic testers bearing recessive mutants, the following abnormal segregations have been observed:

Marker	Chromosome	Number of examined F ₂ progenies	Number of Italian populations	Segregations	
				>3:1	<3:1
<u>lg</u> ₁	2	80	74	4	1
<u>sh</u> ₂	3	8	8	0	0
<u>su</u> ₁	4	91	85	4	1
<u>bt</u> ₁	5	9	8	9	0
<u>y</u>	6	75	71	4	0
<u>su</u> ₂	6	2	2	0	0
<u>gl</u> ₁	7	67	63	2	0
<u>wx</u>	9	88	82	2	4

The mean number of ears examined per F₂ is about 7 for kernel markers and 4 for seedling characters.

Abnormal segregations can be, at least partially, interpreted as a consequence of the presence of gametophyte factors. The deviations for the markers of chromosomes 4, 5 and 9 could be attributed to the ga factors known for such chromosomes.

C. Lorenzoni
M. Pozzi

3. Somatic segregation in plants from X-ray and Ethyl-methane-sulphonate (EMS) seed treatments.

In plants derived from seed of a multi-ear popcorn variety treated with X-rays and EMS, the number and relative position of ears segregating mutants have been reported:

Treatment	Number of plants examined	Ears per plant (mean)	Plants with segregating ears				
			A	B	C	D	E
0	209	4.0	-	-	-	-	-
XR 5000 r	154	3.6	-	1	1	2	-
EMS 0.8%, 12h	70	3.6	3	1	-	1	2
1.0%	69	3.0	-	-	-	-	1
1.2%	21	3.0	-	-	1	-	-
1.4%	157	3.1	5	-	2	1	6

- A. One ear per plant
 B. Two ears at successive nodes
 C. Two ears at alternate nodes
 D. All ears
 E. Not classified, being plants with 2 ears, one of which is segregating, or with 3 ears and segregation at the second of them.

The presence of mutations was detected through pollination by the TB-9b translocation line. Consequently, the reported data refer only to mutants located on the distal part of chromosome 9.

The mutants have been observed at the seedling stage (chlorophyll deficiencies, abnormal growth, dwarfism).

C. Lorenzoni

4. Further data on location of a ga factor in chromosome 9.

Self-pollination of plants heterozygous for gametophyte factors and genetic markers on chromosome 9 yielded the following data in 1967:

Linkage phase	Genetic factors							
	<u>W</u>		<u>Wx</u>		<u>Sh</u>		<u>C</u>	
	Total No. of kernels	% of <u>w</u>	Total No. of kernels	% of <u>wx</u>	Total No. of kernels	% of <u>sh</u>	Total No. of kernels	% of <u>c</u>
C	4637	2.2	8736	4.0	533	9.9	533	37.0
R	-	-	10168	40.2	4709	38.8	4709	14.5

The rate of the recessive markers in F_2 's homozygous for gametophyte factors in our stocks was as follows: $w = 25.4\%$; $wx = 22.9$; $sh = 24.5$; $c = 25.1$. If we assume that the ga pollen has a null or almost null functioning in heterozygotes (as suggested by various considerations), the "non-Mendelian" percentages of the markers may be a dependable indication of the crossover per cent between the gametophyte factor and the marker itself, with the exception of wx . Here, actually, the percentage in the C-phase should be increased to compensate for the reduced transmission of the wx gamete, but decreased because part of the ears showing zero wx kernels are known to be nevertheless $Ga Wx/ga wx$. The two factors practically neutralize each other. In the case of R-phase the wx per cent should be $40.2 \times 25/22.9 = 43.9$.

Consequently the crossover distance obtainable from the data reported above may be inferred as follows:

C-phase	R-phase
$w - ga: 4.4$	-
$wx - ga: 8.0$	$Wx - ga: 12.2$
$sh - ga: 19.8$	$Sh - ga: 22.4$
$c - ga: 26.0$	$C - ga: 29.0$

The values obtained with the coupling phase are lower than those derived from the repulsion phase. Whereas there is no explanation for this fact, if not due only to chance, some cytological observations made by M. Vetturini did not reveal the presence of any abnormality.

From the calculations reported, the linkage map of the genetic factors of chromosome 9 appears now as follows:

C 6 Sh 21 Gag 5 W 4 Wx

(Two estimates of the crossover rate between Wx and W in 1967 were 4.1 and 3.4).

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1. Early sweet-dent hybrids for summer food.

Twenty years ago Singleton, Jones and Everett described a new type of corn, sweet-dent silage, which was developed at the New Haven Station in Connecticut. Their studies showed that it was higher in animal feeding value than regular silage. Furthermore, some farmers reported that cows

preferred the sweet-dent silage to straight field corn silage.

In recent years we have used inbred CO-106 in some nutritional studies and during this period a curiosity developed to determine its potential when combined with certain sweet corn inbreds. CO-106 is a very early inbred and combinations were tested with a few early sweet corn lines such as Ma 21547, C5NT, and Ma 51.408.

Some of these hybrids and particularly CO-106 x Ma 21547 have demonstrated a remarkable degree of vigor considering the season of maturity. From plantings on May 11, the stalks develop to a height of about seven feet and the relatively large ears have developed to the dough stage during the latter part of July during the past two years. The plant habit allows for very close planting with subsequent high plant population.

This preliminary investigation dealing with the feasibility of early sweet-dent hybrids indicates they would not be competitive for silage but do give promise as a source of "summer-food" when many farmers are looking for nutritive and palatable forage for dairy cows.

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1. String cob maize.

Grobman et al (1961) described the cob (rachis) of the tiny-eared, primitive Peruvian race, Confite Morocho, as having small, shallow cupules and as being no thicker than the rachis of the tassel. We have named this feature "string-cob" and we have transferred it to a sweet corn inbred which is designated Sc 51 because of its relationship to Purdue 51. The string-cob ears in their new background are longer than their normal P51 counterparts, uniquely slender and above average in tenderness and flavor.

This evolutionary retreat to recover the string cob condition may have certain economic advantages in modern sweet corn. The string cob ear is ideal for whole ear canning and freezing, the cob is easily disposed in a garbage grinder and the ear is more dainty for eating on the cob.

W. C. Galinat

2. Inheritance of string cob.

After the string cob feature was transferred to a sweet corn inbred (Sc 51) and then outcrossed to three other inbreds, its expression was

found to be controlled by two incompletely dominant genes, as indicated by F_2 ratios of 1:14:1. In some cases the phenotypic effects of these genes are measured best in terms of rachis diameters (cob minus glumes) and in other cases rachis internode length is the more important criterion. In the F_2 segregation from self pollinating a hybrid between a string cob inbred and the sweet corn inbred Ia 5125, the distribution of rachis diameters was trimodal with a good fit to the 1:14:1 ratio. The fit was less distinct for rachis diameters with G29 and not at all apparent with Wilburs Flint. The reverse was true regarding rachis internode length. Only Wilburs Flint yielded a trimodal distribution for rachis internode length fitting a 1:14:1 ratio. The segregation involving Wilburs Flint was homozygous for the eight-rowed condition.

Crosses with the collection of A-B translocation testers yielded a few extreme string-type specimens in hybrids with TB4S and TB9S which indicates that the two major genes involved may be located on the short arms of these two chromosomes. Other studies indicate that teosinte also has loci affecting rachis internode length on these chromosomes.

W. C. Galinat

3. Non-cupulate pistillate rachises in maize.

The F_2 segregation of Iowa 5125 x string cob yielded a few pistillate rachises which were like the staminate rachises in being barren of cupules. This condition, apparently not previously observed in maize, may result from a recombination of parental factors for small cupules.

The small cupules of the string cob inbred stem from their vestigial nature in their "pure-maize" source, Confite Morocho.

The small cupules of 5125 may also be associated with pure maize germ-plasm or, at least, from a low-level of teosinte introgression. Because 5125 is fasciated and because teosinte introgression is known to reduce or eliminate fasciation (Galinat, MNL 37:35-36. 1963), this inbred is presumed to have a low-level of such introgression. While cupules appear to be rudimentary in maize, they do have a function in the formation of the cupulate fruit case of maize's close relatives, teosinte and *Tripsacum*. The well-developed cupules of modern "tripsacoid" maize appears to stem from the introgression by these relatives.

W. C. Galinat

4. Recessiveness of hairy sheath in *Tripsacum* species hybrids.

The gene for hairy sheath (H_s) on the short arm of corn chromosome 7 acts as a dominant (Tavcar, 1932). Yet when either of two species of *Tripsacum* with hairy sheaths, *T. maizar*, *T. pilosum*, are hybridized with species having glabrous sheaths, *T. dactyloides*, *T. floridanum*, *T. zopilotense*, and a glabrous form of *T. australe*, the F_1 hybrid is glabrous. The results of this study of the inheritance of hairy sheath in chiefly diploid species of *Tripsacum* may be useful in determining the parentage of various tetraploid species believed to have had an allopolyploid origin.

The F_1 hybrid of the diploid species, T. maizar (FTG65-1237) and T. floridanum, in addition to having glabrous sheaths is highly pollen sterile and at meiosis in the microsporocytes there are varying numbers of paired and unpaired chromosomes which are irregularly distributed to the microspores.

These Tripsacum species hybrids are being grown for additional genetic tests and cytological studies at the Fairchild Tropical Garden in Miami, Florida along with an extensive live plant collection of the perennial relatives of corn.

W. C. Galinat
L. F. Randolph

5. Irregular transmission of the Su^d marked chromosome from Tripsacum in an addition monosomic stock of corn.

The starchy-marked (Su^d) chromosome, derived from Tripsacum as an addition chromosome on corn, gave a wide range of transmission rates in reciprocal crosses made on 112 ears (rows 66-1029 to 1064) grown under somewhat adverse conditions in Florida in January 1966. Transmission through the female ranged from less than 1% to 77% with an average of 6%. Transmission through the male ranged from less than 1% to 85% with an average of 15%.

Whenever the male and female transmission of the Su^d chromosome differed by more than a few per cent, it was always the male transmission which was higher. Sudden large increases in the rate of male transmission of this extra chromosome do not appear to be inherited. The cross su gl x 66-1038-3 Su^d gave 66% starchy kernels while the reciprocal cross with the same two plants gave only 11% starchy kernels. These wide differences between male and female transmission in reciprocal crosses disappeared in 16 selfed progeny ears (67-266, 267) with a range of 13.8% to 25.2% Su^d and an average of about 19% derived from both crosses. There is, however, a slightly higher rate of male transmission over female transmission which, under good pollinating conditions at least, does appear to be inherited (see next item).

W. C. Galinat
B. G. S. Rao

6. Consistent low female and high male transmission of the Su^d Tripsacum addition monosomic of corn.

Reserve seed of the 66-1038 stock which gave 11% Su^d female and 66% Su^d male transmission in Florida was grown in Massachusetts in 1967 and reciprocally crossed to the sugary background. In the cross with Su^d as the male parent, two sugary seed parents were used with one from the related su seed and the other from an unrelated su tester stock. The results follow:

<u>Su^d</u> Source	No. Kernels	% <u>Su^d</u> as female	Related No. kernels	% <u>Su^d</u> as male	Unrelated No. kernels	% <u>Su^d</u> as male
67-268-2	526	6.85	638	14.3	378	11.9
-3	408	6.40	199	15.6	---	----
-4	771	8.85	486	19.1	124	13.7
-5	611	6.37	911	16.6	252	11.5
-6	575	7.25	---	no tassel	---	----
-7	486	7.20	500	13.0	237	17.3
-8	644	6.06	461	15.4	259	17.7
-9	564	4.97	533	18.3	387	10.3
-10	639	5.31	548	17.1	---	----
Ave. female = 6.58%		Ave. male = 16.0%		Ave. male = 13.7%		

The expected frequency of Su^d on selfing would be the sum of male and female transmissions minus half of the frequency of double unions of Su^d. Using the above data from reciprocal crosses with the combined average for the two Su^d male crosses, we have as follows:

$$\begin{aligned} \text{Expected on selfing} &= 15 + 6.6 - \left(\frac{6.6 \times 15}{2}\right) \\ \text{Expected on selfing} &= 18.8\% \end{aligned}$$

This expected frequency of the Su^d phenotype on selfing is almost identical to the observed (19%) reported in the previous item for self-pollinations.

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7. Morphology of the Tripsacum chromosome carrying the homeolog for su of corn.

Galinat and Mangelsdorf (MNL 40:99-100) have reported a genetic comparison of some of the 18 possible addition monosomics of corn in which the different Tripsacum chromosomes carry the dominant alleles for some of the known recessives on different corn chromosomes. This report represents the preliminary cytological comparison of one of these Tripsacum chromosomes (referred to as the Su^d chromosome) that covers the recessive allele at the su locus on the short arm of chromosome 4 of corn. As already reported the Su^d chromosome does not cover either la on the short arm or gl₃ on the long arm (MNL 41:119). Additional evidence now indicates that gl₂ on the long arm also is not covered by the Su^d chromosome.

The material studied was from two related lines of addition disomics (67-258 & 67-259 in this item and 67-260 following). The homozygosity for the extra chromosome pair from Tripsacum in these two stocks originated independently within the selfed progenies of 20+1 families stemming originally (six generations back) from a single 20+1 plant. The Su^d chromosome was selected among the segregates from the second backcross of (4n su g corn X 4n T. dactyloides Fla.) X su gl₃ corn to su gl₃ corn.

The morphological features of the Su^d chromosome ascertained from 10 pachytene nuclei are given in Table 1. Synapsis at pachytene between the two homologues is complete, regular and normal. With a total length of 29.28 microns, the Su^d chromosome is shorter than chromosome 10 of corn but is similar to it in its arm ratio of 2.8:1.0. The relative lengths and arm ratios of the ten corn chromosomes from these nuclei agree fairly well with the data of Longley (see Rhoades, J. Heredity 41:59-67; 1950).

Table 1
Morphology of the *Tripsacum* chromosome at pachytene in the corn-*Tripsacum* hybrid derivatives: Stock 67-258 & 259 ($2n = 20+2$)

Sl. No.	Length in Microns			Arm ratio
	Short arm	Long arm	Total	
1	7.2	24.8	33.3	3.4:1.0
2	5.4	17.1	23.9	3.3:1.0
3	5.4	18.0	24.8	3.3:1.0
4	6.8	18.0	27.0	2.7:1.0
5	6.8	18.0	26.1	2.7:1.0
6	7.7	20.3	29.3	2.7:1.0
7	9.0	22.5	33.8	2.5:1.0
8	9.0	24.8	34.7	2.8:1.0
9	6.8	18.0	26.1	2.7:1.0
10	9.0	22.5	33.8	2.5:1.0
Mean	7.31	20.40	29.28	2.79:1.0
SE	0.432	0.951	1.34	---

Longley (J. Agric. Res. 54:835-862; 1937), Ting (Bot. Mus. Leaflet. Harvard Univ., 19:97-108; 1960), Chaganti (Bussey Institution of Harvard Univ., Cambridge, Mass. 1-93; 1965) and Tantravahi (MNL 41:52-57; 1967) have reported the pachytene chromosome morphology in four *Tripsacum* species in all. The Su^d chromosome derived from a $4n$ *T. dactyloides* and described now in the corn genome, when compared on the basis of the two diagnostic criteria (total length and arm ratio) with the above reports, has no similarities with any of the chromosomes described for *T. floridanum* by Longley (1937) and Chaganti (1965). However, it resembles chromosomes 11 and 12 described for *T. australe* by Ting (1960) and chromosome 10 of *T. maizar* and chromosome 9 of the tetraploid *T. laxum* recorded by Tantravahi (1967) as can be seen from data presented in Table 2.

Table 2

Morphological features of the pachytene chromosomes of different *Tripsacum* species comparable to the Su^d chromosome in corn-*Tripsacum* addition disomics ($2n = 20+2$)

Species	Chromosome No.	Length (microns)			Arm ratio	Author & Year
		Short arm	Long arm	Total		
<u>T. australe</u>	11	7.98	23.52	32.60	2.9:1	Ting, 1960
<u>T. australe</u>	12	8.19	21.36	31.00	2.5:1	Ting, 1960
<u>T. maizar</u>	10	7.81	21.87	31.25	2.8:1	Tantravahi, 1967
<u>T. laxum</u>	9	10.40	21.84	33.80	2.5:1	Tantravahi, 1967
<u>T. dactyloides</u>	*	7.31	20.40	29.28	2.8:1	Present study

* Su^d chromosome

B. G. S. Rao
W. C. Galinat

8. Synaptic affinities and altered morphology of the *Tripsacum* chromosome from addition disomics of corn.

The morphological features of the Su^d chromosome present in plants of a second addition disomic line (67-260) are given in Table 1 together with similar data from the related stocks (67-258 & 259) reported earlier.

When these two Su^d chromosomes, occurring in different but related 20+2 stocks are compared, a change in the position of the centromere from submedian (arm ratio 2.8:1.0) to nearly subterminal (arm ratio 4.4:1.0), thus altering the chromosome morphology, becomes evident. Considering that these two types of Su^d chromosomes had a common origin from 20+1 addition monosomics, the altered morphology could be ascribed to (a) a deletion of a part of the short arm or (b) a possible crossing over and chromatid exchange between the *Tripsacum* chromosome and any one of the corn chromosomes in one or more of the preceding generations. From the regular and complete pairing at pachytene as well as the occurrence of only bivalents in the later stages of meiosis I, it appears that in either case, the chromosome is homozygous for the alteration, the situation in this case being different from the 20+1 stocks of Maguire (Genetics, 45:195-209 & 651-664; 1960) where she found evidence of complete synapsis in chromosome 2 heterozygous for the ZT interchange segment. In the absence of readily distinguishable markers like, for example, the terminal knob for the *Tripsacum* chromosome isolated by Maguire (Genetics, 42:473-486; 1957), it would be difficult to readily locate the corn chromosome involved in

Table 1
Morphology of the *Tripsacum* chromosome at pachytene in the corn-*Tripsacum*
hybrid derivatives; Stock 67-260. ($2n = 20+2$).

Sl. No.	Length in Microns			Arm ratio	
	Short arm	Long arm	Total		
1	4.5	22.5	29.3	5.0	
2	4.5	20.3	26.1	4.5	
3	5.4	22.5	29.3	4.2	
4	5.4	23.4	30.6	4.3	
5	4.5	18.0	23.9	4.0	
6	5.4	22.5	29.3	4.2	
7	4.5	22.5	28.8	5.0	
8	4.5	18.9	24.8	4.2	
9	4.5	20.3	27.0	4.5	
10	4.5	22.5	28.4	5.0	
11	4.5	18.0	24.3	4.0	
12	4.5	18.0	24.8	4.0	
13	4.5	20.3	26.1	4.5	
14	5.4	22.5	29.7	4.2	
15	5.4	24.8	32.0	4.6	
16	5.4	22.5	29.3	4.2	
17	4.5	23.4	29.3	5.2	
18	4.5	18.0	24.3	4.0	
19	4.5	18.0	24.3	4.0	
20	4.5	22.5	28.8	5.0	
	Mean	4.77	21.07	27.52	4.42:1.0
Stock	SE	0.094	0.499	0.557	--
67-257	Mean	7.31	20.40	29.28	2.79:1.0
& -258	SE	0.432	0.951	1.34	

suspected interchange leading to the altered morphology of the Su^d chromosome. An obvious recourse is to analyze the entire chromosome complement in this material and compare the data obtained with that of Longley (in Rhoades, 1950) for each of the 10 corn chromosomes.

Preliminary studies on these lines indicate that chromosome 4 remains morphologically unaltered. The possibilities of the *Tripsacum* chromosome having equal, if not greater, synaptic affinities with chromosomes other than 4 of corn, therefore, have to be considered. In at least two of the nuclei observed at pachytene, in which some of the corn chromosomes could also be identified, chromosome 8 shows an arm ratio of 4.5 against the expected 3.2 while the other chromosomes correspond fairly well with the data of Longley. Detailed studies to verify the possible implications of the variation are in progress.

It may be of additional interest to mention that consistent with the otherwise regular course of meiosis, these $Su^d Su^d$ plants yielded 100% Su kernels when backcrossed with the recessive female parent both in the present and the preceding generations.

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9. Meiosis in some addition disomic corn-Tripsacum hybrid derivatives carrying the Su^d chromosome.

The source of the cytological materials for this report is the same as that referred to previously in items 7 and 8.

In a large majority of the pollen mother cells, the 22 chromosomes behave normally at meiosis and yield functional spores with 11 chromosomes each. In these, the two extra chromosomes undergo regular synapsis in prophase I and show normal disjunction at anaphase I and anaphase II. However, in a low percentage (about 5%) of cells, the Su^d chromosomes deviate from the normal in the course of their meiotic behavior as outlined below:

- (a) Occurrence as univalents at diakinesis and metaphase I, which probably is due to ineffectual synapsis at pachytene (pairing not followed by chiasma formation) between one of the *Tripsacum* chromosomes and a pair of corn chromosomes;
- (b) Occurrence of higher associations at diakinesis (types 7, 11 & 17);
- (c) Precocious second meiotic division of the chromosomes at metaphase I;
- (d) Unequal segregation (3:1 half-chromosomes or chromatids) at anaphase I;
- (e) Occurrence of chromatin bridges at anaphase I with a chromatin 'knot' on the equatorial plate (arrested terminalization?) involving one of the corn bivalents and independent of the *Tripsacum* chromosomes, and
- (f) Probable deletion-duplication in the corn chromosome pair involved in the bridge formation.

Any or all of the above cytological phenomena would alter the constitution, chromosomal as well as genetic, of the resultant microspores. Considering those listed from (a) to (d), three microspore types, i.e., with none, one or two Su^d chromosomes could be expected. Assuming a similar meiotic behavior in megasporogenesis, the functional egg could belong to one of the three nuclear phenotypes. It appears fusion between male and female gametes, each carrying more than one of the Su^d chromosomes, is eliminated as is to be inferred from the absence, so far as is known, of plants with 3 or 4 extra chromosomes in the derived progeny.

The transmission frequencies for the Su^d allele in the different test crosses made during 1966 and 1967 are listed in Table 1. While in some the observed data agree with the expected, in certain others they are not in agreement. The variable rates from identical crosses probably are related to the meiotic phenomena (a) to (d) and the consequences of those listed under (e) and (f) are not yet understood.

Table 1
Transmission frequencies of the Su^d allele in the different test crosses of addition disomic corn-Tripsacum hybrid derivatives

Year	Plant No. and cross	Observed <u>Su^d</u> kernels (Per cent)
1966	66-1026 : Selfed . . .	100
	66-1026-2 X <u>su gl₃</u> . . .	100
	66-1026-4 X <u>su gl₃</u> . . .	100
	<u>su gl₃</u> X 1026-5 . . .	99
	<u>su gl₃</u> X 1027 . . .	96
1967	<u>su bm₃</u> X 248 . . .	97
	<u>su bm₃</u> X 251 . . .	75

Plant row numbers 1026, 1027 for 1966 and 248, 251 for 1967 have the same source as 67-258 & 259.

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10. Emergence of "Pseudo-substitution" stocks of corn carrying the Su^d allele derived from Tripsacum.

Among the selfed progeny of the 20+1 addition monosomics of corn marked by the phenotypic expression of the dominant Su^d allele of Tripsacum, certain Su^d plants with 2n=20 chromosomes were isolated by cytological studies

(stock 67-262). Both at diakinesis and metaphase I there appeared to be 10 bivalents uniformly in all the nuclei examined. Disjunction was normal and segregation equal at anaphase I and II. Fertility adjudged from kernel set on the ear was near full. It was therefore expected that these plants would represent "substitution" stocks in which the Su^d locus from *Tripsacum* had been successfully transferred to one of the corn chromosomes followed by an elimination from the genome of the *Tripsacum* chromosome, now deficient for the Su^d locus. To verify this as well as to locate the newly introduced *Tripsacum* chromosome segment in the corn genome, studies were extended to analyses of the pachytene nuclei. The preliminary observations are recorded below.

In about 10 pollen mother cells in which the pachytene chromosomes could be analyzed with acceptable clarity of detail, a small extra fragment of chromosome material is found to be present. Like the other chromosomes, the fragment appears to be of a double nature and is always found in association with the centromere of any one of the corn chromosomes in the different analysable cells. In none of them was it possible to identify this fragment pair independent of the other corn chromosomes or as a discrete entity. By virtue of its constant association with the centromere of any one of the corn chromosomes, it is suspected to represent (a) a telocentric fragment pair (similar to a B chromosome or its C, D, etc., derivative) or (b) an iso-chromosome with inside pairing, derived in either case from the extra Su^d chromosome present in the parents. Its occurrence as a lateral "appendage" to the centromeres of any one of the corn chromosomes might be interpreted as due to the association of the centromeres of nonhomologous chromosomes, a phenomenon of not an infrequent occurrence. Due to its extremely small size (about 5 to 8 microns at pachytene), the extra chromosome fragment is not apparent at diakinesis and metaphase I. In a solitary metaphase I plate, one of the bivalents was noticed to have a small spherical knob-like extension at one of its polar ends (centromere) which is believed to represent the condensed fragment pair still in association with the centromere of the concerned bivalent. It would appear that this moves to one pole in its entirety without the anaphase I separation, and its fate during the subsequent course of meiosis is not yet ascertained.

The irregular meiotic behavior of this fragment pair of chromosomes is considered to be related to the inconsistent and unexpected (19% in the parent stocks of 1966) transmission frequencies observed for the Su^d allele in the different progenies derived on selfing the parent stock (Table 1).

From its present form and meiotic behavior, the fragment chromosome pair may not be expected to be stable. Little is known of the causes underlying the metamorphosis of a normal *Tripsacum* chromosome to the state of a small fragment, without apparently affecting the expression of the Su^d allele.

Table 1
Transmission frequencies of the Su^d allele in selfed pseudo-substitution stocks of corn-Tripsacum hybrid derivatives (Stock 67-262)

Plant No.	Total kernels	Observed Su^d kernels	Percentage of Su^d
67-262-1	120	29	24.2
-2	170	10	5.9
-3	264	33	12.5
-4	287	52	18.1
67-263-1	308	51	16.5
-2	317	72	23.5
-3	310	39	12.6
-4	298	21	7.1
-5	304	64	21.0
-6	319	59	18.5
-7	372	29	7.8

Both the stocks 67-262 and 67-263 were derived from selfing of 66-368-8

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1. Effect of temperature on the rate of crossing-over in maize.

Preliminary experiments have been carried out in the Phytotron at the Plant Industry Division, C.S.I.R.O., Canberra, to estimate the effect, if any, on the rate of crossing-over in stable stocks of $C\ Sh$ maize. Homozygous recessive $c\ sh/c\ sh$ plants kept at 27°C were used as female parents, while heterozygotes in coupling phase were used as pollen parents after being grown to anthesis in glasshouses set at 24°C, 27°C, 30°C, 33°C and 36°C. The daylight length was 16 hours and the humidity was kept constant for all glasshouses.

At anthesis the plants were transferred to the 27°C glasshouses and crosses made. Subsequently, the ears were harvested and calculations of recombination values from 17 ears in each treatment were made. The results were as follows:

	1	2	3	4
Treatment	27° x 24°	27° x 27°	27° x 30°	27° x 33°
Mean value for recombination between <u>C</u> and <u>Sh</u>	10.10	9.31	9.04	6.21

No ears were collected from the 27° x 36°, as the pollen was inviable, possibly an effect of the constantly high temperature in the 36° glasshouse.

"t" tests showed no significant differences between treatments 1 and 2, between 2 and 3 and between 3 and 4, but a significant difference was observed between treatments 1 and 4 and 2 and 4. It was therefore considered worthwhile repeating the experiment with more sophistication, and this is being done.

The possibility of B chromosomes producing these differences was considered but eliminated when, on examination, no B chromosomes were found to be present in any of the stocks. These stocks have been grown for 15 years in Melbourne, where the fluctuation in recombination values seemed to be correlated with fluctuation in temperatures from year to year, and this prompted the carrying out of the experiment under controlled conditions.

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1. Phenotypic stability in maize.

Phenotypic stability in maize has been the subject of many investigations. It has been demonstrated that the degree of phenotypic stability exhibited by various genotypes in response to environmental variations is not the same for all characters considered. Furthermore differences in phenotypic stability between genotypes may change after exposure to different environmental variations.

In a previous report (M.N.L., 1967) we presented data on phenotypic stability of eight inbred lines and all their F₁ crosses in relation to the effect of plant spacing. These results indicated that various plant characters are affected by spacing in the field. The degree of change is under genetic control in two of the four characters studied. No significant genetic differences in stability were observed in the flowering time

and leaf length. A single plant randomization design was adopted, so that the genotypes were in competition.

The experiment has been repeated in 1967 using seven of eight inbred lines and all their single crosses, without reciprocals. The field lay-out was in two blocks, each divided into three subunits, one for each level of plant density. Each family was grown in 3x7 plots with two replications per subunit. The measurements were taken on plants guarded by plants of the same genotype. Three plants were used for each plot. In this way the competition effect between genotypes was avoided.

The levels of plant density were the same as in the previous experiment, namely 5(I₁), 7(I₂) and 9(I₃) plants per m². The characters studied are shown in Table 1. In this table the overall F₁ and parental means for each treatment are reported. The analysis of variance indicates that the increase of plant density modifies the expression of the characters studied. The only difference not significant is that of leaf length.

Table 1

Characters	I ₁	I ₂	I ₃
Flowering time* \bar{F}_1	9.08	9.91	10.11
\bar{P}	15.70	15.30	16.06
Plant height \bar{F}_1	154.76	155.05	156.83
\bar{P}	117.58	114.97	111.29
Stock diameter \bar{F}_1	25.04	23.08	21.52
\bar{P}	19.21	18.41	16.23
Leaf length \bar{F}_1	73.15	72.84	73.08
\bar{P}	54.31	54.25	52.05
Ear weight \bar{F}_1	129.29	110.24	93.20
\bar{P}	46.93	36.64	31.05
Plant weight** \bar{F}_1	72.67	60.34	52.87
\bar{P}	40.39	33.64	26.04

* male flowering time

** dry weight

Differences in stability between F_1 and inbred lines are observed for plant height and ear weight. Considering plant height, it appears that the increase of plant density results in an increase of the mean value in the F_1 and a decrease in the inbred lines. A similar behavior was also observed in the previous experiment. Analysis of variance of combining ability has been performed for each character at each level of plant density.

The general combining ability (g.c.a.) and specific combining ability (s.c.a.) mean square are reported in Table 2. Both the items are highly significant ($P < 0.01$) for all the characters studied. The combined analysis provided a test for genetic-environmental interactions (genetic-density).

Table 2

Characters	Items	D.F.	Variances		
			I_1	I_2	I_3
Flowering time*	g.c.a.	6	58.973	71.308	62.570
	s.c.a.	21	29.165	22.728	23.676
Plant height	g.c.a.	6	1337.355	1207.588	1428.914
	s.c.a.	21	982.533	1076.176	1367.365
Stock diameter	g.c.a.	6	95.849	95.299	77.424
	s.c.a.	21	20.106	15.090	17.094
Leaf length	g.c.a.	6	1252.473	1624.187	1537.047
	s.c.a.	21	252.061	250.850	322.916
Ear weight	g.c.a.	6	8152.269	7322.777	4528.067
	s.c.a.	21	4493.349	3390.590	2374.413
Plant weight	g.c.a.	6	2784.826	1562.708	1063.460
	s.c.a.	21	853.637	589.001	488.621

*All the items are highly significant ($P < 0.01$)

Considering plant height and ear weight we notice a significant genetic-environmental interaction for the s.c.a. Proportional variation of the two components of genetic variation has been observed for plant weight. Further analyses will be accomplished in order to obtain a better evaluation of the observed effects and yield information about the genetic control of phenotypic stability.

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S. Conti

2. The smoky and light smoky derivatives of R^{st} .

A. Genetic control of their phenotype

These two stippled alleles were first isolated on ears obtained from the cross $R^{st}R^{st}1M_p \times r^{st}r^{st}$. Both the alleles, designated R^{sk} and $R^{l.sk.}$, breed true and are highly paramutagenic. They are easily distinguishable on the basis of their aleurone phenotype and of their frequency of reversion to R^{sc} in the germinal tissues (8.4×10^{-4} for R^{sk} and 0×10^{-4} for $R^{l.sk.}$). Since both these alleles were first isolated from an R^{st} line carrying M^{st} , the possibility arises that they are the result of a change of M^{st} rather than a change at the R locus. M^{st} is a Modifier of R^{st} , lying 5.7 units distal to it, whose presence increases the number of dark spots in the stippled aleurone. Loss of M^{st} through crossing over leads to a reduction in the number of spots, thus giving rise to a light stippled phenotype.

This possibility has been tested by crossing both R^{sk} and $R^{l.sk.}$ with a homozygous $\underline{g} R^{st} \underline{m}^{st} / \underline{g} R^{st} \underline{m}^{st}$ tester. The two heterozygous combinations have then been crossed with $\underline{g} r \underline{e} \underline{m}^{st} / \underline{g} r \underline{e} \underline{m}^{st}$ plants. If the smoky and light smoky phenotypes reflect a change at the R locus, rather than a change of M^{st} , they should still carry an unchanged M^{st} . The presence of this Modifier can be inferred from the production of a few stippled recombinants among the light stippled segregants in the testcross ears. The results of the crosses performed are reported in Table 1.

Table 1
Results of the crosses performed to establish whether R^{sk} and $R^{l.sk.}$ carry an unchanged M^{st} along their chromosome

Pedigree	Pistillate parent genotype	Observed segregations	Estimated $R-M^{st}$ c.o. value (%)
gl314 x gl201A	$\underline{g} R^{st} \underline{m}^{st} / \underline{G} R^{sk} (\#)$	137 smoky 140 l.st. 10 st.	6.6
gl315 x gl313	$\underline{g} R^{st} \underline{m}^{st} / \underline{G} R^{l.sk.} (\#)$	276 l.smoky 257 l.st. 23 st.	8.2

$\#M^{st}$ presence to be tested.

The appearance of the recombinant stippled kernels, besides the two parental types, shows that both R^{sk} and $R^{l.sk.}$ carry along their chromosome an unchanged M^{st} in its standard position. It then appears that the alteration in phenotypic expression of these two stippled derivatives reflects a change at the R locus. These observations also disclose a specificity of action of M^{st} .

B. Germinal transmission of the smoky derivatives of $\underline{R}^{1.sk.}$

The aleurone of kernels carrying two or three doses of the $\underline{R}^{1.sk.}$ allele often shows sectors of variable size exhibiting the darker phenotype conditioned by $\underline{R}^{sk.}$. In some cases these sectors cover the whole endosperm.

In order to test the germinal transmission of the smoky derivatives of $\underline{R}^{1.sk.}$, the following cross has been performed: $\underline{G} \underline{R}^{1.sk.} / \underline{g} \underline{r}^{\underline{g}} \times \underline{g} \underline{r}^{\underline{g}} / \underline{g} \underline{r}^{\underline{g}}$. This cross gave 6739 light smoky kernels and 51 with the whole aleurone phenotypically smoky. Twenty-one of them have been progeny tested. The results of the progeny tests are shown in the table below.

Table 2

Sporophyte constitution of 21 smoky derivatives of $\underline{R}^{1.sk.}$ obtained from the cross $\underline{G} \underline{R}^{1.sk.} / \underline{g} \underline{r}^{\underline{g}} \times \underline{g} \underline{r}^{\underline{g}} / \underline{g} \underline{r}^{\underline{g}}$ as determined by progeny tests.

$\underline{g} \underline{R}^{1.sk.}$	$\underline{G} \underline{R}^{1.sk.}$	$\underline{g} \underline{R}^{sk.}$	$\underline{G} \underline{R}^{sk.}$
5	10	2	4

These results indicate that:

1. Germinal transmission of the smoky derivative is very low (28.57%). This suggests that a large percentage of the smoky derivatives shows a phenotypic change limited to the endosperm tissues. After correction for lack of germinal transmission the rate of origin of smoky in the sample tested is 0.21%.
2. The data also provide some evidence of the lack of association between the change from light smoky to smoky and proximal crossing over. In fact, assuming a crossover value of 14% between \underline{g} and \underline{R} , the expected number of recombinants in the sample tested would be 2.94 (the upper and lower limits of expectation, according to Stevens method, being 3.06 and 11.7). The observed number of recombinants is 7 out of 21.

The smoky derivatives of $\underline{R}^{1.sk.}$ could be envisaged as either the result of a change at the \underline{R} locus or the consequence of recombination between $\underline{R}^{1.sk.}$ and a closely linked Modifier. One needs suitable markers flanking \underline{R} to discriminate between these two possibilities and stocks are being prepared for this purpose.

Giuseppe Gavazzi

3. Further evidence about the smoky modifier.

In the 1966 News Letter it was reported that when $\underline{R}^{\text{sk}} \underline{r}^{\text{g}}$ plants are crossed with $\underline{r}^{\text{g}} \underline{r}^{\text{g}}$ some of the resulting ears show, besides the expected colorless kernels (genotypically $\underline{r}^{\text{g}} \underline{r}^{\text{g}}$), two kinds of smoky, darker and lighter, often in equal frequency. Such results could be explained by assuming that the lighter smoky phenotype results from the interaction of $\underline{R}^{\text{sk}}$ with a Modifier of the smoky expression that assort independently of $\underline{R}^{\text{sk}}$.

The validity of this assumption can be tested by crossing plants derived from colorless kernels (obtained from the previously mentioned cross) with their dark smoky sibs. In fact, if the smoky Modifier assort independently of $\underline{R}^{\text{sk}}$, approximately one-half of the colorless kernels should carry it. Its presence can be proved by the appearance of two phenotypic classes of smoky, lighter and darker, in the ears obtained from the above mentioned cross. Twenty-four ears so obtained have been scored. Twelve of them segregate only dark smoky and colorless kernels, eight exhibit a clear segregation of dark and light smoky kernels, besides the expected 50% colorless, while the remaining four ears have been discarded because of scoring difficulties. These results clearly indicate that the two classes of smoky are due to the segregation of a smoky Modifier.

Giuseppe Gavazzi

4. Differential response of the R subunits to the paramutagenic action of \underline{rst} .

Plant and seed pigments are controlled by the two subunits of the \underline{R} locus, respectively symbolized \underline{P} and \underline{S} . If paramutation is not confined to the \underline{S} component but affects the \underline{R} locus as a whole, its expression should be observable also in the sporophytic tissues. In a previous test (M.N.L. 1966) we tried to establish this point by comparing the concentration of pigment extracts of $\underline{r}^{\text{g}} \underline{R}^{\text{r}}$ and $\underline{r}^{\text{g}} \underline{R}^{\text{r}'}$ roots grown on filter paper. The spectrometric determination of root pigments failed to disclose a significant reduction in pigmentation level of paramutant $\underline{r}^{\text{g}} \underline{R}^{\text{r}'}$ roots. These data seemed to suggest that the \underline{R} component conditioning pigment formation in the roots is not significantly affected by its association with a paramutagenic allele. Alternatively, they could simply indicate that no suitable growing conditions for pigment formation were used in our test. In fact, it is likely that the establishment of growth conditions leading to an increased anthocyanin biosynthesis in the sporophytic tissues may make observable even small differences in pigment concentration between $\underline{r}^{\text{g}} \underline{R}^{\text{r}}$ and $\underline{r}^{\text{g}} \underline{R}^{\text{r}'}$ roots.

Such conditions are obtained by allowing seeds to germinate on a medium containing agar and sucrose (0.25%). Furthermore it is possible, using this medium, to extend the measurements of pigment concentration to other tissues like the coleoptile and mesocotyl. This medium has been used to obtain the data that are here presented.

Table 1
Comparison of mean anthocyanin content between:
I. $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}}$ and $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$ roots II. $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}}$ and $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$ internodes (1)

Pedigree	Pistillate parent	Genotype	No. individuals tested	$\bar{X}_{(2)}$	s.e.
I. Internodes					
g607 X g1018	W23	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}}$	85	1.55	0.28
g607 X g1018	W23	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$	85	1.29	0.14
g1013 X g1027	W22	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}}$	60	1.64	0.46
g1013 X g1026	W22	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$	60	1.19	0.35
II. Roots					
g607 X g1018	W23	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}}$	50	1.08	0.13
g607 X g1018	W23	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$	50	1.13	0.14
g1013 X g1027	W22	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}}$	80	0.60	0.04
g1013 X g1026	W22	$\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$	80	0.58	0.07

(1) first leaf sheath

(2) expressed as mean O.D. at 530 m μ

The following crosses were performed to produce the seeds used in this experiment: first, sibs $\underline{R}^{\underline{st}}\underline{r}^{\underline{r}}$ plants were crossed with a homozygous $\underline{R}^{\underline{r}}\underline{R}^{\underline{r}}$ stock. Plants of the two genotypic classes so obtained were then used as staminate parents in a cross with $\underline{r}^{\underline{g}}\underline{r}^{\underline{g}}$ plants. The resulting $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}}$ and $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$ kernels were germinated and the amount of pigment in their sporophytic tissues was determined spectrometrically. The results obtained are reported in Table 1. They show that the concentration of pigment extracts from either primary roots or internode tissues of the two classes of seedlings does not differ significantly. The same observations have been extended to $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$ seedlings. In this second experiment two different $\underline{R}^{\underline{st}}$ alleles have been used. They represent two sublines derived from two seeds isolated from a homozygous $\underline{R}^{\underline{st}}/\underline{R}^{\underline{st}}$ stock. The two alleles differ in their capacity to induce paramutation. The former ($\underline{R}^{\underline{st}}-1$) is a strong inducer, while the latter ($\underline{R}^{\underline{st}}-2$) is almost completely devoid of paramutagenic capacity. The comparison in pigment concentration between seedlings of the two classes (see Table 2) shows a significant decrease in only the internode tissues of $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$ ($\underline{R}^{\underline{r}}$ ex $\underline{R}^{\underline{st}}-1$) seedlings.

The last table (Table 3) refers to a test which was performed to establish whether \underline{R} gene action in the aleurone and in sporophytic tissues is correlated. This test has been performed by choosing, in a sample of $\underline{r}^{\underline{g}}\underline{R}^{\underline{r}'}$ kernels of common origin, those with the lighter and darker phenotype. Kernels of the two classes, 0-2 nearly colorless and 5-7 nearly colored respectively, have been germinated on the usual medium with addition of Kinetin, and the amount of pigment in their root extracts has

Table 2

Comparison of the mean anthocyanin content between $\underline{r}^G \underline{R}^{r'}$ roots and internodes⁽¹⁾ obtained from $\underline{r}^G \underline{r}^G \times \underline{R}^{r'} \underline{R}^{st}$ 1 and $\underline{r}^G \underline{r}^G \times \underline{R}^{r'} \underline{R}^{st}$ 2 matings.

Pedigree	Genotype	Aleurone color classes	No. individuals tested	\bar{X} (2)	s.e.	t value
I. Internodes						
g1222 X g1219-1	$\underline{r}^G / \underline{R}^{r'}$	0.2	60	0.79	0.06	2.29*
g1222 X g1218-1	$\underline{r}^G / \underline{R}^{r'}$	5-7	60	0.99	0.06	
II. Roots						
g1222 X g1219-1	$\underline{r}^G / \underline{R}^{r'}$	0.2	60	0.79	0.04	0.89 ^{n.s.}
g1222 X g1218-1	$\underline{r}^G / \underline{R}^{r'}$	5-7	60	0.75	0.02	

(1) first leaf sheath and coleoptile

(2) expressed as mean O.D. at 530 m u

*significant at the 5 per cent level.

Table 3

Determination of the mean anthocyanin content of $\underline{r}^G \underline{R}^{r'}$ roots obtained from seeds exhibiting different levels of aleurone pigmentation

Genotype	No. individuals tested	Aleurone color classes	Roots mean score (1)	s.e.	t value
$\underline{r}^G \underline{R}^{r'}$	70	0-2	1.41	0.05	0.77 ^{n.s.}
$\underline{r}^G \underline{R}^{r'}$	70	5-7	1.46	0.05	

(1) expressed as mean O.D. at 530 m u.

been determined. The results of this test indicate that the two kinds of roots do not differ significantly in their pigment content.

The data so far obtained can be summarized as follows:

1. A significant decrease of R action in the plant tissues is obtained only after exposure of paramutable R to the repressive activity of a paramutagenic allele for two successive generations. These results suggest that paramutation, in the plant tissues, is weak and progressive in nature.
2. No corresponding decrease of pigmenting potential is observed in the roots even after two generations of R^r Rst heterozygosity.
3. The level of R gene action in the aleurone of paramutable rstR' individuals is not correlated to its level of action in the roots.

These data suggest that the R locus does not react as a whole to the action of an inducing allele. Rather, it seems that different R subunits react in different ways to the repressive activity of Rst. However, the interpretation of these results requires a deeper knowledge of the structural organization of the R region and of the biosynthesis of anthocyanins.

Giuseppe Gavazzi
Anna Maffioli

5. Chromatographic analysis of pigments of the various plant tissues.

A chromatographic analysis of the various pigments has been undertaken with the aim of investigating the following points:

1. Distribution of different pigments in the sporophytic and aleurone tissues of a W22 A₁ A₂ C₁ C₂ Pr R^r b pl stock, hereafter referred to as A C R^r.
2. Chromatographic analysis of the anthocyanins extracted from different tissues of A C R^r plants in order to establish whether they are the same or undergo changes in their chemical composition.
3. Variation in pigment distribution of A C R^r plants carrying various allelic combinations at the R locus.

Table 1 shows the variation of spots in different parts of the tissues of plants genotypically A C R^r. The chromatogram was run first downwards with Butanol--Acetic acid--water (4:1:5) and then from left to right with Acetone--Hydrochloric acid (1:3). Spots 1, 2, 3, 4 are anthocyanins. Spots 5 and 7, faint yellow at the visible light, turn blue after spraying with FeCl₃ solution. Spots 8 and 9 react positively with p-toluene sulfonic acid (dark yellow). Spot 10 turns light blue after spraying it with Na₂CO₃ solutions. Spots 15 and 16 react positively with both AlCl₃ and Na₂CO₃.

Table 1
The variation of spots in different parts of plants genotypically A C R^r

Part	Spots present												n(*)	Total No. of spots	Spots missing	
	1	2	3	4	5	6	7	8	9	10	15	16				
Aleurone	+	+	+	+				+	+					48	6	5, 6, 7, 10, 15, 16
Roots	+	+	+		+	+	+	+	+	+				16	9	4, 15, 16
Internode	+	+	+			+	+	+	+					15	7	4, 5, 10, 15, 16
Anthers	+	+	+			+	+	+	+			+	+	8	7	4, 5, 10

(*) n = No. of chromatograms analyzed.

Table 2
Rf values of the anthocyanins present in various parts of plants genotypically
A C R^r

Spot No.	Rf ₍₁₎	Rf ₍₂₎	Rf ₍₃₎	Rf ₍₄₎
1	0.25	0.28	0.27	0.24
2	0.30	0.34	0.34	0.29
3	0.39	0.42	0.42	0.39

(1) Internode (n = 8)

(2) Aleurone (n = 50)

(3) Roots (n = 20)

(4) Anthers (n = 16)

Table 3
Rf values of the anthocyanins found in plants genotypically A C R^r Pr and
A C R^r pr

Genotype	Spot No.	Rf values in			
		BAW (n = 50)	BuHCl (n = 18)	1% HCl (n = 18)	HAc-HCl (n = 16)
<u>A C R^r Pr</u> (seeds)	1	0.28	0.17	0.03	0.27
	2	0.34	0.27	0.04	0.33
	3	0.42	0.40	0.04	0.36
	4		0.68	0.13	0.54
		(n = 30)	(n = 10)	(n = 10)	(n = 10)
<u>A C R^r pr</u> (roots)	1	0.31	0.18	0.06	0.31
	2	0.41	0.28	0.08	0.38
	3	0.51	0.35	0.08	0.40

Table 4

The variation of different spots in the roots and aleurones of plants homozygous and heterozygous for different R alleles

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Part	Genotype	Spots present																n	Total No. of spots
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Roots	$\underline{r}^g \underline{r}^g$					+	+	+		+	+							4	5
Roots	$\underline{r}^g \underline{R}^r$	+	+	+		+	+	+	+	+		+	?	?				32	11
Roots	$\underline{r}^g \underline{R}^{r'}$	+	+	+		+	+	+	+	+								32	9
Roots	$\underline{R}^r \underline{R}^r$	+	+	+		+	+	+	+	+								16	9
Roots	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{1.sk}$					+	+	+	+	+					+	+		14	7
Roots	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{sk}$					+	+	+	+	+								8	5
Roots	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{st}$					+	+	+	+	+								8	5
Roots	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{sc}$					+	+	+	+	+								8	5
Aleurone	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{1.sk}$	+	+	+													+	8	4
Aleurone	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{sk}$	+	+	+													+	8	4
Aleurone	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{st}$	+	+	+													+	8	4
Aleurone	$\underline{g} \underline{r}^g / \underline{G} \underline{R}^{sc}$	+	+	+													+	8	4

The R_f values of the first 3 anthocyanins determined with the BAW solvent are reported in Table 2. They appear to be rather constant in each of the tissues so far analyzed, thus suggesting that no qualitative change in the chemical composition takes place in the various tissues. On the other hand, qualitative changes are observed after substitution of Pr with pr (see Table 3) and C_1 with c_1 . In the latter case, anthocyanin biosynthesis is blocked in the aleurone while it occurs in the plant tissues leading to three different anthocyanins. Their R_f values in BAW are 0.34, 0.41 and 0.48 respectively. Table 4 shows the pigment distribution observed in plants carrying different R allelic combinations.

We are presently involved in the chemical identification of the various anthocyanins and in the genetic control of their biosynthesis in sporophytic tissues.

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1. Notes on tinged in chromosome 10.

The tinged character (Maize News Letter 40:106) was poorly expressed in all F_2 , backcross, and increase progenies the past summer. In previous years it had been well expressed as seedlings, and still classifiable as adult plants. The character is not allelic to g_1 .

C. R. Burnham

2. Effects of colchicine treatment on multiple interchange heterozygotes.

Stocks homozygous for the T5-7-1-9-10 and T3-2-4-6-8 interchanges were crossed with normal stocks to produce F_1 's with $\odot 10$; and with each other for $2 \odot 10$. The F_1 's were treated as seedlings with colchicine solutions of various strengths. Plants with sectors that extruded anthers and shed pollen were found among the treated F_1 plants from the three crosses. Examination of pollen from these sectors with a pocket microscope indicated that more than half was normal in appearance, and considerably larger than the normal haploid pollen. These sectors are presumed to be $4n$. Some selfed seed was obtained. In untreated plants normal pollen was less than 10% in plants with a $\odot 10$, considerably less in plants with $2 \odot 10$.

The cross giving $2 \odot 10$ may be used to test the effectiveness of agents and conditions for inducing polyploidy.

Helmy Ghobrial

The following reports are based on studies supported by N.S.F. Grant G.B. 5543. We were assisted during the summer by Ken Nordland and presently by Dr. Jane M. Magill.

3. T⁴-6 (C.H. Li).

This interchange was used in earlier studies (Burnham, Genetics 35:446-481, 1950) of chromosome segregation. Pachytene observations showed the break in 6 was at or near the distal tip of the organizer. Photographs of pachytene configurations from the heterozygote were kindly analyzed by McClintock. She concluded it is a 1-6 interchange. Intercrosses with T⁴-6 interchange stocks form a @ 6, confirming the fact that it is not a T⁴-6 interchange.

J. Stout
C. R. Burnham

4. "Double interchange" marker method, progress report.

The procedures which might be used to establish stocks in which the 4 arms of two chromosomes are marked with an interchange were described in last year's newsletter (Maize News Letter 41:137-138). F₁'s and backcrosses of Type 1a intercrossoes (breakpoints in opposite arms in both chromosomes) that had relatively long differential segments were grown again the past summer. The F₁'s had higher sterility than that found in either parental heterozygote, about 65% as compared with the usual 50 per cent for a single interchange. High sterile plants were found among the backcross progeny from certain T1-5, T2-6, and T 4-6 intercrossoes. These plants should carry the crossover that combines the two interchanges. Test crosses to the parents were made to test this possibility, and selfs were made for increase. Stocks for establishing a double interchange series with nine stocks that will mark the ten chromosomes with 9 as the common chromosome were received from the Coop and the intercrossoes were made.

C. R. Burnham

5. Notes on T1-5 interchanges.

The following interchanges listed as T1-5 (Longley, A.E., ARS 34-16, 1961) are T1-2 interchanges: 6178, 8347, 018-5, 024-5 and 8388. Number 4331 now has only a 7-10 interchange.

The one listed as 2-6 (4394) is a 4-6 interchange, and the one listed as 2-6 (6671) is a 5-6 interchange. The above identifications are based on examinations at diakinesis of crosses with the chromosome identification set.

J. Stout
C. R. Burnham

6. Correlation of diakinesis observations with chiasma positions and frequencies.

Crosses between stocks of interchanges that involve the same two chromosomes are being studied with regard to pairing, crossing-over, and disjunction. In one group of crosses the breakpoints of the parents are in opposite arms of both chromosomes. This has been designated the type 1a intercross.

If all homologous parts are synapsed at pachytene in the F_1 , the resulting configuration is a 2-cross complex, with one "cross" in each arm of both chromosomes. Each differential segment is shared by the two "crosses" and is comprised of the non-interchanged segments of the long and short arms of the same chromosome. The other two arms of each "cross," in alternate positions, have the two interchange chromosomes.

Diakinesis configurations should differ depending on the number of segments with chiasmata. If a chiasma occurs in each of the differential segments the associations of four chromosomes at diakinesis may appear as criss-crossed bivalents, normal-looking rings- or chains-of-four, or "pairs," depending on the number and location of chiasmata in the interchanged segments. The "pairs" would be homologous only in the differential (centromere) segments. A chiasma in only one of the differential segments may result in a "figure-eight" configuration at diakinesis. No chiasmata in either differential segment may lead to "pairs" which are associated homologously only at the ends. Occasionally, univalents may appear as a result of no chiasmata in either end or differential segments.

All of the expected diakinesis configurations have been observed in the type Ia series of T1-5 intercrosses. When "pairs" are formed they are usually the type in which homologous ends are paired.

John Stout
C. R. Burnham

7. "All-arms" interchange tester set.

The interchanges listed in Table 1 have been backcrossed to A188, an inbred line with white endosperm, strong stalks, very dark green leaves, and a strong tendency for 2 ears per stalk. After 2 to 3 generations of backcrossing at Minnesota, Dr. M. T. Jenkins added 4 or 5 backcrosses. Following this, additional lines from the Cal Tech collection were added, and the backcrossing continued at Minnesota. If the breakpoints are correct, the series marks every chromosome arm at least twice. Not all breakpoints are at what might be surmised as the ideal position, about .6 in each arm. After at least 8 backcrosses, stocks homozygous for the interchange are established. The chromosomes involved in the interchange are then re-checked by cytological examination of crosses with the chromosome identification set, which includes T1-2a, T2-4d, T3-7c, T5-7c, T8-9a, and T8-10b.

The additional interchanges listed in the second part of the table are being retained until the backcrossing and final checking of all lines are completed.

Seed of these stocks is available for distribution.

Chas. R. Burnham
(Assisted by many over the years)

Table 1
List of translocations comprising the "all-arms" interchange set

Interchange	No. of bkc.	Breakpoints (Longley, 1961)		Interchange	No. of bkc.	Breakpoints	
<u>T1-3(5982)**</u>	9	1S.77	3L.66	T3-8(6373)**	6	3S.53	8L.68
T1-7(4405)	4	1S.43	7S.46	T4-8(6926)	6	4L.60	8L.71
<u>T1-8a*</u>	9	1L.41	8S.52	T4-9(4307)	6	4S.48	9L.55
<u>T1-9b</u>	10	1L.50	9L.60	T5-6(6522)	6	5S.87	6L.70
<u>T2-4L</u>	10	2L.59	4S.40	<u>T5-7(5179)**</u>	9	5L.55	7L.73
<u>T2-6b**</u>	9	2S.69	6L.49	<u>T5-8a**</u>	9	5L.49	8S.58
T2-7c*	9	2L.47	7S.34	T5-10(6760)*	8	5S.78	10S.40
<u>T2-10(6061)**</u>	8	2S.60	10L.57	T6-9(5454)*	7	6 Cent.	9S.75
<u>T3-4(5156)*</u>	11	3S.47	4L.67	T6-10(5253)	6	6S.80	10L.41
<u>T3-7c</u>	9	3L.46	7L.45	T9-10b	6	9S.13	10S.40
<u>Additional interchanges</u>							
<u>T1-3(5883)</u>	10	1S.88	3S.60	T4-6(Conn.)	9	4S.4	6L.6 (Roberts)
<u>T1-8b</u>	11	1L.59	8L.72	T4-7(7108)	8	4S.17	7S.45
<u>T2-4b</u>	11	2L.81	4L.53	<u>T5-7e</u>	8	5S.40	7S.18
<u>T2-6d</u>	10	2L.41	6L.45	<u>T5-10(5290)</u>	8	5L.78	10S.49
T2-9c	7	2S.49	9S.33	T8-10c	6	8L.41	10S.56

*These 5 mark one arm of each of the 10 chromosomes.

**These 7 mark the remaining arms; the 12 constitute the skeleton set.

Homozygous lines are underlined.

UNIVERSITY OF MINNESOTA
St. Paul, Minnesota
Department of Genetics and Cell Biology

1. A preliminary test for chlorophyll in xantha or albina mutants.

The classic property of fluorescence of the chlorophylls and related porphyrins can be employed to screen relatively large populations of new xantha or albina traits for the tentative presence of the chlorophylls. The first seedling leaf is macerated with several milliliters (ideally, 4 volumes) of acetone and the resulting crude extract is viewed under long (366 nm) or short (254 nm) wave ultraviolet light. A red fluorescence serves as a rapid preliminary test for the chlorophylls. Confirmation can

be made chromatographically and spectrophotometrically if one is interested in the actual identity of the pigments.

William D. Bell

2. Plastid pigments in white-1 luteus-1 ($w_1/w_1 \ l_1/l_1$) seedlings.

Seedlings of the genotype $w_1/w_1 \ l_1/l_1$ are of interest in that they would probably be scored as xantha rather than albina by most observers. Extracted pigments from greenhouse-grown $w_1/w_1 \ l_1/l_1$ seedlings yielded at least seven detectable plastid pigments when chromatographed on and eluted from sucrose (powdered confectioners sugar) columns. Tentative identification of the pigments included chlorophyll a, β -carotene, lutein, violoxanthin and neoxanthin but quantities at hand were insufficient for spectral confirmation of the separated pigments.

Pigments detected in leaves of $w_1/w_1 \ l_1/l_1$ seedlings were 10% or less than quantities found in normally green maize leaves at the same age, six days after emergence. Of particular interest is that a similar quantity of leaf tissue (0.5 g.) from homozygous w_1 seedlings with dominant alleles at the l_1 locus did not provide sufficient pigments for separation using the same chromatographic technique.

William D. Bell

UNIVERSITY OF MISSOURI
Columbia, Missouri
Department of Genetics

1. Chemical mutagens in mineral oil very effective on corn pollen.

For a number of technical reasons, chemical mutagens are generally ineffective when applied to corn pollen. However, when mineral oil (white domestic paraffin oil) is used as a carrier for the pollen (Coe MNL 40:108, 41:139) effective concentrations can be brought in direct contact with the pollen grains. The results with ethyl methanesulfonate (EMS) and nitroso guanidine (NG) have been especially impressive.

The procedure with EMS is as follows: Prepare a solution of .01 to .1% EMS in mineral oil. Place fresh pollen in a shell vial and add 10 times its volume of treatment solution. Stir immediately with a #10 camel hair brush. Wait 3-5 minutes to begin pollination. Pollinate by stirring pollen, then applying moderate amounts of the mixture to the silks with the brush. With EMS it is necessary to proceed rapidly as the pollen will be killed in less than 20 minutes. Extreme caution should be used to protect handlers as this is a dangerous chemical especially in mineral oil, which will not wash off easily. Disposable gloves, eye protection, and sanitation are vital.

The same procedure is used with NG except that it is in crystalline form and highly insoluble in mineral oil. Place a small quantity of crystals (.4 gram ±) in 100 ml of mineral oil. Do not use solvents as they will

kill the pollen immediately. Shake until the crystals are widely dispersed in the oil. Allow to stand for several hours until all crystals have settled out then pour off the solution and use as indicated above. However, in this case begin pollination whenever ready and continue as long as necessary. Pollinations made at 90 minutes give the same excellent results as those made at 3 minutes.

With EMS, selfed progeny from 42 treatments have yielded 334 good endosperm and seedling mutants, including many resembling known mutants.

Both chemicals are effective in producing large numbers of endosperm losses in experiments designed to test for them. When $A^b Sh_2 et, Dt$ pollen was treated with NG and crossed on $a^m sh_2 Et, dt$ silks, the following results were obtained.

Frequency ($\times 10^{-4}$) of loss of Components of the $\alpha \beta Sh (A^b Sh)$ segment from treatment with nitroso guanidine in mineral oil

Treatment	Population	$\alpha \beta Sh$ (Colorless, shrunken)	Sh (Colored, shrunken)	$\alpha \beta$ (Colorless, normal)	β (Dilute, normal)
Whole endosperm					
Control	8667	3	0	3	6*
NG	22058	48	4	4	5*
Fractionals 1/8 +					
Control	8667	65	3	13	9*
NG	22058	817	196	130	134

*Progeny tests will probably eliminate most of these as resulting from exchange between α and β at meiosis.

M. G. Neuffer

UNIVERSITY OF MISSOURI
and
UNITED STATES DEPARTMENT OF AGRICULTURE
Columbia, Missouri

1. Map location of c_2 .

The placement of c_2 distal to gl_3 on chromosome 4 is established by the following data from the cross of $+ gl_3 c_2 \times Tu gl_3 +/+ c_2$:

Parental		Reg. 1		Reg. 2		Doub.		T
56	59	13	13	7	9	0	1	161
118		26		16		1		

$$\underline{Tu} - \underline{Gl}_3 = 16.8 \pm 2.9 \quad \underline{Gl}_3 - \underline{C}_2 = 10.6 \pm 2.4 \quad c = 0.35$$

Two-point data from the cross of $\underline{+ +/Gl}_3 \underline{c}_2$ x $\underline{Gl}_3 \underline{c}_2$ give a better estimate of the map distance:

	+	+	+ c	gl	+	gl c	T	%
$\underline{Gl}_3 \underline{C}_2$ CB	468		22	28		440	958	5.2 \pm 0.7

E. H. Coe, Jr.

2. Selective enrichment experiments with pollen.

Tests parallel to those outlined last year (News Letter 41: 139) show some encouraging results. Tests with pollen from a multiple heterozygote for \underline{bz}_2 , \underline{a}_1 , \underline{c}_2 , \underline{a}_2 , \underline{pr} , \underline{c}_1 , \underline{bz}_1 , and \underline{r} (8 markers, 5 chromosomes) have been analyzed. Tests with other markers are in progress.

Pollen from the multiple heterozygote (1 ml) was mixed with 4 ml of aqueous medium (modified according to work of Y. H. Chang; 0.35M sucrose plus 1,200 ppm Ca Cl₂) and applied with a # 8 or # 9 brush to the silks. Each mixture was used on all 8 recessive testers, one ear each. The medium contained one of two concentration levels of one of 28 different agents. The list below gives the class, identity, and concentration of each agent tested. Ratios are given only for tests in which one or more of the ratios for that marked chromosome were significantly deviant (**1%, *5%, + or - 10%). Three hundred and eight of the 450-odd pollinations yielded sufficient seed for statistical test of the ratio; the 10 highly-significant ratios should include several true enriched samples.

The flavonoid relatives appear to be the most promising agents, as might be expected since the markers are flavonoid factors.

Agent	Ppm	Ratio	Locus	Dev.	Chrom.
Acridine orange	1000	0:1	\underline{c}_1		9
	1000	10:2	\underline{c}_1	+	9
	500	11:8	\underline{c}_1		9
<u>Carbohydrate metabolism</u>					
2, 4-dinitrophenol	100				
	50				
Oligomycin	50				
	25				
turanose	5000				
	2500				

Agent	Ppm	Ratio	Locus	Dev.	Chrom.
<u>Flavonoid relatives</u>					
<u>p</u> -methoxy cinnamic acid	5000	58:92	c c	- **	9
	2500	21:27			9
<u>p</u> -nitro cinnamic acid	100	14:84	a a a a a a a a	- **	3
	50	37:30			3
	100	6:153			5
	50	10:7			5
	100	4:4			5
	100	2:0			5
	50	12:9			9
	100	23:11			9
esculin	50	11:11	c c b z	+	9
	50	51:70			1
	1000	2:2			10
	500	40:25			10
hesperidin	1000	0:5	r r b z c c b z	-	1
	500	82:119			1
	1000	3:1			4
	500	48:30			4
	1000	1:0			9
	500	33:21			9
	1000	13:7			9
	500	46:65			9
naringin	100	5:0	r r	+	10
50	50:49	10			
phloridzin	1000	4:5	a a a a	+ **	5
	500	77:28			5
	1000	6:1			5
	500	34:17			5
quercitrin	500	9:4	p r b z b z	+	9
	500	1:1			9
	250	1:0			9
	500	1:3			9
	500	0:6			9
	250	12:6			9
rutin	1000	18:6	a a	+	5
	1000	2:0			5
<u>Hormones</u>					
gibberellic acid	2500				
	1250				
indole-3-acetic acid	500	20:14	r r	-	10
	250	89:114			10
<u>Lysine relatives</u>					
arginine	10000	7:0	a a a	+ *	5
	10000	2:0			5
	5000	20:29			5

Agent	Ppm	Ratio	Locus	Dev.	Chromo.
<u>Lysine relatives (cont'd)</u>					
arginine	10000	2:7	<u>pr</u>	-	5
	10000	0:1	<u>pr</u>		5
	10000	1:1	<u>bz</u> ₁		9
	5000	2:1	<u>bz</u> ₁		9
	10000	17:10	<u>c</u> ₁		9
	10000	8:11	<u>c</u> ₁		9
	5000	9:1	<u>c</u> ₁		9
lysine	10000	7:0	<u>a</u> ₂	+	5
	10000	1:1	<u>a</u> ₂		5
	5000	6:5	<u>a</u> ₂		5
	10000	0:1	<u>pr</u>		5
	10000	3:3	<u>pr</u>		5
	5000	15:15	<u>pr</u>		5
	<u>Methionine relatives</u>				
ethionine	1000	11:4	<u>bz</u> ₂		1
	500	27:6	<u>bz</u> ₂	+++	1
	1000	9:2	<u>a</u> ₁	+	3
	500	15:11	<u>a</u> ₁		3
	1000	5:14	<u>c</u> ₂	-	4
	500	33:22	<u>c</u> ₂		4
methionine	10000	3:4	<u>bz</u> ₂		1
	5000	146:221	<u>bz</u> ₂	-**	1
	10000	1:0	<u>a</u> ₁		3
	5000	9:0	<u>a</u> ₁	+++	3
<u>Tryptophan-niacin relatives</u>					
3-acetyl pyridine	1000				
	500				
anthranilic acid	500				
	250				
5-fluorotryptophan	500				
	250				
5-hydroxy tryptophan	2500				
	1250				
indole	1000				
	500				
kynurenine	500	4:3	<u>a</u> ₁		3
	250	42:21	<u>a</u> ₁	+++	3
	500	1:2	<u>a</u> ₂		5
	250	110:65	<u>a</u> ₂	+	5
	500	9:2	<u>pr</u>		5
	250	20:18	<u>pr</u>		5
	500	83:12	<u>c</u> ₂	+++	4
	250	37:33	<u>c</u> ₂		4

Agent	Ppm	Ratio	Locus	Dev.	Chromo.
<u>Tryptophan-niacin relatives</u> (cont'd)					
nicotinamide	500				
	250				
alpha-picolinic acid	500	0:5	$\frac{c_2}{c_2}$	-	4
	250	0:1	$\frac{c_2}{c_2}$		4
pyridine-3-sulfonic acid	10000	8:11	$\frac{bz_1}{bz_1}$		9
	5000	33:30	$\frac{bz_1}{bz_1}$		9
	10000	17:7	$\frac{c_1}{c_1}$	+	9
	5000	32:38	$\frac{c_1}{c_1}$		9
tryptophan	5000	29:22	$\frac{bz_1}{bz_1}$		9
	10000	24:25	$\frac{c_1}{c_1}$		9
	5000	19:8	$\frac{c_1}{c_1}$	+	9

Deviations in opposite directions for the same chromosome do not necessarily negate each other, nor do insignificant deviations necessarily negate significant ones. The anthocyanin markers themselves may be responsible for physiological differences that are subject to selection, but they may also be linked with unknown factors that were heterozygous in either parent of the hybrid plants that were used as males. Of course possible contaminations and other errors require that the above tests be repeated extensively.

E. H. Coe, Jr.

3. Recombination frequency and coincidence in maize.

The aim of the present study was to examine the effects of translocations on recombination, using coincidence and recombination percentage together as a measure of these phenomena. The backcross data studied, which were compiled by D. R. Knott (1963, Maize News Letter 37:164-172), represent largely the work reported by R. A. Emerson in 1940 and 1941.

Statistical analysis was done on backcross material which was either structurally normal or translocation-bearing in the F_1 generation. The translocations all involved chromosome 1 and differed from one another with respect to their individual breakpoints; not all were located precisely. For convenience the long arm of chromosome 1 was divided into four regions: $\frac{br-f}{br-f}$, designated as region 1; $\frac{f-an_1}{f-an_1}$, region 2; $\frac{an_1-gs_1}{an_1-gs_1}$, region 3; and $\frac{gs_1-bm_2}{gs_1-bm_2}$, region 4. Regions covering more than one of these were designated by 1-2, 1-3, etc. For all samples containing one or more of the intervals 1, 2, 3, 1-2, 1-4, and 3-4, average recombination values were calculated from the raw data by dividing the total number of recombinations for the region, regardless of structural constitution, by the grand total of individuals for the region. These values, which may be symbolized as \bar{P}_t , were used as the means to which individual recombination frequencies were compared to indicate those values which were higher than the average and those which were lower. In addition,

the standard errors were calculated to determine fit, that is, percentage of the time a given recombination frequency higher or lower than the mean would be expected by chance. Finally, coincidence calculations were made on all double exchanges, and the probabilities of these values occurring by chance in a population whose true interference level was zero.

As a result of the statistical analysis several graphs could be constructed. First, fit (P) to a coincidence of one for each sample, separating those with c less than one from those with c greater than one, was plotted against frequency. Such a graph was desirable in order to evaluate whether the population at hand was spread according to theoretical expectations. It is expected that a few samples will have high coincidence, since this is a property of the binomial distribution when q is extremely small as compared to p . What is not expected is any sort of clustering, but this is what has been found.

Of twenty samples carrying a translocation internal to one of the intervals considered, twelve had coincidence in excess of one. Of these, six had P values between zero and twenty per cent, three approaching deviations which might be significant, having P values between five and eight per cent. The eight internal-translocation bearing samples with coincidence less than one had no deviations which even approached significance. When coincidence was considered with respect to immediately adjacent intervals on opposite sides of the translocation cross, all four samples were found to have an interference level in excess of one with P values between zero and twenty per cent. Two of these approached deviations which might be significant, having P values of five and seven per cent. When this observation was extended to include all adjacent intervals involving an internal translocation, seven out of eight were found to have coincidence greater than one.

An examination of those samples involving external translocations also yielded interesting information. Of the twenty samples comprising this population nine had coincidence greater than one, deviations for two samples being significant.

Double exchange in directly adjacent regions free from structural anomalies also merits consideration. Two samples with coincidence less than one were significant. Six out of fourteen samples with coincidence greater than one were significant. Of these six, four were from directly adjacent regions. This may indicate that double exchange within directly adjacent regions is more coincidental than in more widely separated regions.

At this point it became important to determine the effect translocations were having on recombination frequency in this population. Frequency distribution graphs to a fit to \bar{P}_t were made for the six regions. Of the nine translocation-bearing samples involving region 1, seven were below \bar{P}_t . In region 2, eight of the ten translocation-bearing samples were found to have recombination frequencies below \bar{P}_t . In region 3, three out of four translocation-bearing samples fell below \bar{P}_t . The other three regions showed the effect also.

One final figure was plotted which sought to combine the information of the preceding ones. Probability (P) for a given p_i was positioned in the graph according to whether it was higher or lower with respect to its \bar{p}_t . This then was related to the associated coincidence for that region with another region in the same sample (involving either whole regions or segments thereof depending on the position of intervening genes or translocations) with respect to a fit to coincidence equal to one. For a given sample, recombination in an interval may be higher or lower than \bar{p}_t . This should have direct bearing on the interference levels when the doubles involving such a region are considered. The figure generated was divided into four parts. The first zone designated recombination frequencies lower than their respective \bar{p}_t for intervals which yield coincidence less than one. The second zone designated recombination frequencies higher than their \bar{p}_t for intervals which yield coincidence less than one. The third zone designated recombination frequencies lower than their \bar{p}_t for intervals which yield coincidence values in excess of one. The fourth and last zone designated recombination values higher than their respective \bar{p}_t for intervals which yielded coincidence in excess of one.

One of the most striking features of this graph was the distribution of translocation-bearing samples. Since it was demonstrated that the presence of a translocation reduced recombination frequency, it was expected that reduction of p_i for an interval would also reduce the associated doubles, as interference levels are a function of p . Therefore the great majority of aberration carriers is expected to be found in quadrant one. On the contrary, of twenty-four samples with an internal translocation, where p_i for the interval was less than \bar{p}_t thirteen were in quadrant three (coincidence greater than one). Included in these were two cases where the coincidence was greater than one for immediately adjacent regions on opposite sides of the translocation cross. On the one hand, p_i was low for the interval as a whole, whereas on the other, double exchange was high--being significant in one case at the five per cent level. Considering all adjacent regions in the samples with an internal translocation, where p_i for the interval being studied was lower than \bar{p}_t , it was found that of nine samples five were associated with coincidence greater than one. For doubles which were not immediately adjacent and where p_i for the sample was lower than \bar{p}_t , out of fifteen samples eight were associated with coincidence greater than one.

When p_i for a given sample was higher than \bar{p}_t and the sample carried a translocation, its position was always external. Samples carrying external translocations occurred in all four quadrants. This variability presumably reflects the differences in the position of the breakpoints.

Reference must also be made to the distribution of samples carrying no aberration. The area of interest here was quadrant four (high p_i , high c). Of sixteen samples twelve involved exchanges between adjacent intervals. Furthermore, four out of five samples in quadrant three (low p_i , high c) involved exchanges between adjacent intervals. This may be taken as another indication that double exchange in general takes place between more closely associated areas, although this effect is reduced by the presence of an intimately associated translocation.

In summary, the presence of translocations reduced overall single exchange, but multiple exchange was not similarly reduced. In fact, high coincidence values were associated with the presence of a translocation. In addition, in normal samples, coincidental exchange seemed to be more closely associated with directly adjacent regions, in contrast to what has previously been observed. Before definitive statements can be made as to what the above means in terms of mechanism, critical comparisons of double exchange in normal and structurally aberrant populations are needed.

S. I. Goldman
E. H. Coe, Jr.

4. A B-type translocation involving the short arm of chromosome 3.

A new B-type translocation involving the short arm of chromosome 3 was reported last year (News Letter 41:139). The translocation has now been further characterized and can be designated TB-3b.

The segment of chromosome 3 distal to the break carries not only cr₁ and d₁ but also ra₂. Maize linkage maps usually place ra₂ in the long arm of chromosome 3. Since TB-3b uncovers ra₂ in addition to the 3S markers cr₁ and d₁, ra₂ appears to reside in 3S instead of 3L.

J. B. Beckett

5. A translocation complex involving chromosomes 5, 6, and a supernumerary.

Last year it was reported (News Letter 41:139) that the gene pr on the long arm of chromosome 5 appeared to be uncovered by a new A-B translocation. It is now evident that the genes ae, pr, gl₃, lw₂, ys₁, v₂, and v₁₂ are distal to the break and that bt₁ is proximal. Since the gene order is normally given as bt₁ v₃ bv₁ pr, progenies involving v₃ and bv₁ will be tested next summer to locate the breakpoint more precisely.

Preliminary cytological observations indicate the presence of a translocation complex involving chromosomes 5, 6, and a B.

From a cross by pollen from a normal plant, 15 plants were tested for the ability to produce hypoploid sperm (pr test). Eight plants with 60-70% aborted pollen gave about 20% hypoploid endosperms, two plants with 60-70% aborted pollen gave no hypoploid endosperms, and five plants with 10-25% aborted pollen gave no hypoploid endosperms. Therefore, it is still not clear whether the B-type translocation is separable from the remainder of the translocation complex.

J. B. Beckett

6. Duplications from translocations between homologous chromosomes.

A method for the detection of duplications arising from translocations between homologous chromosomes was presented in a previous issue (MNL 38: 101-105). Further work has been done on this problem.

Twelve possible cases of duplications have been isolated on the basis of genetic evidence. Cytological examinations at pachynema for each of these cases have not revealed any observable buckles which would indicate the presence of a duplication. Either the duplications are too small to be seen or they are not there and the genetic data must be interpreted differently. The genetic data are too involved and incomplete (due to a hailstorm last summer) to be presented here. In general, however, the data conform to theoretical expectations. Gene markers which are on presumptive duplication chromosomes show reduced transmission rates through the pollen.

Theoretically, the frequency of translocations between homologous chromosomes should not be uncommon. Roughly, it should be $(1/n-1)$ times the frequency of translocations between non-homologous chromosomes, where n is the haploid number. This neglects complications arising from different chromosome lengths and different arm ratios. The translocation between homologous chromosomes must involve the same arms if a duplication is to be induced. (If the two arms are different, then two duplication-deficient chromosomes are produced; crossing over between them produces a normal chromosome and a pericentric inversion). Since the number of chromosome arms equals $2n$ the frequency with which the desired type of translocation occurs is $(1/2n-1)$ or $1/19$ in the case of maize. Even allowing for the probabilities that one break must be proximal and the other distal to the marker gene if the duplication is to be detected, the observation of duplications does not seem theoretically impossible.

The above discussion rests upon an assumption which is probably not true--that the chromosomes are randomly arranged in the interphase nucleus. It is known from the work of Longley that there is a correlation between the distances from the centromeres to the breakpoints for the two chromosomes involved in a non-homologous translocation. This is believed to be the result of polarized orientation of the chromosomes brought about by the previous telophase. Presumably, there would be a similar correlation in homologous translocations, in which case the duplications produced would tend to be short ones, and there would be a low probability of a given gene being bracketed by a proximal and distal break.

Furthermore, recent work by Feldman and Mello-Sampayo, and by Maguire suggest that homologous chromosomes tend to be associated with each other during the cell life cycle. It is quite possible that homologous chromosomes tend to lie close together during interphase and consequently are more apt to exchange segments with each other than with non-homologous chromosomes following radiation-induced chromosome breakage. The ratio of homologous translocations to non-homologous translocations would be illuminating. However, the technique for detecting homologous translocations needs more work.

G. G. Doyle

7. Pollen: a crude enzyme system.

As part of an extensive research program concerned with the biosynthesis of anthocyanin in maize, efforts were made to extract and identify

phenolic compounds in different parts of the maize plant. Standard organic extraction methods were used and thin layer and paper chromatography techniques were used for identification purposes. Such efforts concerning pollen indicated that quercetin and its 3-glucosylated form, isoquercitrin, were present in pollen with the exception that the anthocyanin mutant bz₁ did not possess isoquercitrin.

If this were true then it seemed quite reasonable that pollen should have an enzyme that could catalyze this reaction. However, efforts to obtain a protein extract after freezing the pollen with liquid air and grinding it with a mortar and pestle failed. If the enzyme could not be extracted, it seemed reasonable to use the whole pollen grain as a crude enzyme system. The reaction system used was typical for the study of such a reaction and included: MgCl₂, uridine-5'-diphosphate glucose (UDPG), quercetin, tris buffer (pH 7.4), distilled water, and whole pollen. Incubations were carried out at 37°C for three hours with shaking. The results of these studies indicated that the conversion was enzymatically catalyzed as the activity could be destroyed by heat, that the UDPG was required as a glucose donor, and that quercetin was required as a substrate. Although the conversion rate in the reaction is low, it is substantially larger than that for control reaction samples. To date the activity has been found to be stable on storage in vacuum in the deep freeze. Studies are in progress at the present time to relate this conversion to anthocyanin synthesis.

R. L. Larson

8. Pollen: a crude enzyme system and genetic studies.

The results obtained in the studies discussed in the previous note strongly suggested further investigations to learn of any possible relationship of this reaction to the genetics of anthocyanin biosynthesis. This was possible since pollen samples were available having the following genes singly recessive: c₁, c₂, r, a₁, a₂, bz₁, bz₂, and pr. Conditions used for the different pollen samples were those given in the previous note. The results obtained indicated that bz₁ was indeed the gene responsible for the glucosylation reaction, as enzymatic activity was found in all mutant pollen except bz₁. Studies are presently in progress to investigate a possible gene dosage relationship using the homozygous recessive and dominant pollen for bz₁ as well as the heterozygote.

R. L. Larson
E. H. Coe, Jr.

9. Knotted leaf mutations.

Two different mutations for the knotted leaf character have been found during the past two years. One occurred in the inbred line Mol4W and the other in a commercial single cross. Both mutants appear to be dominant and similar in phenotype to the original knotted leaf. Allelism tests are in progress.

M. S. Zuber
P. J. Loesch, Jr.

10. Vivipary induced by a fungus.

Several years ago while studying the comparative ear rotting effect of different isolate strains of Diplodia maydis, we noted that two isolates obtained from Dr. Arthur L. Hooker of the University of Illinois produced premature germination (vivipary) of kernels on the diseased ears. In 1967 a replicated experiment was conducted with five isolate strains including the original two isolate strains that induced vivipary. Inoculum laden toothpicks were inserted in the center of each ear of the single cross K4XB2, approximately 20 days after 50% of the plants had silked. At harvest it was found that ears inoculated with the two isolates exhibited 100% vivipary. The strains inducing vivipary also were milder in their attack on these ears. Kernels which germinated were located in the region of recent infection, but not where infection had already destroyed the kernels. We suspect that the two isolates may synthesize some hormone or enzyme which breaks the normal dormancy of the embryo.

M. S. Zuber
O. H. Calvert
M. G. Neuffer

NATIONAL COLONIAL FARM
Accokeek, Maryland

1. The National Colonial Farm.

The National Colonial Farm is destined to become a working colonial farm of about the period 1750, with crops and livestock of the period. Since maize was an important colonial crop it will be one of the main crops of the colonial farm. Items regarding maize grown at the Farm appear below.

2. Reconstitution of Dent corn.

Virginia Gourd seed and Northern Flint varieties are being grown in an attempt to reconstitute by crossing and selection the Dent types grown so widely. Fortunately we were able to obtain Virginia Gourd seed from Dr. William Brown at the Pioneer HiBred Seed Company, and the Northern Flints from the Cornstock-Ferre Seed Company in Wethersfield, Connecticut.

3. A reconstituted Golden Bantam Sweet Corn.

Crosses of Black Mexican sweet corn and Canada Flint are expected to produce a yellow sweet corn similar to the old 8 Row Golden Bantam.

4. Gaspé Flint--world's earliest corn?

In 1966 we obtained seed of Gaspé Flint from Dr. Robert I. Brawn, MacDonald College, Quebec. Plants from an August 2 sowing produced pollen on August 27, just 25 days after putting dry seed in the ground. The F_1 plants between this Flint and Virginia Gourdseed were much nearer the Gaspé than the Gourdseed in maturity. This study is being continued. Efforts will be made to obtain F_2 plants comparable in maturity to Gaspé Flint.

5. Seed irradiation studies.

Seed irradiation studies are continuing. In 1967, seeds of the B14 inbred were treated by Dr. Robert Briggs at the Brookhaven National Laboratory, and an isolated open pollinated field grown at the National Colonial Farm in Accokeek, Maryland. Many self-pollinations will be made in 1968 to test the effectiveness of inducing mutations in maize seed. This method should be applicable to all open pollinated crops.

W. Ralph Singleton

UNIVERSITY OF NEW HAMPSHIRE
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1. A dosage effect at the Ht locus.

The Ht gene conditions chlorotic lesion resistance to northern corn leaf blight. In a program directed toward the study of gene dosage at the Ht locus, we were able to obtain monoploid, triploid, and tetraploid seed from diploid Ht Ht and ht ht stocks. As a result, we were able to test one, two, three, and four doses of both the Ht and ht alleles for disease reaction. The diploid stocks were secured from Dr. Albert L. Hooker of the University of Illinois in Urbana. Lines R223 and 65:225-1 are homozygous Ht. W153R is homozygous ht. Lines 65:225-1 and W153R are isogenic. Most of our data were obtained from R223 and W153R. Experiments now in progress are aimed at testing the dosage levels in the isogenic material.

Putative monoploids were detected by crossing purple embryo marker as pollen parent to the diploid lines. The kernels with purple endosperm and non-purple embryos were saved. Monoploids were confirmed by chromosome counts on growing root tips. Tetraploid seedlings were obtained by means of Shaver's "decapitated root" technique (Maize Genetics Newsletter 38: 21-22). Triploids were obtained from tetraploid x diploid crosses.

Plants on the four dosage levels were inoculated at the three-four leaf seedling stage with spore suspensions of the pathogen, Helminthosporium turcicum. Seedlings were incubated for 18 hours at 20°C and 100% humidity. The degree of infection was determined by measuring the total area of the fourth leaf, and then the area of that leaf covered with lesions. The per cent infection was calculated as follows:

$$\frac{\text{Infected area of leaf}}{\text{Total area of leaf}} \times 100$$

Monoploid and diploid seedlings, carrying one and two doses of Ht, showed no significant difference in degree of resistance. Triploid and tetraploid seedlings, carrying three and four doses of Ht, did not differ significantly. However, three and four doses of Ht conferred a significantly higher level of resistance than did one or two doses. The heterozygote (Ht ht) was less resistant than the n-2n class, although this difference was not statistically significant. However, heterozygous seedlings were significantly less resistant than the 3n-4n class.

Monoploid ht seedlings were significantly more susceptible to leaf blight than were the diploid (ht ht) seedlings. There was no significant difference in susceptibility between $2n$, $3n$, and $4n$ seedlings carrying two, three, and four doses, respectively, of ht.

Ted Namm
G. M. Dunn

2. The use of purple embryo marker in screening for twin embryo seeds.

We have been screening large populations of corn seeds for monoploids by crossing our test lines, as female parent, to purple embryo marker. In a population of about 83,000 kernels, we found four seeds, each of which possessed two distinct embryos. On all four kernels, each of the two embryos showed the purple color. It was apparent in two of these kernels that the twin embryo could not have been detected unless the pigment was present. The female parent was line 65:225-1, which is homozygous Ht.

We suggest the use of purple embryo marker to facilitate the detection of such seeds in programs where the primary purpose of research is to uncover kernels with twin embryos.

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1. Linkage intensities of lutescent-1.

The lutescent mutant in maize has been characterized (MNL 39:146-147). Its expression has been found to result from two recessive genes, lutescent-1 (lu₁) and lutescent-2 (lu₂) (MNL 41:150-152). One of these genes, lu₁, has been located on chromosome 5, and preliminary F_2 studies indicated close linkage with a₂.

A testcross was carried out between plants hybrid for lu₁, lu₂, a₂, bm₁, bt₁, and pr, and one homozygous for lu₁, lu₂, and pr. The latter plant was heterozygous for bm₁ and bt₁, but did not carry a₂. The seeds with colorless aleurone resulted from the segregation of a gene other than a₂ since no linkage could be demonstrated between the aleurone color gene and any of the other chromosome 5 markers. The results of this testcross are presented in Table 1. A high percentage of inviability was encountered among seeds homozygous for bt₁, and the values shown have been corrected to allow for this inviability.

Acceptable 3:1 monohybrid ratios were observed for colored vs. white seeds, full vs. brittle seeds, green vs. brown midrib, and green vs. lutescent leaf. It will be noted that 3:1 represents a testcross ratio for green and lutescent since duplicate genes are involved. A 1:1 ratio was observed for purple vs. red seeds. Dihybrid segregations involving lutescent plant, and brittle seed, brown midrib, or red aleurone all

Table 1

Testcross phenotypes from crosses between lutescent and chromosome 5 markers: a₂ (anthocyanin), bm₁ (brown midrib), bt₁ (brittle endosperm) and pr (red aleurone)

Aleurone	Phenotypes			Number of seedlings
	Endosperm	Midrib	Leaf	
Purple	Full	Green	Green	179
			Lutescent	106
		Brown	Green	1
			Lutescent	0
	Brittle	Green	Green	2
			Lutescent	0
		Brown	Green	55
			Lutescent	0
Red	Full	Green	Green	170
			Lutescent	50
		Brown	Green	3
			Lutescent	0
	Brittle	Green	Green	9
			Lutescent	1
		Brown	Green	128
			Lutescent	4
White	Full	Green	Green	132
			Lutescent	59
		Brown	Green	1
			Lutescent	0
	Brittle	Green	Green	1
			Lutescent	0
		Brown	Green	47
			Lutescent	10
			Total	958

deviated highly significantly from the expected ratios and indicated linkage.

In order to calculate linkage intensities between lu_1 and the other markers, individual recombinant types had to be determined and the frequencies of detected recombinants used to calculate the frequency of the total recombinants. For example, when considering pr and lu_1 , four recombinant gamete types are possible: $Pr Lu_1 Lu_2$, $Pr Lu_1 lu_2$, $pr lu_1 Lu_2$, and $pr lu_1 lu_2$. When crossed to the homozygous recessive, only one of these gametes will produce the recombinant phenotype: red aleurone, lutescent plant. Since 55 of this phenotype were observed, the total number of recombinant gametes produced by the hybrid would be $55 \times 4 = 220$. This represents 31% of the total plants, indicating a map distance of 31 units.

The other distances were calculated on the basis of the recombinant phenotypes observed multiplied by eight, since, out of the eight recombinant genotypes possible, only one will produce the double recessive recombinant phenotype. The distances calculated in this way are: $lu_1 - bm_1$ 12 units, and $lu_1 - bt_1$ 13 units. According to the maps of Neuffer (MNL 40:167-172), these data would place lu_1 at map locus 9 on chromosome 5.

David K. Shortess

2. Inheritance patterns and distribution of the low temperature-chlorosis genes in Oh51A.

A low temperature-chlorosis effect in maize inbred line Oh51A has been described (MNL 41:152-153). F_1 and F_2 populations were on hand which involved this line of Oh51A as one parent and material which displayed no low temperature response as the other parent. Seeds from these populations were germinated at $10 \pm 1^\circ\text{C}$ and were scored for the chlorotic condition. All F_1 seedlings were green. In the F_2 population 710 seedlings were green and 53 were chlorotic, a good fit to a 15:1 ratio ($p=0.45$). These results indicate a bigenic pattern of inheritance. It appears that two recessive genes are required for this expression. These genes have tentatively been designated cold-chlorotic-1 and -2 (cc_1 and cc_2).

In an effort to determine whether or not this was a universal trait in Oh51A, seeds of this line were obtained from a number of agricultural experiment stations across the country. The results of germinating these seeds at $10 \pm 1^\circ\text{C}$ are presented in Table 1. It is apparent from these data that this trait is not universal with this line. It was indeed fortuitous that the original material used for this work, obtained from the Pennsylvania Agricultural Experiment Station, was homozygous for these genes.

Table 1
The distribution of the cold-chlorosis genes

Oh51A Source	Seedlings (at 10 ± 1°C)	
	Green	Chlorotic
Illinois	0	17
Indiana	0	3
Iowa	4	16
Minnesota	2	18
New Jersey	6	10
New York	11	10
North Dakota	8	9
Ohio	8	12
Pennsylvania	0	182
South Dakota	2	5
Wisconsin	12	6
Wisconsin (outcrossed and recovered)	12	0

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1. Studies on cytoplasmic male sterility in maize.

It was of interest to determine if cytoplasmic male sterility in corn is specific for male florets or if it could confer sterility in both male and female floret born on the tassel. To study this problem the inbred line T 204 ms, which is male sterile due to the Texas type cytoplasm, was crossed with stocks heterozygous for the dominant tassel seed gene, either Ts₅ or Ts₆. Ts₅ and Ts₆ plants produce tassels which contain silks and anthers. Some kernels usually develop on the tassel, although the tassel is very susceptible to smut. Seed from the cross was grown in the field. Approximately half of the plants segregated for the tassel seed phenotype, that is, silks were born on the tassel. All plants were completely male sterile. Tassel born silks were allowed to open pollinate and then covered with bags to prevent bird predatoriness. At maturity the seeds set on the tassels were harvested and planted in the greenhouse. These seeds

germinated and the resulting plants were grown until after tassel emergence. Again all plants were male sterile and segregated about 50% for the tassel seed characteristic.

These observations clearly showed that fertile female florets do exist on tassels where all male florets contain aborted pollen. Thus, sterility conferred by this cytoplasm is specific for male florets and is not associated with the tassel location since female florets are fertile.

Studies^{1/} have shown that stamen-less tomatoes can be induced by gibberellin A₃ to form anthers and viable pollen. This report suggests that gibberellin may play a determinative part in the development of male gametophytes. Therefore, the male sterile (Texas type) and normal cytoplasmic versions of the corn inbred line T 20⁴ were selected for treatment with plant hormone to ascertain any effect on pollen fertility. Five treatments were chosen, a control (no hormones), low GA₃, high GA₃, IAA, and kinetin and GA₃. Hormones were pipetted twice weekly into the plant whorl in 1 ml volumes. Treatments were begun shortly after the plants emerged from the ground and were terminated at the time of tassel emergence. Thirteen treatments were applied in this interval. Total amounts of hormones applied to each plant were; low GA₃, 130 ug; high GA₃, 260 ug; IAA, 130 ug; and kinetin and GA₃, 130 ug of each.

After termination of treatments, tassels were observed with regard to pollen shed. T 20⁴ with normal cytoplasm shed its normal viable pollen regardless of hormone treatment. T 20⁴ ms with male sterile cytoplasm shed no pollen in spite of hormone treatments. Consequently, these hormones were ineffective in altering the expected pollen fertility or sterility. GA₃ treatments did increase early height of plants, caused a yellowing in plant color and in general gave a less thrifty looking plant. The latter observations have been reported previously.

^{1/}Phatak, S. C., S. H. Wittwer, S. Honma, and M. J. Bukovac. Gibberellin-induced Anther and Pollen Development in a Stamen-less Tomato Mutant. *Nature* 209:635-636. 1966.

C. S. Levings, III

2. Genetic variability for coleoptile elongation.

It has been known for years that coleoptile elongation in grasses is mediated by auxin. It is reasonable to assume that auxin production and/or regulation is under genetic control. Preliminary studies with corn, which were conducted primarily to develop experimental technique, involved a number of different inbred lines, including a sample of random inbreds of the varieties, Jarvis and Indian Chief. Due to limited seed supply, studies with inbreds have been postponed until seed increases are available. However, these studies suggested that inbred lines differ somewhat with respect to coleoptile elongation and response to exogenous IAA. Critical statistical tests of these differences were not possible, but differences were of sufficient magnitude to strongly suggest genetic differences among inbreds.

An experiment was then conducted in a varietal composite (Jarvis x Indian Chief) F_5 to provide a statistical test for genetic variability for three traits related to auxin regulation of coleoptile elongation. First, dark grown coleoptiles were measured six days after planting. Second, oat coleoptile sections, grown in the presence of corn coleoptile tips, were measured after 19 hours. Oat sections, therefore, served as a bioassay for auxin produced by the corn coleoptile tips. Last, corn coleoptile sections in vitro were provided exogenous auxin, IAA, and their response measured. The first trait gives an in vivo measure of coleoptile growth which should reflect auxin production as well as responsiveness to auxin. The second and third traits provide in vitro measurements of auxin production and responsiveness to auxin, respectively. The above description of the system is recognized to be an oversimplification and therefore, other factors contributing to growth may be confounded with our measures of auxin response.

Seeds used in these studies were treated with the fungicide thiram and planted in plastic shoe boxes on 6 layers of paper towel and covered with tissue paper. One hundred ml of distilled water were added to each box. All seed and coleoptiles were grown in a dark room maintained at approximately 72°F. This room was kept dark at all times except for a safe light which emitted non-stimulatory wave lengths. After five days growth, coleoptile length was measured on ten plants. In addition, plants whose coleoptile length was between 1.0 and 1.49 cm were selected. Three mm tips and five mm sections were cut from the coleoptiles. Five mm sections were taken by first removing the 3 mm tip and then cutting off the next 5 mm. Fifteen 3 mm corn tips were placed in a shell vial with 5 sections of oat coleoptile (5 mm long) and 3 ml of phosphate buffer (pH6.0) plus 1% sucrose. The oats were grown and coleoptiles were cut in the same manner as the corn. After 19 hours growth, oat section elongation was measured. For the third trait, 5 corn coleoptile sections (5 mm long) were placed in a Petri dish with 10 ml of phosphate buffer (pH6.0) plus 1% sucrose and 20 ug of IAA and 1 ml of water. In addition, a control was run identical to the above except no IAA was added. After 19 hours growth, corn section elongation was measured. The difference between IAA treated and the untreated sections was determined and used as a measure of response to IAA.

The genetic material consisted of progeny of randomly controlled crosses involving 36 plants used as male parents each with 2 plants used as females. The 36 male groups comprising a total of 72 full-sib families were randomly assigned to sets of 6 male groups each. The 12 families of a set were grown together in the darkroom during the same time period. Three such sets were planted on consecutive days each week, so that the entire experiment was completed within a two week period. The three sets which had been grown the first week of the experiment were then repeated on the third week to provide a measure of repeatability.

Data of each set were analyzed separately and then pooled to provide an overall analysis. For those sets which were involved in two runs, a combined analysis was computed to remove effects of Run x Family interaction.

Results from the analysis of coleoptile length indicated significant variability due to females, but variability attributable to male

differences was non-significant. Analysis of response of oat sections to corn tips and corn sections to exogenous IAA indicated that differences due to females or due to males were non-significant. These results suggest that the genetic variability of coleoptile elongation after six days in darkness is primarily non-additive genetic variance. Magnitude of genetic variability for auxin production (as assayed by oat coleoptiles) and responsiveness to auxin (as determined by exogenous IAA application to corn coleoptile sections) is insufficient to be detected by these experiments.

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1. Diffuse: a dominant pigment inhibiting gene.

A. Distribution of Diffuse in indigenous populations of Peru

At the time of the initial report on Diffuse (Brink and Greenblatt, Jour. of Hered. 1954) the gene was known to have come from Peru and was thought to be rare. A recent (January 1968) search for the Diffuse (Idf) gene in Peru has disclosed that it is not rare, but is rather widespread throughout the country. In Table 1 are listed the Districts of Peru in which the mutable form of the gene was identified. The principal source of this information was the collection of indigenous races of maize kept at the Universidad Agraria, La Molina, Peru. In addition, a search was made of rural market places, farmers' seed supplies and phenotypes of plants currently being grown.

The recovery of 23 distinct sources from 9 districts represents a minimum estimate of the distribution of Idf. Since only the mutable form of the gene in a colored pericarp background is unique enough for absolute recognition by phenotype alone, the two other forms or states of Idf, fully active and relatively inactive would have gone undetected. The disproportionately high number of locations in the Ancash Districts most likely means a more intense collecting from this region rather than a higher gene frequency. The single collection from Puno may be erroneous. The ear type expressing the Idf mutable phenotype, I was told, does not correspond to the races known from Puno and is most likely a mislabeling of the museum sample.

B. Field search for Diffuse in Peru.

While in Peru, an effort was also made to discover the presence of mutable forms of the gene by searching during or after the time of pollination for stripes on plants with colored stems. In the Cuzco region, while 97% of the plants exhibited intense plant color, no striped plants were found, nor were any totally green plants (the fully active form of Diffuse) found. The same was true for the Huancayo District--96% full pigmentation and no striping and no totally green plants.

Table 1
 Frequency of mutation of R^{st} to R^{sc} when homozygous and when heterozygous
 with several other alleles and in male and female
 gametes

Allelic combinations	No. of tests	R^{sc} frequency	Rate $\times 10^{-4}$	Limits of expectation (P=.05)	
				lower	upper
<u>Female Gametes</u>					
$R^{st} M^{st}/R^{st} M^{st}$	2	41/ 23,830	17.2	12.3	23.3
$R^{st} + /R^{st} +$	3	129/ 60,576	21.3	17.8	25.3
$R^{st} M^{st}/R^{st} +$	1	67/ 30,898	21.6	16.8	27.5
Pooled		237/115,304	20.5	18.0	23.3
$R^{st} M^{st}/R^r +$	3	53/ 28,545	18.6	13.9	24.3
$R^{st} M^{st}/r^r +$	1	1/ 2,055	-	-	-
$R^{st} + /r^r +$	3	23/ 23,406	9.8		
Pooled		24/ 25,461	9.4	6.0	14.0
$R^{st} M^{st}/r^g +$	1	14/ 19,239	7.3	4.0	12.2
$R^{st} + /r^g +$	2	10/ 13,078	7.6	3.7	14.1
Pooled		24/ 32,317	7.4	4.8	11.0
<u>Male Gametes</u>					
$R^{st} M^{st}/R^{st} M^{st}$	3	390/ 92,122	42.3	38.2	46.7
$R^{st} M^{st}/R^r +$	1	59/ 30,400	19.4	14.8	25.0
$R^{st} M^{st}/r^r +$	1	53/ 30,236	17.5	13.1	22.9
$R^{st} M^{st}/R^{st} M^{st}/r^g +$ (trisomic)	1	132/ 29,710	44.4	37.2	52.7

somewhat blunted leaves which are wider than normal siblings. The mature compact plant develops to at least three feet in height, or to nearly 50 per cent of the height of normal siblings; the internode number is the same, but the average internode length is two inches shorter than in the normal siblings. The shortened internode length is evident throughout the mutant plant and is not confined to those internodes below the ear node. The mutant plants have enlarged stalks. The ear is reduced in size and the tassel is compacted, but pollen is shed abundantly and ears are produced on most plants.

Intercrosses were made with thick-tassel-dwarf (ttd) in chromosome 5, Hy-rd and ct obtained from Dr. O. E. Nelson, none of which gave a positive allele test. Phenotypically the mutant appeared not to be similar to d₁ or d₂ in chromosome 3, or br₁ in chromosome 1.

The mutant was crossed to a waxy marked chromosome-nine translocation series involving all chromosomes and F₂ waxy seeds were screened. All F₂ populations showed normal 3:1 segregation except those involving the wx 1-9c (1S.48, 9L.22) and wx 1-9⁴⁹⁹⁵ (1L.19, 9S.20) interchanges in which the following data were collected. Waxy seeds from 4 families involving the cross with wx 1-9c gave 57 normal : 7 compact plants, and waxy seeds from 3 families involving the cross with wx 1-9⁴⁹⁹⁵ gave 74 normal : 14 compact plants. These data gave indications of linkage only with chromosome 1. This mutant is tentatively designated codw.

Dwarfs such as midget 8043, tiny 8446, dwarf 4963 and others have been associated with chromosome 1, but the codw phenotype is not similar. A test of allelism with br₁ has not been made. Its position in relation to the known markers on this chromosome is at present uncertain.

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1. Frequency of R^{st} to self colored (R^{sc}) aleurone mutations.

Early in the studies of paramutation at the R locus it was observed that Rst mutated to R^{sc}, and the frequency of such mutations has been reported by several investigators. The results from recent tests, together with some previously reported, are brought together in Table 1. The frequencies shown are the number of verified R^{sc} mutations over the number of stippled gametes tested. Where verification of self colored kernels was not accomplished the number of stippled gametes tested was proportionately reduced. The number of kernels scored was determined by actual counts or estimated from the weight of a sample of 500 to 1000 kernels from each family.

Certain of the allelic combinations have been tested more than once, and the number of tests and pooled frequency are shown in Table 1. Prior to pooling, the limits of expectation were calculated for each test and in

It is hypothesized that a deoxyribonucleotide which forms a complex with the DNA via a specific agent is responsible for the peculiar base composition. The synthesis of the atypical DNA from Black Mexican Sweet Corn appears to depend on the stage of kernel development and probably coincides with the time of pigment development. The abnormal base ratios reported previously are presumably not related to heterochromatin content.

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1. A new striate mutant on chromosome 10.

A new mutant type was isolated which has longitudinal white stripes that parallel the leaf venation from early seedling stage onward. This mutant arose in M_2 segregating material from the combined chemical mutagen treatment of ethyl methanesulfonate followed by diethyl sulfate to the seed of a multiple marker stock used in mutation experiments. This character is recessive and very similar to Waseca stripe (sr_2) in chromosome 10 which was described by Joachim and Burnham (1953) MNL 27:66. Classification is good in the seedling and mature plant stage and pollen and ears are produced on most plants.

Intercrosses were made with homozygous recessive $sr_2 sr_2$ stocks obtained from Dr. R. A. Brink. There were no striate individuals among 193 F_1 plants from eight crossed ears.

The mutant was crossed to a waxy marked chromosome-nine translocation series involving all chromosomes and F_2 waxy seeds were screened. All F_2 populations showed normal 3:1 segregation except those involving the wx 9-10b interchange (9S.13, 10S.40) in which the following data were collected in ten families. Waxy seeds gave 349 normal : 12 striate plants. These data indicate that the mutant is located close to the interchange point on the short arm of chromosome 10, whereas the Waseca stripe (sr_2) gene has been placed distal to R on the long arm of chromosome 10. The symbol, sr_3 , has been assigned tentatively to this new mutant.

David V. Glover

2. A compact plant gene located on chromosome 1.

This mutant was given to this station by Allan Caspar of the Blandy Experimental Farms. The mutant produces seedlings which have very wide and

On the other hand the inbred Pa 422P appears to have two genes exhibiting complementary action for resistance to the MDM virus. When the cross Pa 422P x Pa 887P was selfed, the F_2 segregated in a ratio that appeared to fit a ratio of 9 resistant to 7 susceptible. A population of 400 seedlings gave 216 resistant and 184 susceptible. The X^2 probability for a 9:7 ratio for this is .30 - .50. Another inbred, Oh 7B, also appears to have 2 genes with complementary action for resistance. Further tests of both Pa 422P and Oh 7B are being conducted to verify the validity of these results.

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1. DNA from Black Mexican sweet corn.

We reported previously that the base ratios of DNA from young kernels of lines of Black Mexican sweet corn with and without B-chromosomes differed from those of a white inbred line. Other workers have failed to find these abnormal ratios using leaf material. Our determinations were repeated using material from young seedlings and leaves and mature husks in addition to kernels. The methods used are described in detail elsewhere.

The results are given in Table 1.

Table 1
Base composition of DNA-preparations of three
lines of Zea mays

Composition in moles per cent (Average of 2 or more determinations)	
Material	% C + G
K64--commercial white dent inbred	44.0
Black Mexican Sweet Corn with no B-chromosomes; leaves	44.0
husks	45.0
seedlings	42.0
kernels (10 days after pollination) colorless	44.5
kernels (>14 days after pollination) colored	55.0
Black Mexican Sweet Corn with B-chromosomes	
leaves	46.0
husks	42.0
seedlings	43.0
kernels (10 days after pollination) colorless	46.0
kernels (>14 days after pollination) colored	70.0

5. Transformation of sex?

The C^I inhibitor mutant was grown for seed multiplication on 19th August 1967. The seed set was poor. It is interesting to note that 29.4% of the plants (47 out of 153) showed tassel seed, which is quite unusual and could be due to photoperiodism.

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1. Inheritance of resistance to strain A of Maize Dwarf Mosaic Virus.

In Maize Genetic Coop News Letter 40, 1966, p. 121 Wernham and Mackenzie reported on monogenetic control of resistance to M.D.M.V. (Strain A) in the inbred line Pa 11. The double cross Pa 444 selfed (Pa 54 x Pa 11) (Pa 32 x Pa 33) did not reveal a recognizable ratio in that 362 seedlings were symptomless whereas 454 susceptible plants could be separated into 4 distinct groups.

In the 1967 season an F_2 population of inbred Pa 405 (resistant) x 63-604 (susceptible) was inoculated with strain A of MDMV. A population of 570 seedlings gave 426 resistant: 144 susceptible. An analysis of the data revealed the X^2 probability for a 3:1 ratio to be .90. The data support a single gene hypothesis for MDMV control in Pa 405.

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2. Further studies on the inheritance of resistance to MDMV.

Additional data in support of the single gene hypothesis for MDMV resistance in Pa 11 has been obtained. In greenhouse inoculated tests the cross Pa 11 x W153R gave all resistant plants. The F_2 segregated in a ratio of 3 resistant to 1 susceptible and the backcross to the susceptible parent, W153R, segregated in a 1:1 ratio. The data as observed:

	<u>Resistance</u>	<u>Susceptible</u>	<u>Total</u>
1. Pa 11 x W153R	92	1	93
2. (Pa 11 x W153R) \otimes	71	31	102
X^2 probability for 3:1 segregation is .20 - .30			
3. (Pa 11 x W153R) W153R	52	49	101
X^2 probability for 1:1 segregation is .70 - .80			

A negative correlation was found between methionine content of protein and the protein content for 16 varieties. The correlation co-efficient (r) is -0.54909 and the regression line was found to be $y = 3.2139 - 0.1142 x$. However, when the six hybrids along with opaque-2 and local varieties were considered these values were found to be $r = - 0.873$; $y = 4.2159 - 0.2287 x$.

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2. A correction for lysine content.

In last year's Maize News Letter (1967) seven varieties of maize were analyzed for protein and amino acid composition. The lysine content was reported higher (ranging from 0.52-1.18) due to the use of lysine hydrochloride as a standard for bioassay and should have been multiplied by 0.8 to get the following correct lysine content in each variety, Opaque-2 4.74, Deccan hybrid 2.82, Ranjit 2.36, Ganga 101 2.08, Ganga-3 2.80, Hi Starch 2.21, Ganga sufed-2 2.40, Local variety 2.35.

G. M. Reddy

3. Incorporation of the opaque-2 gene into Indian inbred lines.

A breeding program was initiated in collaboration with the Maize Research Station (Amberpet, Hyderabad) to incorporate the opaque-2 gene in the desired background of Indian inbred lines and to ultimately recover the varieties with the opaque-2 gene. Thirteen inbred lines were used, including CM 105, CM 109, CM 110, CM 111, CM 201 and CM 202 which went into the leading Indian hybrids.

The opaque-2 gene in the yellow and white background was recovered by selfing the F_1 of these inbreds. The work is being continued with back-crossing the selected F_2 seed to their respective recurrent inbred lines. These varieties will be further tested for protein and lysine content as well as other amino acids in order to evaluate the performance of the opaque-2 gene in different genotypic combinations.

G. M. Reddy
K. Vaidyanath

4. Reversion of opaque-2 by diethyl sulfate (DES).

Opaque-2 white seeds (667) were treated with a 0.05 M concentration of DES for 8 hours by changing the treatment with fresh solution every hour. Besides a few chlorophyll sectors, five yellow seeds were obtained on independent selfed ears. Further tests will eliminate the possibility of contamination.

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V. S. Bharathi
K. Vaidyanath

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1. Protein and amino acid content studies in single crosses of Indian hybrids.

The eight single crosses of Deccan hybrid, Ranjit, Ganga 101, Ganga-3, and opaque-2 as control have been analyzed for crude protein and for lysine, tryptophan, and methionine content. The procedure and technique used were the same as described earlier (M.N.L. page 162, 1967). The results are presented in Table 1.

Table 1
Protein and amino acid composition of single crosses of maize.

Variety	Single cross	Crude protein %(Nx6.25)	Amino acids (gms/16gms N)		
			Lysine	Methionine	Tryptophan
Deccan hybrid	CM 104x105	11.42	2.90	1.94	0.42
	CM202x201	10.55	2.82	1.97	0.45
Ranjit	CM 103x104	12.69	2.70	1.86	0.43
	CM 202x106	11.47	2.57	1.76	0.44
Ganga-101	CM 103x104	12.69	2.70	1.86	0.43
	CM 201x105	11.53	2.54	1.91	0.47
Ganga-3	CM 202x111	8.94	2.84	2.35	0.33
	CM 109x110	9.20	2.82	2.83	0.43
Opaque-2		9.90	4.74	1.74	0.55

3. An alternative to Vavilov's thesis of the meaning of the center of diversity.

Vavilov proposed that the center of diversity of a species might well be the center of origin of the species. The insight being that the peripheral areas represent the migration of the population to less selectively compatible environments--pioneering, as it would be called.

An alternative to this idea is simply that the center of diversity represents solely that region which exerts the least negative selection on the gene pool and heterogeneity can accumulate. This does not suggest center of origin. It is easier to picture the major evolutionary steps taking place in peripheral areas (not necessarily geographic but rather environmental). With introgression, selected mutation, etc. representative migrants return to the non-selective environment and join with other "returning migrants" to interbreed in an "open" non-selective environment where genetic diversity is not eliminated as in the more selective peripheral environments.

Irwin M. Greenblatt

4. In defense of the thesis that R^{nj} is an R gene plus a pattern gene.

Both Brink (M.N.L. 34:122) and Kermicle (M.N.L. 41:199) have presented evidence that R^{st} (a spotting R allele) can recombine with R^{nj} (aleurone pigmented only at top of kernel) and result in an aleurone phenotype of spots limited to the top portion of the aleurone and the base colorless, typical of a Navajo pattern. This contrasts with the Navajo and Stippled elements in repulsion which yield a typical R^{nj} phenotype at the top of the kernel, and R^{st} spotting at the base of the same kernel.

What follows argues for the compound nature of R^{nj} on a functional basis rather than on a recombinational one.

In a mating of $R^{nj}/R^{nj} \text{ } \text{f} \times R^r/R^r \text{ } \text{m}$ all of the resultant kernels were fully and uniformly pigmented. If the R component of R^{nj} was non-functional in the basal cells, kernels with full pigment at the crown and mottled pigment at the base would have been expected (single dose of R via the male). Finding only fully pigmented kernels suggests that the R (aleurone pigmentation) component of R^{nj} , though not producing pigment in the basal region of the kernel itself, interacts with the R^r gene (a potential mottle) which then produces uniform pigment development. Most likely the R^r allele is responsible for the basal pigment rather than the activation of the R component of R^{nj} . In R^{nj}/R^{st} heterozygotes, only spots are found in the basal portion of the kernel and not uniform pigment. The R component of R^{st} does not stimulate R^{nj} to express itself at the base of the kernel but the R component of R^{nj} does stimulate a potential R-mottled allele to produce uniform pigment.

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growing conditions. When air temperatures are low for the 8 month growing season the frequency of heavily pigmented plants is about 96% (pigment is present in the leaf sheath, stem and ear husk but not obvious in the leaf). The ears are so close to the ground that the ear nodes may be below ground. When air temperatures are not limiting (12 month growing season), the colored plant frequency falls to approximately 65% and the plant and ear heights are considerably higher (ears three feet above ground).

2. Colored anthers (dark red or purple) are present in low frequency in contrast to the high colored plant frequency.
3. Agricultural practices are such that no human selection is made for or against plant color directly. Strong and varied selections are made for kernel (endosperm, aleurone and pericarp) pigmentations. The active form of the major pigment inhibitor, Diffuse, would be the only recognized factor selected for kernel effects which would also modify the plant coloration significantly. This active dominant pigment inhibitor was expressed in 23% of the plants in the field where temperatures were not limiting and was totally absent in the two "cold grown" fields. Plant size and planting density under native growing schemes are such that the whole body of the plant is exposed to direct sunlight.
4. Direct temperature measurements by thermister probes (sensitive to 0.10°C) of intensely colored and nearly green plants were made at the same time. The data involve measurements from, (a) the center of the ear at the time of pollination and one month after, (b) the husk surface, (c) the internal stem at the height of the ear, and (d) the stem surface. At all positions of the plant the dark colored plants were warmer than the near green. Dark colored plants were from 0.5°C - 3.5°C warmer than the near green when the sun was on the plant. In early morning both kinds of plants were the same. At night or when air temperatures were dropping the center of the ear and center of the stem were warmer in the colored than in near green plants. When the sun was heating the plants they were above air temperatures and remained so for long periods after heating stopped.
5. Conclusion: When air temperatures are low during the day the plants obtain the needed heat directly from the sun and the dark colored plants do this better than the near green plants. The larger the plant structure, the ear especially, the longer the higher temperatures remain elevated as compared with the more exposed portions of the plant. Thus, the genes for dark plant color (B and Pl) appear to be selected for by this greater heat uptake. While this appears to be the case for plant color, the opposite seems to be the case for anther color.

Night temperatures at these very high locations are very low for corn culture. It is believed that the short plant and low ear height are adaptive features which bring the plant closer to the major heat sink available--the soil.

Table 1
Districts of Peru where the Diffuse gene has been identified

District	Map locations*	Number of locations
Ancash	9° - 78°	13
Apurimac	14° - 73°	1
Cajama	7° - 79°	1
Huancavelica	13° - 75°	1
Huanuco	9° - 74°	2
Junin	11° - 75°	2
La Libertad	8° - 79°	1
Piura	5° - 80°	1
Puno	16° - 70°	1

*Degrees latitude south of the equator - degrees longitude west of Greenwich

In the Ancash District this was not the case. Totally green plants constituted a large proportion (approx. 20%) of one population examined. The mutable form of Idf was found on only one plant. These results from Ancash were unexpected, based upon laboratory experience. The most frequently found state of Idf is the mutable form, unless selection for other states is made. The fully active form is highly unstable, progressively reverting to the mutable form in subsequent generations. It was found that the farmers in Ancash region prefer two types of ears--"Blanco" (white) and highly colored (every pericarp color gene known). One way to have "Blanco" is to lose the major dominant pigment conditioning genes (but they are almost always present), or to select for a dominant pigment inhibitor--the fully active form of Diffuse. Such a selection by the farmer would account for the high frequency of fully active Diffuse.

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2. A possible selective advantage of plant color at high altitudes.

Field observations and temperature measurements of three high altitude corn fields in Peru have disclosed the following (statistical analysis of the temperature measurement is not as yet complete):

1. There seems to be a very high correlation of both (a) high frequency of plant color, and (b) low ear and plant size with low temperature

no case did different tests of the same allelic combination give significantly different estimates of the mutation rate.

The general procedure in all tests of female gametes was to pollinate with an $\underline{r} \underline{r}$ stock carrying a marker gene or genes at other loci to aid in identifying mutant phenotypes attributable to pollen contamination. The self colored kernels were grown out to verify a germinal mutation. In tests of female gametes only about 50% of the self colored kernels proved to be germinally $\underline{R}^{\text{sc}}$, the remainder being $\underline{R}^{\text{st}}$. $\underline{R}^{\text{sc}}$ mutations also are known to occur only in the germ, giving a kernel with an $\underline{R}^{\text{sc}}$ germ but a stippled aleurone; no mutations of this exceptional type are included in the Table 1 data. It is assumed that self color mutants borne singly on an ear result from mutations in the basal megaspore, or during or just prior to megasporogenesis, and that kernels with noncorresponding endosperms and germs result from mutations which occur during formation of the female gametophyte. Rarely, mutations to $\underline{R}^{\text{sc}}$ occur in somatic tissue to give ear sectors of $\underline{R}^{\text{sc}}$ tissue; such sectors were not included in the mutation rate data.

$\underline{R}^{\text{sc}}$ ear sectors were readily apparent on all but $\underline{R}^{\text{r}} \underline{R}^{\text{st}}$ ears but tassel sectors were not and are a possible source of inflated mutation rate estimates in tests of male gametes. In five of the six tests of male gametes, numbered plants were tested individually without bulking pollen, and mutation rates could be calculated for each plant. The number of gametes tested from each plant was necessarily small, and, consequently, the limits of expectation were large. No significant differences were found between individual male plants in any of the tests, but one $\underline{R}^{\text{r}} \underline{R}^{\text{st}}$ plant gave a mutation rate sufficiently greater than that of other plants in the test so as to make tassel mosaicism suspect; mutations from this plant were not included in the data reported.

Indications of $\underline{R}^{\text{sc}}$ ear sectors on $\underline{R}^{\text{r}} \underline{R}^{\text{st}}$ ears can be obtained by noting the frequency of $\underline{R}^{\text{sc}}$ mutants recovered from each ear. Ears scored in these three tests totaled 191 from which 53 mutants were recovered; one ear yielded three mutants, six ears yielded two, and the remaining 38 mutants occurred singly. There is no evidence in these data of large somatic sectors, and if it were assumed that all mutants had an independent origin, the frequency of ears with more than one mutant does not appear to be greater than would be expected from chance alone.

The tests involved both "stippled" ($\underline{R}^{\text{st}} \underline{M}^{\text{st}}$) and "light stippled" ($\underline{R}^{\text{st}} \underline{+}$), which differ only in the presence or absence, respectively, of a modifier six units distal to the \underline{R} locus. Early data suggested that $\underline{M}^{\text{st}}$ had no effect on the frequency of germinally recoverable $\underline{R}^{\text{st}}$ to $\underline{R}^{\text{sc}}$ mutations, and this is substantiated by the data in Table 1: compare lines 1 and 2, and lines 9 and 10. In the absence of any effect of $\underline{M}^{\text{st}}$ the data from tests of stippled and light stippled were pooled and used to compute a single mutation rate for the various allelic combinations.

In female gametes, $\underline{R}^{\text{st}}$ mutated to $\underline{R}^{\text{sc}}$ more frequently when homozygous than when heterozygous with \underline{r}^{r} or \underline{r}^{g} (lines 4, 8, and 11). The rate in $\underline{R}^{\text{r}} \underline{R}^{\text{st}}$ plants (line 5) was about the same as in $\underline{R}^{\text{st}} \underline{R}^{\text{st}}$ plants and greater than in $\underline{R}^{\text{st}} \underline{r}^{\text{r}}$ and $\underline{R}^{\text{st}} \underline{r}^{\text{g}}$ plants, although the difference between $\underline{R}^{\text{r}} \underline{R}^{\text{st}}$ and

$\underline{R}^{st} \underline{r}^r$ was not quite statistically significant.

The frequency of \underline{R}^{sc} mutations was significantly greater in male than in female gametes in $\underline{R}^{st} \underline{R}^{st}$ plants (line 4 vs. 12), approached significance in $\underline{R}^{st} \underline{r}^r$ plants (line 8 vs. 14), and was clearly not significant in $\underline{R}^r \underline{R}^{st}$ plants (line 5 vs. 13).

The frequency of \underline{R}^{sc} mutations was the same in male gametes from $\underline{R}^{st} \underline{R}^{st} \underline{r}^g$ (trisomic) as from $\underline{R}^{st} \underline{R}^{st}$ plants (line 12 vs. 15), which indicates that the greater mutability of \underline{R}^{st} when homozygous than when heterozygous with \underline{r}^g is due to interaction between \underline{R}^{st} alleles in the homozygote rather than to a mutation inhibiting action of \underline{r}^g in the heterozygote.

R. B. Ashman

2. Gene linkages in translocation T9-10a heterozygotes.

Translocation T9-10a (9L.14--10L.92) has been used as a distal marker for \underline{R} in several of our genetic studies, and a test was made to determine the linkage between the translocation and \underline{R} and \underline{M}^{st} on chromosome 10 and \underline{Wx} on chromosome 9.

Kernels from the following cross were classified for stippled and waxy:

$$\frac{r + N \underline{wx}}{r + N \underline{Wx}} \quad X \quad \frac{\underline{R}^{st} \underline{M}^{st} \text{ T9-10a } \underline{wx}}{r \quad + \quad N \quad \underline{Wx}}$$

The number of kernels in the two parental classes was 1067 $\underline{R}^{st} \underline{wx}$ and 989 $\underline{r} \underline{Wx}$, and in the two crossover classes 291 $\underline{R}^{st} \underline{Wx}$ and 204 $\underline{r} \underline{wx}$. An excess of \underline{R}^{st} kernels was noted in both the parental and crossover classes. The uniformity of the data was tested in a 2 X 2 contingency table, and the chi-square value was highly significant, indicating inconsistencies within the class frequencies. The excess of $\underline{R}^{st} \underline{Wx}$ kernels is very likely due to the functioning of some $\underline{R}^{st} \underline{Wx}$ duplicate-deficient gametes, since these gametes are deficient for only about 8% of 10L. The $\underline{r} \underline{wx}$ duplicate-deficient gametes would be much less likely to function, since they are deficient for about 86% of 9L. If it is assumed that $\underline{r} \underline{wx}$ duplicate-deficient gametes do not function, the $\underline{r} \underline{wx}$ kernels result only from crossing over, and the percentage of $\underline{R}^{st} \underline{Wx}$ kernels resulting from the functioning of duplicate-deficient gametes can be estimated as $291-204/291=30\%$. Also, if alternate and adjacent-1 disjunction are assumed to occur with equal frequency, it can be estimated that about 7% of the $\underline{R}^{st} \underline{Wx}$ duplicate-deficient gametes produced function through the pollen, even though in competition with normal gametes.

Kernels in the two crossover classes were grown out and the ears classified for the translocation (semi-sterility). Ears from the $\underline{R}^{st} \underline{Wx}$ kernels were also classified for \underline{M}^{st} , which is scorable only in the presence of \underline{R}^{st} . Kernels in the two parental classes ($\underline{R}^{st} \underline{wx}$ and $\underline{r} \underline{Wx}$) were not grown out so no data were obtained on the frequency of double crossovers.

Two adjustments were made in the data before crossover per cents were calculated: (1) the total population was adjusted for the proportion of

crossover kernels verified in the classifications for the translocation and \underline{M}^{st} , and (2) the frequency of kernels in the \underline{R}^{st} \underline{Wx} crossover class was reduced to the same proportion as the \underline{r} \underline{Wx} crossover class to correct for the presumed transmission of duplicate-deficient gametes. The data from the \underline{R}^{st} and \underline{r} kernel classes are presented separately in the tabulation below, and where data for a particular chromosome segment were obtained from both classes a pooled value is shown.

The $\underline{R} - \underline{M}^{st}$ distance has been measured in nontranslocation stocks (Ashman, Gen. 45:19-34) and was found to be 797/13,881 or 5.7 crossover units. The difference between this value and the one shown in the table, 0.4, represents the crossover suppression effect of the translocation. The $\underline{R} - \underline{T}$ distance has been estimated by others to be about 5 crossover units; our data estimate this distance to be, at most, 2.9 units, and the pooled data gave a value of 2.3 units.

Chromosome region	\underline{R}^{st} kernels		\underline{r} kernels		Pooled	
	Frequency	%	Frequency	%	%	
R - \underline{Wx}	-	-	204/1193	17.1	-	
R - \underline{M}^{st}	5/1136	0.4	-	-	-	
R - \underline{T}	19/1136	1.7	31/1070	2.9	2.3	
\underline{M}^{st} - \underline{T}	14/1136	1.2	-	-	-	
$\underline{T} - \underline{Wx}$	161/1136	14.8	144/1070	13.5	13.8	

R. B. Ashman

3. The location of miniature seed (mn).

The location of the miniature seed (\underline{mn}) character has been shown to be on chromosome 2 (MGCNL 39: 158, 1965). Evidence of the position of miniature seed (\underline{mn}) on chromosome 2 comes from the following testcross data.

W22 was crossed by a chromosome 2 tester carrying \underline{lg}_1 , \underline{gl}_2 , \underline{v}_4 and \underline{mn} and backcrossed to the chromosome 2 tester. A total of 452 normal kernels from this cross was planted and scored for \underline{lg}_1 , \underline{gl}_2 , and \underline{v}_4 with the following results:

		Marker	Total	% Recombination
+	+			
+	+	\underline{lg}_1	219	48.5
+	\underline{gl}_2	\underline{gl}_2	172	38.0
+	\underline{gl}_2 \underline{lg}_1	\underline{v}_4	72	15.9
\underline{v}_4	+			
\underline{v}_4	+	\underline{lg}_1	10	
\underline{v}_4	\underline{gl}_2		1	
\underline{v}_4	\underline{gl}_2 \underline{lg}_1		21	
			<u>452</u>	

This would indicate that miniature seed (mn) is located between v₄ and gl₂ having 16% recombination with v₄ and 38% recombination with gl₂.

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1. Variable reaction of the monogenic resistant varieties Lady Finger Pop Corn and G.E. 440 to H. turcicum.

Lady Finger Pop Corn (PI. 217407) and inbred G.E. 440 have been reported by Hooker (1963) to carry dominant monogenic resistance to H. turcicum, the causal organism of the northern leaf blight of maize. He further observed that the genes controlling restricted chlorotic type of lesions in the two cultures were either alike, allelic or very closely linked. Recently Sharma and Aujla (1966) for the first time observed that the variety Lady Finger Pop Corn was highly susceptible to leaf blight in the northern hills of India (Kulu Valley). Since this observation differed from the report by Hooker, it was thought that the H. turcicum culture prevailing in India may be a different biotype than the isolates used by Hooker for studying the reaction of the variety. Prompted by this, it was thought desirable to study the reaction of G.E. 440 also to the prevailing biotype in the field inoculum.

The observations were taken on Lady Finger Pop Corn as well as on G.E. 440 at two different locations, viz. Bajaura (Kulu Valley) and Hyderabad. The reaction type on the two cultures was characteristically distinct at both places. On G.E. 440 the lesions were typical as described for the inbred by Hooker giving a restricted chlorotic type, while in the case of Lady Finger Pop Corn the lesions were clearly of the susceptible type as reported earlier by Sharma and Aujla. Later on these findings were confirmed under controlled conditions at the seedling stage in pots at Bajaura.

This sort of differential reaction of the two varieties indicates the presence of either different alleles at the same locus or different closely linked genes. The study is in progress and an F₂ population from the cross between Lady Finger Pop Corn and G.E. 440 will be studied for reaction to leaf blight in the 1968 rainy season.

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1. Determination of amino acids in maize through a microbiological method.

Since the discovery that varieties of maize containing the opaque-2 (o-2) gene have higher amounts of certain amino acids, mainly lysine, there is

an increased interest in the analysis of other varieties of maize for their content of lysine and other amino acids. As it is frequently not possible to have access to an amino acid analyzer, other methods, such as microbiological ones, can be used for this purpose. We have developed a simple microbiological method for the assay of methionine, lysine and phenylalanine which can be used for maize. The three amino acids were assayed through nutritional deficient mutant strains of the fungus Aspergillus nidulans by a technique first developed by Princivale and Caradona (Rend. Inst. Sup. Sanità, 26, 75) for the microbiological assay of vitamins. In our case, the diameters of the growth zones for the amino acids were linear functions of the logarithms of the doses for solutions of 15.625 ug/ml to 500 ug/ml for methionine and 156.25 ug/ml to 5000 ug/ml for lysine and phenylalanine. Using an acid hydrolysate of endosperm of opaque-2 maize, the results obtained were comparable with those found through the use of amino acid analyzers. In conclusion, the method presents several advantages: the media used are simple inexpensive preparations; the strains can be maintained for years without changing properties; the standard deviation obtained was never more than 9%; the fungus is resistant to penicillin so that this antibiotic can be added to the medium reducing the danger of contamination during the assay; responses are highly specific and are not influenced by other substances. The method can be useful for those people who have no facilities for analyzing their local varieties of maize for amino acid content through an amino acid analyzer.

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2. A preliminary report on the literature related to the history of the races of maize in Brazil.

Although in certain areas of Latin America the variation among the races of maize is very great, the races of maize from the lowland regions of southeastern South America encompassed by eastern and southern Brazil, Uruguay, Paraguay, eastern Argentina and certain parts of Bolivia have relatively little variability. The region was occupied by several different Indian groups which are known to have cultivated maize and from which Brieger et al (1958) managed to obtain collections. The descriptions of maize encountered in the literature are remarkably consistent with those of ethnologists who have observed the remnant Indian populations in recent years. Although there appears to have been some transposition of names, the collections of maize described by Brieger et al seem to correspond quite well to the early descriptions also.

The earliest descriptions¹ (Thevet, 1556; Lery, 1578; Souza, 1587) indicate that the maize commonly encountered in the coastal regions of Brazil was a

¹The non-twentieth century dates cited are those of the earliest known editions except in case of extreme delay in publication for which the actual year that the article was written is cited.

white flint, and that there were cream, black (or purple), and/or red (or purple) variants in lesser proportions. In addition, a floury maize was cultivated (which was probably also predominately white).

The white flint corn was generally called either "Avati tupi" or "Avati ata" or variants thereof (Montoya, 1639; Azara, 1809; Dobrizhoffer, 1784; Graty, 1865), although these may be generalized names for flint corn rather than specific names for the white flint under discussion. The white flour corn was generally called either "Avati ti" or "Avati moroti" (Montoya Azara, Dobrizhoffer, Graty). Yellow corn was generally called "avati yu" (Graty, Montoya), while the first description of what appears to be the pointed popcorn of the region was presented by Dobrizhoffer, who called it "bisingallo."

Most of coastal Brazil was inhabited at the time of its discovery by various groups of Tupi (the Portuguese name) or Guarani (the Spanish name) Indians to whom the maize types described above belonged. Much less is known about the other groups of Indians of Brazil. The group called the Ge Indians is particularly important, but reports about their agriculture are quite conflicting. They have been reported to have been non-agricultural until being pacified during the present century (Silva, 1930 referring specifically to the Caingang tribe); as having been agricultural at the time of the discovery, but as having quit the pursuit of agriculture soon thereafter, re-adopting it recently (Henry, 1941, also referring to the Caingang); and as having been agricultural continuously since well before the discovery (Nimuendaju, 1946). The maize of the Caingang tribe described by Brieger et al. and Barboza (1913) appears to differ from the maize of the Timbira tribes of central Brazil described by Nimuendaju only in having larger kernel size. The Suya Indians, another Ge tribe from central Brazil, are reported to have had a small-eared maize with golden colored kernels at the time of their discovery (Steinen, 1894).

In general the long-eared, late-maturing floury corn of the deep interior was called "pururuca" or "saboro" (Hunnicuttt, 1933; Roquette-Pinto, 1935). It has been called "interlocked" by Brieger et al. and is closely related, if not identical to the race Corioco described by Cutler (1946) and Ramirez et al (1961). Unfortunately very little is known about the distribution of (or about the variation present within) this race.

There seems to be little indication in the early literature that the race "cateto" (or "catete") could have been indigenous to southeastern South America. No mention of an orange-colored flint corn has been encountered, although Souza and Azara mention light yellow-colored flints and Souza also mentions a red-colored flint corn. Although cateto is sometimes said to be "red" ("vermelho"), Lery left no doubt when he said "rouge, . . . bled farrazin". Even the early descriptions of cateto emphasize that it was limited to the coastal regions (Velloso, about 1800), and whereas good descriptions are available for atati ti, avati ata, avati moroti, avati tupi, and "bisingallo" at very early dates, nothing is available for cateto until after 1800.

The word "tupi", as used in the name "avati tupi", has been accepted by Serrano (1936), Schaden (1954), and Brieger et al. (1958) to signify "enemy"

or "something strange or foreign", and Schaden reported that "avati tupi" is said to be a type of maize obtained by the Guarani from their neighbors, the Caingangs, whom they call "tupis". However, at least two other hypotheses exist. Luccock (1820) translates "tupi" as "the excellent people" while Bertoni (1914) says that "tupi" signifies everything that is not civilized, everything that has not evolved from its inferior state; when applied to objects, plants, or animals, he says it always means the most rustic and primitive. Obviously the correct interpretation is important in the case at hand.

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1. Methylation of DNA as the molecular basis for paramutation in maize.

Several parallels can be drawn between the phenomenon of host-controlled modification of phage, and that of paramutation in maize. Recent evidence has established that the basis of the host-controlled modification process, at least in some instances, is a specific enzymatic alteration of DNA. This realization that alteration of DNA, by either glucosylation or methylation, has a specific biological effect prompts consideration of these processes as the possible molecular basis for paramutation in maize.

It is clear from recent reviews [Srinivasan and Borek, Prog. in Nucleic Acid Res. and Mol. Biol. 5: 157 (1966); Borek and Srinivasan, Ann. Rev. Biochem. 35: 275 (1966)] that the enzymatic alteration of nucleic acids is a highly specific process. In particular, the process of methylation of DNA is apparently of universal biological distribution and has the specificity to reasonably accommodate the specific allelic interactions which occur in some paramutation systems. Moreover, the biological effects of methylation of DNA, as exemplified by host-controlled modification, suggest that certain of the properties distinguishing paramutation systems (invariability of occurrence, occurrence in somatic cells, reversibility of paramutant, and metastabilized states) could be expected as consequences of the process of specific methylation of DNA.

Although an hypothesis of paramutation based on specific methylation of preformed DNA is completely speculative, it may nevertheless be of interest to examine the principal postulates of such a hypothesis applied to the case of paramutation at the R locus:

1. Paramutation results from the effects of substitution of methyl groups on the DNA of a specific segment at or near the R gene. Paramutagenic alleles carry this DNA segment receptive to methyl groups, whereas non-paramutagenic alleles do not.
2. Methylation of the DNA segment is mediated by specific methylases, whose synthesis is controlled by a genetic region at or near the R gene

of paramutagenic alleles (\underline{R}^{st} , \underline{R}^{mb} , \underline{R}^{sc} , etc.).

3. Introduction of methyl groups produces an alteration in the structure of DNA. The biological consequence of this alteration is repression of \underline{R} action, i.e., the paramutant phenotype, perhaps by interference with the transcription process.
4. The level of repression of \underline{R} action is proportional to the extent of methylation of the DNA segment involved. This postulate is necessary to account for different levels of paramutation of the \underline{R}^F gene. In host-controlled modification, there is evidence that T_1 -DNA may be methylated to different extents, depending on its host specificity.
5. The reversion of paramutant $\underline{R}^{r:st}$ alleles has its basis in the specific but incomplete demethylation of the methylated DNA segment of the \underline{R}^F allele. Chemically induced complete reversion may involve complete demethylation of the DNA segment.
6. Persistence of the paramutant state requires replication of the methylated form of DNA. This postulate is questionable, but is necessary to account for replication of the paramutant state following removal of the paramutagenic allele.

The hypothesis, as presented, bears many similarities to Brink's metamere hypothesis. Perhaps translation can be effected by substituting "methyl group" for "metamere" and the "process of specific methylation of DNA" for "under and over replication of metameres" in Brink's hypothesis.

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1. The effect of synaptic partner change on crossover frequency in adjacent regions of a trivalent.

Genetic markers \underline{B} and \underline{sk} flank the point of an interchange between maize chromosome 2 and a *Tripsacum* chromosome. In plants which carry a normal maize chromosome 2 as well as the reciprocal products of this interchange, synapsis is virtually limited to homologous maize segments so that a trivalent configuration is usually formed. Stocks were constructed to measure recombination in regions near the interchange point and beyond with the following results:

<u>Region</u>	<u>% Recombination</u>
\underline{B} -interchange	8.8
interchange- \underline{sk}	1.6
\underline{sk} - \underline{v}	40.9

Crossover frequency appears near normal or slightly increased in the vicinity of the region of partner exchange in trivalent configurations (in

spite of frequent synaptic failure of these portions) but markedly increased in a large adjacent region (sk-v) which includes the centromere.

Marjorie Maguire

2. Mechanism of high transmission frequency of a Tripsacum chromosome in maize.

An extra chromosome derived from Tripsacum in an otherwise apparently normal maize complement has been found to be transmitted to about 90 per cent of the progeny through the egg (Genetics 48: 1185-1194). Since high ovule abortion accompanied this high transmission (and other possible explanations were ruled out or seemed relatively improbable), it was suggested that zygotes or female gametes lacking the additional chromosome from Tripsacum may be selected against (killed) in the presence of a maternal background which contains it. In more advanced backcross generations to maize of this stock, a line has appeared in which transmission of the Tripsacum chromosome seems to approximate 50 percent, and seed set approaches normal in 21 chromosome plants. This is consistent with the interpretation that female gamete or zygote selection is indeed the mechanism of the high transmission mentioned above and that capacity for selection has been lost from the exceptional line. Further tests are in progress.

Marjorie Maguire

3. Normarski interference contrast microscopy of maize chromosomes.

An appearance of longitudinal doubleness in maize diakinesis chromatids as viewed with bright field light microscopy has been reported (P.N.A.S. 55: 44-50. 1966). This effect was seen in bivalents in which unusual decondensation or uncoiling had been induced, thought to be the result of heat treatment of the living material but since shown to be due to rapid and immediate chilling of material fixed in alcohol acetic acid 3:1 mixture. More recently, structures resembling half chromatids have been found to be visible in normal maize diakinesis microsporocytes fixed in alcohol acetic acid 3:1 mixture at room temperature and stained with acetocarmine in the usual way. These can sometimes be resolved with bright field optics (with planapochromat objective), but are commonly and clearly visible with the Zeiss Normarski interference contrast system (with planapochromat objective). The apparent structural subunits have a diameter only slightly greater than the theoretical limit of resolution of light optics and do not seem to become visible until mid to late diakinesis.

Marjorie Maguire

TUFTS UNIVERSITY
Medford, Massachusetts
Department of Biology

1. Modification of expression of the gene "rootless."

During 1966 and 1967, further studies were carried out utilizing treatments of growing plants with various combinations, strengths and frequencies of application of the growth substances naphthalene acetic acid (NAA), tri-iodo benzoic acid (TIBA), gibberellic acid (GA), and indole butyric acid (IBA). Dimethyl sulfoxide (DMSO) was found to enhance the efficacy of TIBA treatments in inducing rooting, providing the amount used was .5 ml per liter of TIBA solution. Above this amount, tissue death occurred in the margins of leaves.

Ontogenetic studies were done during 1967 on the number of roots initiated per node per week for 7 weeks for each of 38 different treatments. All plants were derived from seed obtained by self-pollination of "really rootless" plants in 1966, and mixed before planting.

Control plants showed 10-15 roots on 6 nodes initiated by 7 July; by 1 August, sister plants had only 1-7 roots on 1-3 nodes. By 15 August the total number had climbed to 15-22 on 5-7 nodes, but by 21 August had declined to 2-20 on 1-6 nodes, with several to all roots completely dead, yet with plants showing no signs of water shortage. NAA treatments, by contrast, showed 23 roots on 7 nodes in early July and 79-85 roots on 9 nodes by 21 August, with no decline around August 1. GA, normally a root inhibitor, showed no decreases in number of roots formed nor in nodes initiating roots, and actually caused slight increases consistent over the growing season (17 roots initiated by 10 July on 5 nodes; 15 roots on 7 nodes on August 21, all living). TIBA-treated plants showed 14 roots on 5 nodes on 8 July, an increase up to 34 on 9 nodes by August 1 and a drop back to 30 roots on 9 nodes by 21 August. IBA-treated plants showed a generally larger number of roots when compared with controls, with 20 roots from 6 nodes on 10 July, a drop to 11 on 4 nodes on 1 August and 13-17 roots on 6 nodes by 21 August.

Treatments with TIBA at 3-day intervals showed a higher number of roots on 1 August (26-31 at 7-8 nodes) than on 10 July (15 at 4 nodes) or on 21 August (3-20 on 5-7 nodes). Treatments with GA and IBA showed similar increases in numbers of roots per node without a dropoff around 1 August.

Combinations of treatments and spacings of application in general followed these basic findings. The most extreme development of roots occurred under a regimen of NAA and GA on alternate days, followed by two days without treatment; 231 roots were formed on 8 nodes by 21 August. TIBA-NAA on an every-other-day treatment regimen also produced high root numbers (80 roots on 9 nodes by 21 August).

Fresh weight-dry weight comparisons of roots and shoots show the clear relationship between treatments and root system size, as well as the general relationships between shoots and roots.

Family 66-10 (really rootless)

Treatment	Root Weight (g)		Shoot Weight (g)	
	<u>Fresh</u>	<u>Dry</u>	<u>Fresh</u>	<u>Dry</u>
Dist. water	8.5	3.2	694.4	110.0
TIBA	41.0	15.7	455.0	82.7
GA	98.9	15.1	709.2	113.5
NAA	244.2	49.4	693.3	105.5
IBA	4.3	.9	683.8	104.4
Control	3.6	1.6	1671.5	244.2

Norton H. Nickerson

2. Races of maize in Panama.

As a part of the Bio-Environmental effort to appraise effects of a sea-level canal in Darien, Panama, I was asked to make a study of cultivated crops among the Choco Indians this past fall. Internodal and tassel data on 5 - plant samples from each of 10 Indian fields were obtained. Soil and kernel samples were obtained for mineral analysis by the University of Florida. Seventeen collections of maize, totalling 118 ears, were obtained from various locations on the major drainages of the area, Rio Sabana, Rio Chucunaque, Rio Tuirra, and Rio Balsas (Rio Tucuti). Preliminary study shows evidence of more complex race relationships than had previously been assumed.

These may be tentatively summarized as follows:

Race designation	# of Collections in which racial characters are present	# of ears exhibiting racial characteristics
Nal-Tel	8	33
Cuban Flint	4	13
Chococeño	7	24
Coastal Tropical Flint	8	51
not the same { Negrito	4	14
{ Negro	1	7
Cariaco	1	8
Pollo (?)	1	2
Capio	1 (plants measured; no ears obtained)	

The slash-mulch cultivation method reported for the Province of Choco in northern Colombia is also practiced in the valleys of the upper Rio Balsas drainage system.

Norton H. Nickerson

TULANE UNIVERSITY
New Orleans, Louisiana

1. Terminal loop configurations in maize x teosinte hybrids.

Teosinte stocks collected in Mexico and Central America (MNL 37, 1963; MNL 38, 1964) have been crossed with a knobless New England flint (Wilburs Flint) and the pachytene chromosomes studied cytologically (Teosinte--The Closest Relative of Maize, Bussey Institution of Harvard University, 159 pp. 1967). Unique terminal loop configurations of the pachytene chromosomes have been observed in maize x teosinte hybrids using three of the recognized races of teosinte (Guatemala, Chalco, Central Plateau). These loops are very similar to inversion configurations and they are terminal, but it is questionable if they are, in fact, terminal inversions. These terminal loop configurations are seen in the short arms of chromosomes 8 and 9 and are not found in every cell prepared from a single anther in plants known to possess them. Some cells show a non-paired segment on the short arm, while the majority appear to be perfectly normal and pair with no observable inversion configuration. Working on the hypothesis that these loops represent several small chromosome rearrangements or small non-homologous segments in the short arm that, depending on condition at pachytene, may act as a single rearrangement, I have begun genetic studies with maize tester stocks for chromosomes 8 and 9 to test the suppression of genetic crossing-over and chiasma formation. Also, because these loops do not appear with regularity in lines known to show loop configurations attempts are being made to select teosinte lines in which the expression of the loop is complete in every pachytene cell. Genes unique to teosinte are hypothesized to reside isolated from maize introgression in these terminal inversion-like configurations. It is hoped that once these non-homologous segments are established in a high penetrance, loop forming line and genetically mapped, their transfer to a maize background will help us in identifying the genes which separate teosinte from maize.

H. Garrison Wilkes

2. Teosinte x maize hybrids, Nobogame, Mexico.

Naturally occurring teosinte x maize hybrids have been reported from Mexican maize fields, but seldom, if ever, are these plants thought to have been purposely planted by the cultivator. Most maize x teosinte hybrids are attributed to teosinte fruitcases containing hybrids naturally disseminated in the field. Of all the sites where maize and teosinte are known to hybridize naturally the maize cultivation pattern at Nobogame has probably changed the least over the last 150 years (MNL 39, 1965). Therefore, a detailed analysis of the maize from a field where teosinte x maize hybrids were present was undertaken. A careful study

of the entire harvest was made and tripsacoid cobs selected. Also collected were 8 ears said by the cultivator to be the type he would use as seed ears. Four of these ears were studied. The cobs were shelled and 100 seeds from each ear grown. Three cobs yielded all maize plants but the fourth produced 3 maize x teosinte hybrids. Morphologically maize x teosinte seed could not be distinguished from the pure maize seed on these selected ears. Yet several ears from the field, which had been selected because they possessed smaller than usual seeds, all yielded uniformly teosinte x maize hybrids. There appears to be a chemical feedback mechanism (growth hormone) between the developing seed and the cob because if the ear is pollinated only by teosinte the hybrid seeds are smaller than the few maize x teosinte hybrid seed found on a predominately maize-pollinated ear. This hormone must act to stimulate the conduction of food through the cob to the developing seed.

In the seed corn at Nobogame, Mexico, the frequency of 3 maize x teosinte hybrids/400 plants compares well with the abundance of highly tripsacoid cobs found in the total harvest. These very productive seed ears are not highly tripsacoid, but they too show evidence of teosinte introgression (rigid cob, straight rows and indurated glumes).

H. Garrison Wilkes

TULANE UNIVERSITY
New Orleans, Louisiana
and
HARVARD UNIVERSITY
Cambridge, Massachusetts

1. Tripsacum studies.

Tripsacum studies have begun to delineate the species lines and evolution of the genus Tripsacum from central Mexico north through the United States. Field collections have been made from the Mississippi Valley and from the Gulf Coast, and established in a genetic garden at the Riverside Research Laboratories of Tulane University. The discovery of a more widespread distribution of a narrow-leaved Tripsacum along the Gulf Coast has raised some question about the endemism of T. floridanum in southern Florida. These plants occur in both open and shade habitats, but always in very moist soils. Diploid T. dactyloides is not limited to wet environments, while tetraploid T. dactyloides often is found growing in wet soils. Field studies to date have tended to bear out the hypothesis put forward by Tantravahi (Tripsacum Newsletter 1968) for the hybrid origin of tetraploid T. dactyloides. Prior to the present study diploid T. floridanum and T. dactyloides were thought to be allopatric, but field studies of the Gulf Coast Region from Texas (Orange-Jefferson, Liberty, Harris, Fort Bend and Brazoria counties) to Florida (Highland, Polk and Hillsborough counties) shows that there are pockets of narrow-leaved T. floridanum plants which are probably remnants of a once more extensive Pleistocene distribution.

H. Garrison Wilkes
Tulane University
Ramana Tantravahi
Harvard University

UNITED NATIONS SPECIAL FUND (UNESCO) PROJECT
Mindanao Institute of Technology
Kabacan, Cotabato, Philippines

1. MIT--UNESCO Corn Program.

The strengthening of agricultural training at the Mindanao Institute of Technology is the overall program of the plan of operations for this UNSF/UNESCO Project. In the area of agronomy, of seed production and distribution and of corn improvement, seed and field corn production for food, feed and fodder is one of the objectives. Since September 1966, crop oriented production goals and the ways and means to accomplish them were drawn. Vigorous direct action and high pay-off corn improvement for Mindanao island is vital to the Filipinos. This new virgin land of promise and hope, so suitable for the corn crop, will offer a great opportunity in socioeconomic growth of new settlers from Luzon and Visayas.

B. S. Sidhu
C. Marasigan

2. Corn improvement studies.

Pyramiding the corn yields through controlled biological plant development is a complex undertaking because many varietal-soil plant-environment-management interactions are involved in successful crop production. This is especially true for the humid tropics where sufficient adaptive agronomic research is yet lacking.

Several local, exotic and intercrossed composites and varietal collections are being test screened. Some new experimental synthetics of white and yellow flinty types are under observation. A new MIT variety satisfactorily resistant to downy mildew disease has been released. The use of opaque-2, brachytic and prolific corn materials is being made in the breeding program.

B. S. Sidhu
R. Gloria

3. Genetic studies on resistance to downy mildew (*Sclerospora* sps.).

This is a very serious disease not only in the Philippines but also in the neighboring countries of Indonesia, Malaysia and Nationalist Chinese Republic.

A 43 entry downy mildew screening regional trial nursery under the International Corn Program has been planted and resistant lines will be used in the breeding program. Simultaneously, on the basis of preliminary investigations regarding the resistance to the disease among native varieties such as Mimis and Tinigib, a study is underway on the investigation of inheritance of resistance to the disease organism.

R. Gloria
B. S. Sidhu

4. Inheritance studies on cut leaves.

Observations on single or double subterminal marginal leaf blade cut(s) in the first leaf and or subsequent leaves in certain races of corn are being studied for some clue as to the genetic behavior. Any information on this complex character and availability of seed materials will be highly welcomed.

B. S. Sidhu
R. Gloria

5. Adaptive agronomic corn research.

Several more experiments involving plant breeding, soil-plant relations and problems of pathological and entomological studies are in progress.

Thanks are due to the MIT and UNESCO administration for providing research facilities in this corn program.

C. Marasigan
R. Gloria
B. S. Sidhu

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Soil and Water Conservation Research Division
Mineral Nutrition Laboratory, Beltsville, Maryland

1. Soil responses of the yellow-stripe maize mutants.

Inbred M1⁴ yellow-stripe-1 ($\underline{ys}_1/\underline{ys}_1$) and Early Butler yellow-stripe-3 ($\underline{ys}_3/\underline{ys}_3$) were crossed twice to Pa5⁴ and recovered as the homozygotes. Differences in response of $\underline{ys}_1/\underline{ys}_1$ and $\underline{ys}_3/\underline{ys}_3$ to foliar applications of 0.5% ferrous sulfate in water were largely eliminated following recovery. Other traits such as brown midrib and aleurone colors in the former and a segregating glossy in the latter were also eliminated. Both recovered mutants displayed a strong interveinal chlorosis when grown on Millville loam (pH 7.8), a calcareous soil, at various times of the year in a greenhouse or in a controlled environment chamber.

The recovered genotypes also responded similarly on Bladen soil (pH 4.6) by producing leaves that were entirely green and indistinguishable from those of similarly cultured Pa5⁴. Complete greening on Bladen soil and similar responses to ferrous sulfate sprays indicate that the metabolic blocks in both mutants seem to reside in the iron uptake mechanism. This would appear to eliminate the possibility of a block in iron transport as was earlier suggested (MNL 36: 72, 1962).

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1. Further observations on an "opposite leaf" phenotype.

In MGCNL 41: 197 we described briefly a plant with opposite leaves. Pre-mature comments were presented concerning the heritability of this phenotype; during the 1967 winter and summer crops, an additional 61 plants presenting the "opposite leaf" phenotype have been studied.

The progeny data include:

	1967 <u>planting</u>	Number of plants	
		<u>"Opposite leaf"</u>	<u>Normal</u>
Original plant, (X)	ear A winter	6	25
	summer	6	38
	ear B winter	2	29
	summer	9	30
subtotal		23	122
Normal sibs X "opp. leaf", summer		25	166
Normal sibs X Normal sibs, summer		3	69
Open pollinated "opp. leaf", summer		10	16

Random distribution of the respective "opp. leaf" kernels on the ear has been observed in all cases.

Progeny from outcrosses to unrelated stocks, using "opposite leaf" plants as males and/or females have not yet been classified.

Sporocyte analysis of two "opposite leaf" plants has yielded:

Opposite leaf plant number	Number of meiocytes								% Abnormal meiocytes	% Sterile pollen	
	Normal				with bridges		with laggards				
	M _I	A _I	M _{II}	A _{II}	A _I	A _{II}	M _I -A _I	M _{II} -A _{II}			
5402-45	91	45			12		30			29	26
5402-50			71	66		7		52		40	33

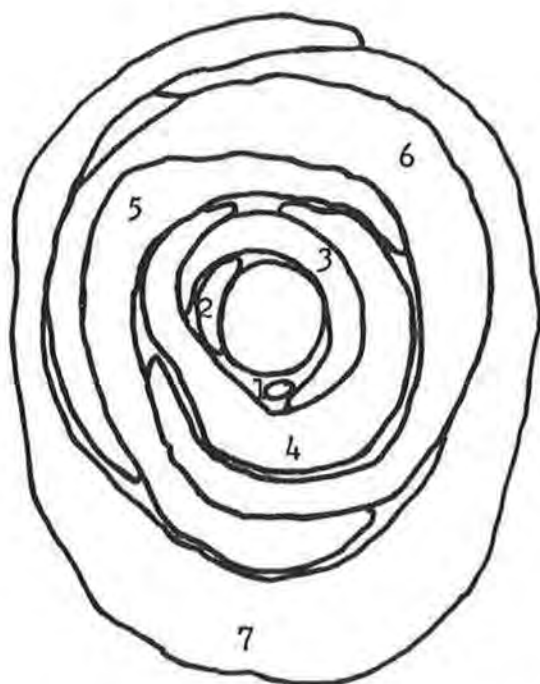


FIGURE 1

0.5 mm

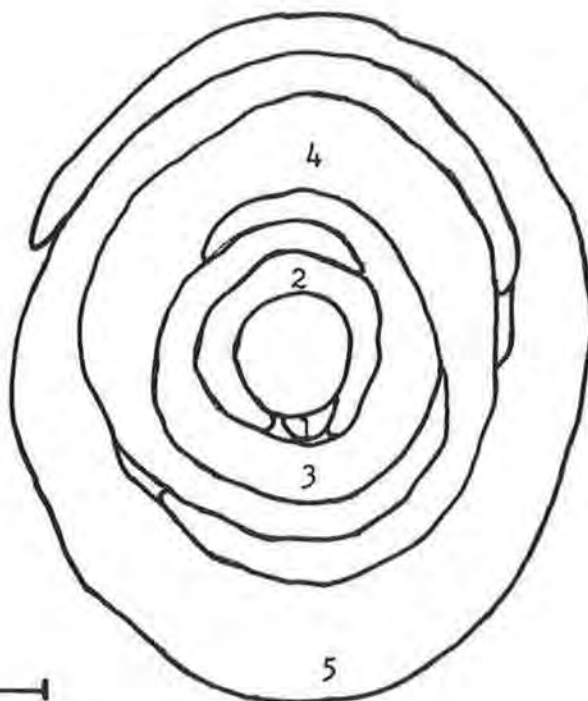
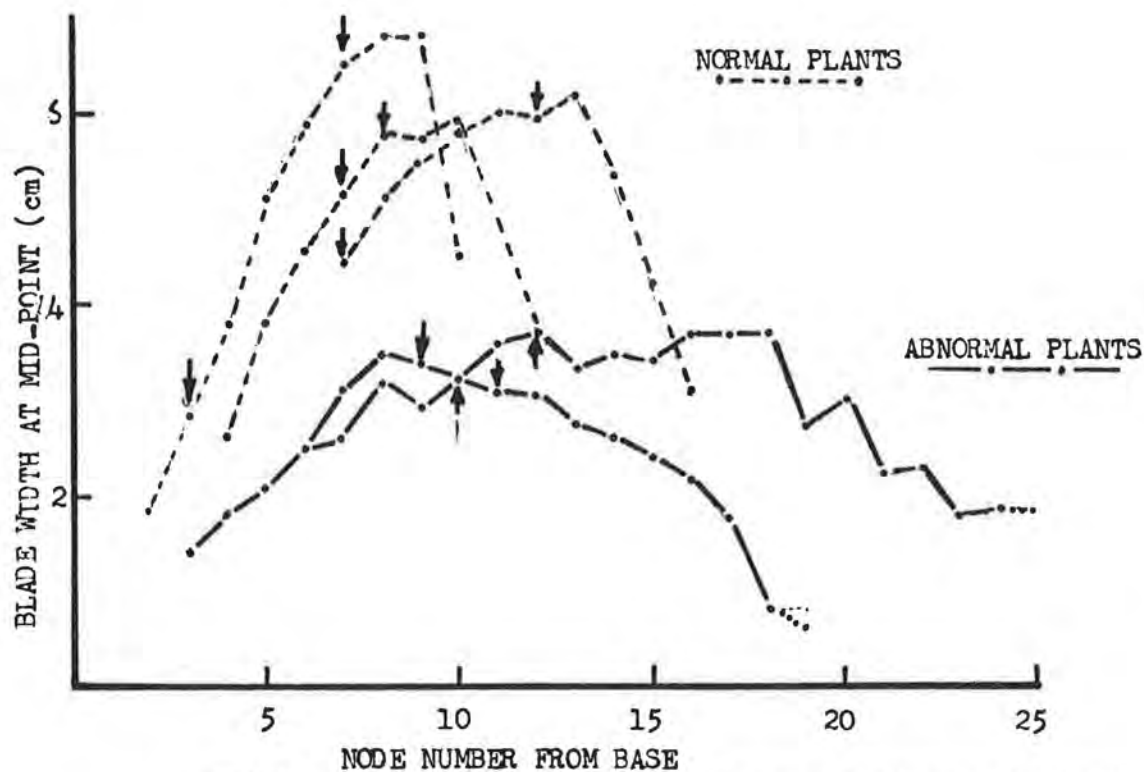


FIGURE 2

LEAVES ARE NUMBERED BEGINNING WITH YOUNGEST PRIMORDIUM



ARROWS INDICATE NODES BETWEEN WHICH EAR SHOOTS ARE LOCATED

FIGURE 3

Pachytene analysis has failed to reveal the chromosomes involved in a possible aberration and aneuploidy. Mitotic karyotyping has not been initiated. No B chromosomes were observed in pachytene and the parental stock had no previous history of any aneuploid condition.

In addition to the production of opposite leaves, these "opp. leaf" plants exhibited other modifications of leaf arrangement and structure. These include special arrangement of leaves usually accompanied with some internodal fusions, reduction in leaf width, and increased number of nodes. One plant produced a number of spirally arranged leaves with "between-leaf-fusions" prior to the production of pairs of opposite leaves above the ear shoot. Another, however, had opposite pairs inserted at nodes 7 and 8 followed by individual leaves, spirally arranged, to the base of the tassel. A number of plants produced a series of spirally arranged leaves with rather serious internodal fusions. The possibility that the varied phenotypic expressions were a product of environmental interactions seems worthwhile for future study.

Fig. 1 represents a cross section below the tip of the youngest leaf primordium (1) from an abnormal plant. This apex was collected prior to the tassel initiation. This section clearly illustrates the spiral arrangement of the leaves and can be compared with the distichous arrangement of a similar section from a normal plant (Fig. 2). Besides this spiral phylotaxy, the width of the sheath and the amount of sheath overlap seem considerably less in the "opp. leaf" plant than in the normal plant.

Leaf width of "opp. leaf" plants is generally considerably less than in normals and can be used to identify abnormal plants (at the 4-5 leaf stage) before the recognition of other characters is possible. In Fig. 3, blade width taken at the midpoint of the leaf blade is plotted at each node, for 3 normal and 2 abnormal plants.

Measurements were made on plants that had reached maturity under similar conditions and over the same period of time. Arrows indicate the nodes between which rudimentary or mature ears were located.

On all plants a certain number of the youngest leaves were dead and lost. These data suggest that in most cases leaves from "opp. leaf" plants were up to $\frac{1}{2}$ the width of comparable normal leaves. Further it appears that the node number at which ears were produced is not altered appreciably in these abnormal plants.

The "opposite leaf" phenotype appears to include a broad spectrum from the largely spiral phylotaxy possessing a few nodes with true opposite leaves to an almost completely opposite leaf plant. The conversion which has been seen in almost every specimen from alternate to opposite leaf insertion may be influenced by environment as our winter grown (greenhouse) materials have thus far presented the more extreme spiralling. The availability of a large seed source will permit further study of these morphogenetic aspects.

The idea that specific genetic information controls leaf insertion and thus the phylotaxy of the plant is not new. However, we are presented with a system for the study of other problems which in turn may provide suggestions on how the architecture of the corn plant may be further modified. The applied applications of this project are being considered.

R. I. Greyson
D. B. Walden

2. Acrylamide gel electrophoresis of maize pollen protein.

Pollen proteins have been separated by electrophoresis on acrylamide gel columns in pairs of large polyethylene reservoirs holding fourteen tubes per pair. The gels were prepared according to the method of Ornstein and Davis; the best travel and separation was obtained using a tris-glycine buffer of pH 9 in both upper and lower reservoirs and a current of 5mA per tube. The gels were stained with amido black and destained electrophoretically in an apparatus which allowed the current to be applied across the gel column.

Several preparations, obtained by subjecting the extracted protein to such preparatory treatments as ammonium sulfate precipitation and dialysis gave poorer resolution than the crude extract. The latter was prepared by homogenizing the pollen in cold mannitol buffer, 0.5 M pH 8.0, centrifuging at 20,000 g for 10 minutes and adding the supernatant to the columns.

Pollen and other parts of several different genotypes are being examined by this method.

D. B. Hayden
F. S. Cook

3. The separation and detection of some dehydrogenase isozymes in maize pollen.

The electrophoretic separation on acrylamide gels described above was used to detect several dehydrogenases in the pollen of "Seneca 60" (su_1/su_1). Soluble protein extracted from fresh pollen in the mannitol buffer of pH 8.0 was separated and detected on the gels using a system containing nitroblue tetrazolium (Fine and Costello, Methods in Enzymology, Vol. VI).

The method used differs from that of Ornstein and Davis in that the protein sample was mixed with a solution of 50% sucrose and was added directly on top of the large pore gel without any further polymerization. When this method is used, care must be taken to add the buffer in such a way that there is a minimum amount of agitation.

Two or more isozymes of the following NAD-dependent dehydrogenases were detected: malic, glutamic, lactic and alcohol. Five distinct isozyme bands were found for malic dehydrogenase.

D. B. Hayden
F. S. Cook

4. Relationships between maize and human cytogenetics.

Considered in its broadest context cytogenetics has undergone a resurgence during the last ten years. In part, this renewed interest has been generated by advances in cell biology; likewise, the investigations in human cytogenetics have provided an additional stimulus. Particularly noteworthy from this latter area of interest are the workers, techniques and the problems. From the vantage point of an association with a medical school, there appears to be a paucity of discourse among groups of cytogeneticists. While professional isolation may in part account for this lack of interdigitation, certainly some of it arises merely from the ignorance that the other fellow exists. Intriguing cytogenetic problems are emerging amongst the mammalian and, in particular, the human cytogenetic areas: e.g. quantification of the variation in homologues; asynchronous replication of chromosome organelles; transmission of aneuploidy; the relatively high frequency of adjacent segregants from translocation heterozygotes (more often spoken of as the unbalanced carrier condition from a balanced carrier by the human cytogeneticist); as well as the more newsworthy association of chromosome abnormalities with disease (cause or effect?) and congenital abnormalities associated with aneuploidy.

It is to be hoped that there will develop a greater exchange between the largely research oriented cytogeneticists, employing predominantly corn and Drosophila technologies and the medically oriented, and undoubtedly motivated, human cytogeneticists. The case for understanding meiosis in man has been well put and considerable interest generated recently in attempting pachytene-diakinesis analysis, particularly during spermatogenesis. Without doubt, the extensive studies available from corn will provide a basis for human meiotic cytogenetic investigations.

Perhaps not so obvious is another area of joint interest. Since human cytogenetic studies have been almost exclusively a karyotype analysis of somatic material, the problem concerning the value of the karyotype as a predictor of meiotic behavior needs to be examined. Likewise, the accuracy of extrapolation from corn studies to humans can also be questioned. To provide evidence on these two questions, clearly mitotic karyotype procedures are needed in maize. Investigations of the mitotic karyotype and meiotic configurations in single plants or populations of plants could then be undertaken. Contributions from several laboratories, as noted in the last three Newsletters, have provided the necessary information and techniques so that it is now possible to undertake studies in maize employing a mitotic karyotype from root tips and the meiotic analysis of the same plant.

It is the purpose of this communication to bring these features to the attention of maize geneticists and to encourage this approach to specific problems in maize cytogenetics. Already we are impressed in our laboratory with several features of the mitotic karyotype-meiotic analysis protocol. Noteworthy at this time are: (1) the variability between homologues; (2) alterations in mitotic-meiotic arm ratios; (3) the use of chromosomal aberrations for experimental studies.

The availability of the large number of structural rearrangements and aneuploids in maize suggests that specific experimental procedures can be applied to problems of somatic cell cytogenetics in corn once the specific evidence on the relationship between the corn system and the human system has been presented. It can be anticipated that there could be a broad application of such corn studies. Not beyond a reasonable probability is

the promise of synthesizing a maize system to mimic a human chromosomal abnormality such that data would be available from progeny testing of maize prior to the attainment of reproductive age by the carrier of the abnormality.

We have been developing during the last 2½ years in this laboratory a catalogue of the karyotypes of various standard stocks--both hybrid materials and genetic tester stocks. We would be particularly interested to receive from other laboratories stocks containing known structural abnormalities not already available through the Co-op or stocks possessing chromosomes which appear cytologically "abnormal."

D. B. Walden

5. Root tip squash technique.

The following is the procedure of root tip squash technique presently used in our laboratory.

1. Pretreat the excised root tips in 0.002 M 8-hydroxyquinoline for three hours. Root tips can be obtained by germinating seeds in petri dishes or by growing plants in pots. In both cases, we usually keep the seedlings at 28-30°C.
2. Fix in a mixture of one part of acetic acid and three parts of absolute alcohol overnight.
3. Wash with 70% alcohol and store in the same fluid in a refrigerator until needed.
4. Hydrolyze in N HCl at 60°C for 8 minutes.
5. Rinse in distilled water for three changes, 2-3 minutes each change.
6. Stain in leuco-basic fuchsin for 1-2 hours.
7. Treat with 5% pectinase for two hours.
8. Put in 45% acetic acid for about 10 minutes before squashing to clear the stain in cytoplasm.
9. Squash the meristematic regions in 45% acetic acid.

We found this technique quite satisfactory for the somatic chromosomes of maize. The ten pairs of chromosomes can be identified without much difficulty by their relative lengths, arm ratios and presence or absence of a satellite at metaphase. The eu- and heterochromatic regions and even some knobs can be differentially stained at prophase. Using this technique, we are studying the karyotypes of several diploid strains and translocation stocks and identifying the extra chromosomes of different trisomics.

C. C. Chen

6. Computerized karyotype analysis.

A meaningful analysis of the variation in arm lengths and arm ratios of chromosomes requires a large number of measurements and repetitious calculations. Preceding such an analysis a survey was initiated to investigate the possibilities of computerized analysis.

Computerized analysis of human chromosomes is being attempted in several laboratories. The Chloe film scanner, F.I.D.A.C., and the Cydac system are three devices being used. The first two systems provide as computer output the image of the chromosome spread; analyzed as to arm ratio and total length of each chromosome. Negatives of the cells to be analyzed must be fed into the Chloe scanner and F.I.D.A.C. whereas the Cydac system takes the image directly from the prepared slide. Cydac appears to be the most efficient system for an analysis of the proposed type since it would both collect and analyze the data.

Manual measurements of chromosome lengths and arm ratios can be done with good precision. A computerized project would also permit the determination of mean arm lengths and ratios with a small standard error. Whether any greater precision can be obtained using the computer, has not been established. Thus, the merit of computer use appears to lie in its relative speed and precision and removal of the limitation of small sample size.

Computerized chromosome analysis could be useful in any area where somatic chromosomes are to be examined. For instance, development of an aneuploid series could be accelerated. As well, trisomic and nullisomic analysis could be computerized. Since the computer can be programmed to identify, record and recall, normal and abnormal chromosome complements can be determined. Computerization, then, could increase quantity without a sacrifice on quality.

Computerized analysis of corn root tip preparations have been attempted using the Chloe film scanner. Many technical problems unique to the corn material, have yet to be overcome. Whereas the human leucocyte cultures are in a monolayer due to the air drying process used in slide preparation, corn root tip smears are thicker and therefore result in a more dense background. As well, the corn chromosome arms are not spread as widely as the human material. The net result of these technical differences is the inability to locate accurately the centromere and define the limits of the arms. Attempts are being made to correct the technical problems and hopefully a satisfactory solution can be found.

W. G. Filion

7. Nuclear cycle in maize root tips.

An investigation of the nuclear cycle, and its components, in corn was undertaken in preparation for evaluation in specific chromosomes of the pattern of incorporation of H^3 - thymidine during DNA synthesis.

Seeds of "Seneca 60" hybrid ($\frac{su_1}{su_1}$) were germinated and grown at 28°C on filter paper kept moist with distilled water. Germinated kernels with roots at least 1 cm in length were placed for 0.5 hours in a solution of H^3 - thymidine (6.3 c/m M diluted to 1.0 uc/m M distilled water) after which they were incubated in distilled water (without chaser). Root tips were fixed subsequently at hourly intervals. Liquid emulsion autoradiographs (Kodak KTB-2) were prepared from Feulgen squashes of this material, and slides were scored for the frequency of labelled division figures. Over 6000 division figures were examined.

Per cent prophase and metaphase figures were plotted against time after labelling (Fig. 1). The adjusted prophase line (short dashes) indicates the line for the end of prophase.

Following Wimber's (1960) interpretation, the duration of G_1 , S, G_2 and mitosis was calculated from the data. Dissatisfaction with the lack of precision of these estimates obtained by extrapolation from the curve led to a probit regression analysis, yielding more appropriate calculations of the mean and standard deviation of the durations of the various phases of the nuclear cycle.

The results are tabulated below, and are compared with other information on the nuclear cycle of corn (Clowes, 1965).

Duration (hours) of nuclear cycle of maize			
Phase	Mean	Standard Deviation	Clowes (1965)
G_1	-0.75	0.66	3
S	6.02	0.07	11
G_2	3.08	0.09	3
M - Prophase	0.97	0.66	
Metaphase	0.37		
Anaphase	0.13		
Telophase	0.37		
sub total (M)	<u>1.84</u>		<u>2.5</u>
Total	10.19	0.67	19.5

The difference between Clowes' report and the present study in the total nuclear cycle time can be attributed to differences in the S and G_1 . Clowes' seed was germinated and incubated at 20°C, whereas the present study was conducted at 28°C. We suggest that the difference in the duration of S is due to the accelerated rates of biochemical processes at the higher temperature. The negative value calculated for G_1 indicates that G_1 cannot be accommodated within the total mitotic cycle.

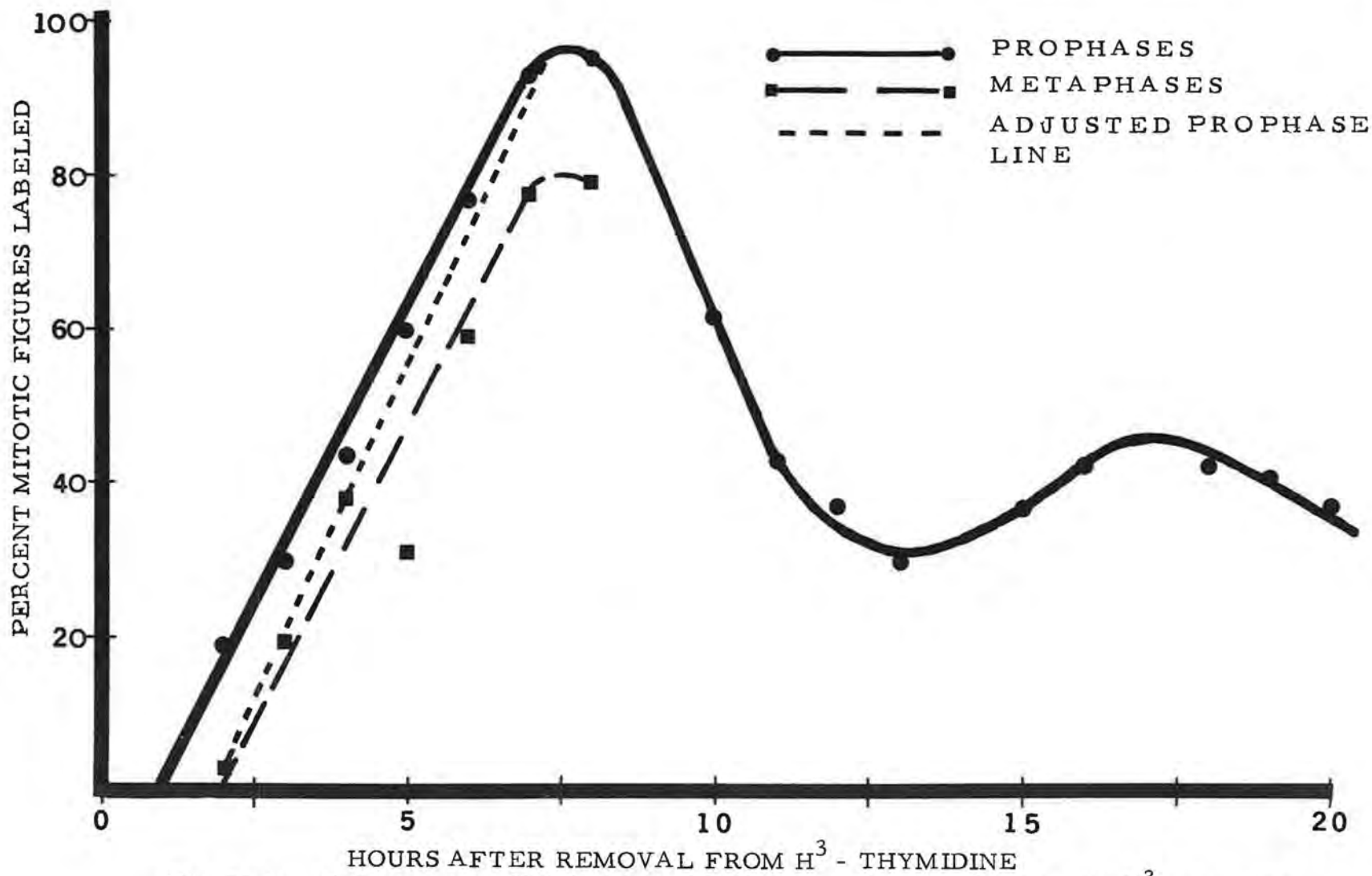


Figure 1. Percentage of divisions labeled vs. time after treatment with H³ thymidine

Clowes (1967) has shown that in rapidly dividing cap initials of corn, G_1 is telescoped such that DNA synthesis can start as early as telophase. In our case the short cell cycle indicates that most cells of the root tip were rapidly dividing, leading to an overlap of the S period with the end of telophase, and thus, effectively eliminating G_1 .

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1. Effects of ionizing irradiation on paramutation at the R locus in maize.

A. Effects of X-irradiation on pigmenting potential of standard R^r and paramutants from standard R^r

Standard R^rR^r seeds and pollen from R^rR^r plants were irradiated with 10,000r and 1,200r, respectively, following which testcrosses were made on $r^s r^s$ plants. The data obtained by evaluating the one dose $R^r r^s r^s$ aleurone of the testcross ears indicated that these treatments produced no effect on the pigmenting potential of R^r . On the other hand, the pigmenting potential of strongly repressed $R^{r''}$ alleles (passed through heterozygotes with the R^{st} allele for three generations) was partially restored, and to different levels, after X-irradiation of seeds (10,000r) and pollen (1,400r). Complete restoration, however, occurred only rarely. The frequencies of detectable changes were 21 per cent for treated seeds and 12 per cent for treated pollen. Other paramutant alleles at different levels of repression were tested by the same methods; all gave a response similar to that of the $R^{r''}$ alleles. Further tests showed that the X-ray-induced increases in pigmentation were heritable, and that all the paramutant alleles whose pigmenting ability had been increased by X-irradiation were still paramutable. The high frequency of X-ray-induced changes support the suggestion (Brink 1964) that there is a component at or near the R locus responsible for repression, and that this component, rather than the R structural gene itself, is involved in the response to irradiation. That the increases in paramutant pigmenting ability were to different levels also supports the postulate that more than one unit of the genetic component is involved in repression.

B. Effects of X-irradiation on the paramutability of standard \underline{R}^r

Homozygous $\underline{R}^r \underline{R}^r$ seeds and pollen from $\underline{R}^r \underline{R}^r$ homozygotes were X-irradiated with 10,000r and 1,200r, respectively, and then used as staminate parents in crosses with unirradiated $\underline{R}^{st} \underline{R}^{st}$ plants. The paramutability of the irradiated \underline{R}^r alleles was then measured by testcrossing the $\underline{R}^r \underline{R}^{st}$ heterozygotes on $\underline{r}^g \underline{r}^g$ plants. The \underline{R}^r alleles showed no detectable changes in paramutability after seed X-irradiation. On the other hand, the data showed a highly significant reduction in the paramutability of \underline{R}^r alleles in 28 per cent of the plants resulting from pollen treatments. This result is similar to that reported by Linden (1963) in a study with gamma rays. There was no case, however, in which paramutability was completely lost after X-irradiation. The frequency of changes induced was far higher than that characteristic of mutation in general, which suggests that irradiation leads to inactivation of the component conditioning paramutability rather than to an induced gene mutation at the \underline{R} locus. That paramutability was reduced but never lost suggests that more than one unit of the repressor component is responsible for paramutability, and that this component is closely associated with the \underline{R} structural gene.

C. Effects of X-irradiation on the paramutagenicity of \underline{R}^{st} , \underline{R}^{sc} , and $\underline{r}^r(I)$

The paramutagenic alleles tested were first crossed to standard $\underline{R} \underline{R}$ homozygotes used as females, and the heterozygotes thus obtained were then testcrossed on $\underline{r}^g \underline{r}^g$ plants. The pigmentation of the testcross paramutants was examined for changes in the paramutagenicity of the tested alleles. \underline{R}^{st} showed no detectable changes in paramutagenicity after X-irradiation of $\underline{R}^{st} \underline{R}^{st}$ seeds with 10,000r. Pollen from seven $\underline{R}^{st} \underline{R}^{st}$ plants was also irradiated. Forty-nine per cent of the treated pollen grains from one plant were significantly reduced in potential paramutagenic action. Nine \underline{R}^{sc} alleles (mutant derivatives of the \underline{R}^{st} allele having different levels of paramutagenicity) were generally found to be insensitive to X-irradiation at the seed stage (14,000r). The alleles derived from two of the 68 irradiated seeds were exceptional, however, and showed an increase in paramutagenicity. Two paramutagenic $\underline{r}^r(I)$ alleles (isolated from the testcross progeny of $\underline{R}^r \underline{R}^{st}$ heterozygotes) were also studied. Paramutation of \underline{R}^g was reduced in $\underline{R}^g / \underline{r}^r(I)_5$ heterozygotes when the strongly paramutagenic $\underline{r}^r(I)_5$ allele was X-irradiated at the seed stage with 14,000r. Such a reduction did not occur, however, in \underline{R}^g heterozygotes carrying the irradiated weakly paramutagenic $\underline{r}^r(I)_3$ allele. Sensitivity to X-irradiation and direction of induced changes in paramutagenicity appears to vary among different paramutagenic alleles.

These results are similar to Linden's findings, which showed that the paramutagenicity of \underline{R}^{st} and \underline{R}^{mb} was reduced in some cases and enhanced in others after gamma-irradiation. The new findings support the interpretation that a paramutagenic allele involves more than one component. The variability of the effects of irradiation on paramutagenicity of paramutagenic alleles suggests that the chromosomal component responsible for paramutagenic action is not equivalent to the component responsible for paramutability, which is consistently and uniformly affected by irradiation. Since the effect of irradiation on paramutagenicity can be either

reduction or enhancement, it is assumed that an inactivation mechanism is not the principal factor involved. It may be that the X-ray-induced changes in paramutagenic properties have the same characteristics as the changes produced by a number of other genetic factors known to influence the level of R action (e.g., Rst or r).

D. Effects of X- and gamma-irradiation on paramutation of standard R^r in heterozygotes with different paramutagenic alleles

Paramutation of R^r was significantly reduced in R^rRst heterozygotes after direct X-irradiation of R^rRst seeds with 8,000r and 10,000r. Aleurone pigmentation was examined in paramutants extracted from testcrosses of treated and control R^rRst heterozygotes made on r^gr^g plants. Thirty-three per cent of the testcross ears in the 8,000r treatment group and 16 per cent of the testcross ears in the 10,000r treatment group were darker than their respective control ears. Similar results were obtained in R^rR^{sc}₂₃ and R^g/r^r(I)₄ seed treatments, although in the latter case treatment induced only small changes. A further test showed that the Rst alleles of the very dark testcross ears derived from treated R^rRst seeds retained the same level of paramutagenic action as the control; and it has already been noted that R^r allele showed no detectable changes in paramutability when treated at the seed stage with 10,000r. Therefore, the dark R^r paramutants induced by treating R^rRst seeds were due either to derepression of the repressed R^r allele, which implies that paramutation has proceeded to a significant extent before seed germination, or to an impairment of the process of paramutation, which indicates that paramutation also occurs during plant development, or to both factors.

Irradiation of plants, rather than seeds or pollen, was another approach used in investigating whether paramutation occurs during vegetative growth. R^rRst plants responded to X-irradiation with different changes in R^r pigmenting action at different growth stages. The data demonstrated that the plants were relatively more sensitive to irradiation during the earlier stages of development than during the later stages close to, and during, meiosis. However, it was not determined whether paramutation proceeds evenly in the cells of the developing R^rRst tassel until the separation of R^r and Rst at meiosis, or whether paramutation proceeds more rapidly in the early stages of tassel development. Data obtained from gamma-irradiation of plants also indicated that R^rRst plants were more sensitive to treatment before meiosis than during meiosis.

This experiment also showed that plants heterozygous for R^r and different paramutagenic alleles, Rst, R^{sc}₁₃₂, and R^{sc}₁₁₀, responded to X-irradiation with bidirectional changes, that is to either an enhanced or a reduced level of paramutation. All three different heterozygotes showed induced repression in the level of R^r pigmenting ability when irradiation was applied between 19 and 21 days of plant growth. On the other hand, R^rRst plants treated at 33 days of growth and 41 days of growth produced R^r paramutants with increased pigmentation. That less pigmented and more highly pigmented R^r paramutants were produced by irradiating plants is additional proof that paramutation can occur during tassel development.

2. Induction of heritable change at the R locus by abnormal chromosome K10.

Abnormal chromosome K10 has a novel effect on the R paramutation system. Following introduction into a K10 chromosome a paramutable R factor becomes relatively insensitive to stippled action in R K10/Rst heterozygotes. It has now been observed that the insensitivity regularly persists, in one or another degree, after return of R by crossing over to a structurally normal chromosome 10. Recent data also show that the change in sensitivity does not occur in plants in which paramutable R and the K10 knob are carried by homologous chromosomes, that is, in repulsion. The large, terminal knob characterizing abnormal chromosome 10 is distal in 10L to striate-2 which, in turn, is distal to R. Since striate-2 and R show about 35 per cent recombination, the R locus and the K10 knob are far apart. The evidence suggests that the observed change in R sensitivity is the result of a stimulus originating in the distinctive, terminal K10 segment that is propagated along the same chromosome to the R locus, at which the heritable alteration in sensitivity to Rst action is then effected.

R. A. Brink

Addendum:

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1. Investigations on WF9 mutants.

In 1960-61 mutants were induced in the WF9 line by X-rays of 7000 R and 15000 R. On the basis of morphological and biochemical investigations, 60 mutants were identified.

In 1965, the protein and oil content was determined in seeds of 37 mutants. The results of these investigations are shown on Table 1.

In 1967 a study of the resistance of 60 mutants was made at our request by the Agricultural Research Institute of the Hungarian Academy of Sciences, where Dr. I. Manninger had the goodness to include our mutants in an experiment together with his own breeding material.

During one series utilizing comparative experiments of standard methodology, in two experiment places (per 10 plants) he studied resistance to the head and the common smut with the provocation method, and resistance to Fusarium in a natural infection.

In the starting material the frequency of infection was 5.26 per cent for head smut, 2.17 per cent for common smut, and 7.3 per cent for Fusarium. Among the 7 kinds of WF9 breeding material (including 3 kinds of male sterile) selected by the Institute or received from abroad, two kinds appeared to be fully resistant to all 3 pathogens.

Table 1
The results of investigations of protein and oil content in 37 WF9 mutants
(M₅ generation, 1965).

Stock	Protein %	Oil %
Control	9.8 ± 1.6	4.47 ± 1.0
561 1	13.01	6.89
517 1	11.8	3.83
518 1	12.1	3.01
520 1	11.8	4.52
522 1	13.3	3.59
523 1	12.7	6.52
524 2	11.5	5.91
527 1	13.8	4.78
528 3	12.7	4.96
530 1	11.5	4.71
530 2	11.5	3.62
530 3	11.8	3.92
531 1	12.7	4.26
532 2	12.9	4.81
533 2	12.1	4.32
534 1	10.7	4.68
537 1	11.8	5.06
538 1	9.4	5.09
538 3	11.6	4.49
539 1	13.3	4.51
542 1	9.8	5.41
542 2	9.0	5.19
547 1	10.7	4.27
547 2	13.3	4.51
548 1	14.4	4.26
549 1	11.6	3.68
549 3	10.0	4.41
553 1	8.9	2.48
553 2	12.8	3.74
555 1	14.2	4.03
556 3	11.6	4.45
558 1	10.0	4.43
559 1	9.0	4.20
559 2	8.0	4.82
135 2	9.4	4.26
136 1	10.6	4.00
137 1	9.8	4.47

Among the mutants, 43 out of 60 showed total resistance to head smut. Fourteen of the mutants, however, had a frequency of infection surpassing the standard value significantly, from 10 to 44 per cent.

Thirty-six lines were fully resistant to common smut in the provocation experiment. Of these, 28 lines were totally resistant in the provocation experiment for head smut; therefore, in 28 mutants the two kinds of resistance were to be found together. Some mutants, however, in the control experiment showed a much higher susceptibility for common smut as well (10-33%). As for *Fusarium*, in two experiment places 14 mutants were fully resistant, and 7 mutants were fully resistant to the two smut and the *Fusarium* damages together.

The investigations will be repeated in 1968. The genetical analysis of resistance in the stably resistant form will be begun after this control only.

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J. Sutka

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1. Lysine microbiological assay.

A procedure has been developed using a microbiological assay to rapidly and accurately determine the lysine content of corn grain samples. Samples are prepared for analysis by hydrolyzing a measured scoopful (approximately 200 mg) of ground corn in 10 ml of 6 N HCl at 110°C for 24 hours. Hydrolysis is carried out in a 10 ml screw cap vial. The caps are fitted with Teflon liners and care is taken to insure that the caps fit tightly. After hydrolysis the sample is filtered into a 50 ml beaker and evaporated on a hot plate to dryness. The samples are stored dry in a freezer until ready for assay. Just prior to assay the samples are resuspended in 25 ml of 0.05 M phosphate buffer pH 6.8.

The assay itself is a modified version of the one described by Difco Laboratories. The organism used is *Leuconostoc mesenteroides* and all procedures are identical except that a 3 ml assay volume is used instead of 10 ml. This results in a considerable saving of media and the assay is read directly in the culture tubes. Growth is determined by increase in turbidity at 660 mμ.

Nitrogen is determined on an aliquot of the hydrolyzed sample by Nessler's procedure. Nitrogen determination on an aliquot of the same sample as is used for the lysine assay permits direct expression of lysine in terms of the nitrogen content of the sample and also does not require that gravimetric procedures be used in sample preparation, i.e., the sample does not have to be weighed and the filter paper need not be washed quite so thoroughly.

Typical results are shown in the table below for whole kernel.

Pedigree	<u>Grams Lysine</u> 100 grams Protein	<u>+ 1 Standard deviation</u>
R109B +/+	3.02	<u>+ .35</u>
R109B o ₂ /o ₂	5.42	<u>+ .41</u>

Work is also progressing in our lab for a single kernel assay.

William A. Feist
James C. Cooper
Dennis Elmore

III. REPORT ON MAIZE COOPERATIVE

Seed requests to the Maize Cooperative for 1967 numbered 153. This is one more request than was received in 1966. The requests were from a wide geographical area with 71 per cent coming from 25 states in the U.S. and 29 per cent from 16 foreign countries. This is a 13 per cent increase in foreign requests over 1966. A further breakdown of requests showed that 75 per cent of the requests were from geneticists, 16 per cent from plant breeders and 9 per cent for educational purposes (class ratios, etc.).

During the summer of 1967 seed increases were made on A-B translocation stocks, virescent seedling traits and certain chromosome tester stocks in need of increase. In addition, increases were made of certain selected chromosome tester stocks needing upgrading. Several single gene traits, e.g., su_1^{am} , j_2 , etc., which have been difficult to classify or are very poorly expressed in their present genetic background, were grown to isolate better expressions of the trait. Increases were also made on primary trisomics 3, 5, 8, 10 and in addition trisomics 2 and 4, received from Dr. Doyle, were increased. Work is in progress to isolate the other trisomic stocks of the series.

About 50, permanently designated, reciprocal translocation stocks of Dr. E. G. Anderson were increased in 1967. This completes the assembling of the material at one location that was started in 1960. At present, the collection consists of 863 different reciprocal translocations of the 1,003 listed in Dr. Longley's publication ARS34-16. The material is being ordered and a listing should be available in March.

Work is also continuing on chromosome location of unplaced genes in the collection. This past summer 108 different mutants, which came from Dr. E. G. Anderson's collection and had been located to chromosome over the past several years, were grown. This work will continue with emphasis on more specific location of those traits which will make good seedling or mature plant chromosome markers.

The attached catalogue of stocks represents a listing of currently available genetic stocks. This list is slightly revised from that published in 1967. A more complete list of reciprocal translocation stocks is published in the 1966 News Letter, Vol. 40 (P. 186-190).

Requests for seed and correspondence relative to the stock program should be addressed to Dr. R. J. Lambert, S-116 Turner Hall, Department of Agronomy, University of Illinois, Urbana, Illinois 61801.

R. J. Lambert

Catalogue of Stocks

Chromosome 1ad₁ an₁ bm₂ad₁ bm₂an₁ bm₂

as

br₁ Vgbr₂bz₂^m; Mbz₂^m; m

Kn

Kn Ts₆lw₁P^{CR}P^{CW}P^{MO}P^{RR}P^{RW}P^{VV}P^{RR} ad₁ an₁P^{RR} ad₁ bm₂P^{RR} an₁ gs₁ bm₂P^{RR} br₁ f₁ an₁ gs₁ bm₂P^{WR} bm₂P^{WR} gs₁ bm₂P^{WW} br₁ f₁ bm₂P^{WW} br₁ f₁ ad₁ bm₂Chromosome 1 (continued)P^{WW} br₁ f₁ an₁ gs₁ bm₂P^{WW} hm br₁ f₁rs₂sr₁sr₁ P^{WR} an₁ bm₂sr₁ P^{WR} bm₂sr₁ P^{WR} an₁ gs₁ bm₂sr₁ zb₄ P^{WW}ts₂ts₂ P^{WW} br₁ bm₂Ts₆

Vg

Vg an₁ bm₂vp₅vp₈zb₄ ms₁₇ P^{WW}zb₄ P^{WW} bm₂zb₄ P^{WW} br₁zb₄ P^{WW} br₁ f₁ bm₂zb₄ ts₂ P^{WW}an⁶⁹²³-bz₂ (apparent deficiency including an₁ and bz₂)bm₂

necrotic 8147-31

Chromosome 2

al lg₁
 al lg₁ gl₂ B sk
 al lg₁ gl₂ b sk v₄
 ba₂
 fl₁
 gl₁₁
 Ht
 lg₁
 lg₁ gl₂ B
 lg₁ gl₂ b
 lg₁ gl₂ b fl₁ v₄
 lg₁ gl₂ b fl₁ v₄ Ch
 lg₁ gl₂ B gs₂
 lg₁ gl₂ b gs₂
 lg₁ gl₂ b gs₂ sk
 lg₁ gl₂ B gs₂ v₄
 lg₁ gl₂ b gs₂ v₄
 lg₁ gl₂ b gs₂ v₄ Ch
 lg₁ gl₂ B sk v₄
 lg₁ gl₂ b sk v₄
 lg₁ gl₂ b sk fl₁ v₄
 lg₁ gl₂ B v₄
 lg₁ gl₂ b v₄
 lg₁ gl₂ b v₄ Ch
 lg₁ gs₂ b v₄
 w₃

Chromosome 2 (continued)

w₃ Ch
 ws₃ lg₁ gl₂ B
 ws₃ lg₁ gl₂ b
 ws₃ lg₁ gl₂ b fl₁ v₄
 ws₃ lg₁ gl₂ B sk
 ws₃ lg₁ gl₂ b sk
 wt
 mn

Primary trisomic 2

Chromosome 3

A₁ ga₇; A₂ C R
 A₁ sh₂; A₂ C R
 A^d-31; A₂ C R
 A^d-31; A₂ C R Dt₁
 A^d-31 sh₂; A₂ C R
 a^P et; A₂ C R Dt₁
 a₁; A₂ C R B Pl dt₁
 a₁ et; A₂ C R Dt₁
 a₁ sh₂; A₂ C R Dt₁
 a₁ sh₂; A₂ C R dt₁
 a₁st Sh₂; A₂ C R Dt₁
 a₁st sh₂; A₂ C R Dt₁
 a₁st sh₂ et; A₂ C R Dt₁
 a₁st et; A₂ C R Dt₁
 ba₁
 Cg

Chromosome 3 (continued)

cl_1
 cr_1
 d_1
 $d_1 Lg_3$
 $d_1 ts_4 lg_2$
 $d_1 ts_4 lg_2 a_1; A_2 C R Dt_1$
 d_2
 $gl_6 lg_2 a_1 et; A_2 C R Dt_1$
 gl_7
 $lg_2 a_1 et; A_2 C R Dt_1$
 $lg_2 a_1 et; A_2 C R dt_1$
 $lg_2 a_1 sh_2 et; A_2 C R Dt_1$
 $lg_2 a_1^{st} et; A_2 C R Dt_1$
 $lg_2 a_1^{st} sh_2; A_2 C R Dt_1$
 $lg_2 pm$
 Lg_3
 $Lg_3 Rg$
 na_1
 pm
 ra_2
 $ra_2 lg_2 pm$
 $ra_2 Rg$
 Rg
 rt
 ts_4
 $ts_4 na_1$
 ys_3

Chromosome 3 (continued)

pg_2
 vp_1
 Primary trisomic 3

Chromosome 4

bm_3
 bt_2
 $bt_2 gl_4$
 $c_2; A_1 A_2 C_1 R$
 fl_2
 $Ga_1 Su_1$
 $Ga_1^s Su_1$
 gl_3
 $la su_1 gl_3$
 $lw_4; lw_3$
 o_1
 st
 $su_1 bm_3$
 $su_1 gl_3$
 $su_1 gl_4$
 $su_1 ra_3$
 $su_1 Tu$
 $su_1 Tu gl_3$
 $su_1 zb_6$
 $su_1 zb_6 Tu$
 su_1^{am}
 Ts_5
 $Ts_5 su_1$

Chromosome 4 (continued)Tu gl₃v₈

Primary trisomic 4

Chromosome 5a₂; A₁ C Ra₂ bm₁ bt₁ bv₁ pr; A₁ C Ra₂ bm₁ bt₁ pr; A₁ C Ra₂ bm₁ pr v₂; A₁ C Ra₂ bm₁ pr ys₁; A₁ C Ra₂ bt₁ pr; A₁ C Ra₂ bt₁ pr ys₁; A₁ C Ra₂ v₃ pr; A₁ C Ra₂ pr; A₁ C R

ae

bm₁ pr; A₁ A₂ C Rbm₁ pr v₂; A₁ A₂ C Rbm₁ pr ys₁; A₁ A₂ C Rbm₁ pr ys₁ v₂; A₁ A₂ C Rbt₁ pr; A₁ A₂ C Rgl₅gl₈gl₁₇ bt₁gl₁₇ v₂lw₂lw₃; lw₄na₂Chromosome 5 (continued)na₂ prpr; A₁ A₂ C Rpr ys₁; A₁ A₂ C Rys₁v₃ pr; A₁ A₂ C Rv₁₂vp₂ gl₈vp₂ pr; A₁ A₂ C Rvp₇vp₇ pr; A₁ A₂ C R

Primary trisomic 5

Chromosome 6at = allele of si₁

Bh

po Y₁ plpo y₁ pl

Pt

si₁

wi

y₁ l₁₀Y₁ pb₄ plY₁ pg₁₁; wx pg₁₂y₁ pg₁₁; wx pg₁₂y₁ Pl Bhy₁ pl BhY₁ Pl sm Pt

Chromosome 6 (continued)Y₁ Pl smY₁ Pl sm py; A₁ A₂ b P^{RR}Y₁ pl su₂y₁ pl su₂y₁ Pl; seg w₁l₄₉₂₀"male sterile-silky" =
allele of si₁

"orobanche" (seedling)

"white 865F" (seedling)

Chromosome 7

Bn

bd

E₂gl₁gl₁ E₂gl₁ ij bdgl₁ slgl₁ Tp₁

Hs

ij

ij bd

in; pr A₁ A₂ C Ro₂o₂ bdo₂ gl₁ slChromosome 7 (continued)o₂ ra₁ gl₁o₂ ra₁ gl₁ ijo₂ ra₁ gl₁ Tpo₂ v₅ gl₁; seg ra₁o₂ v₅ ra₁ gl₁o₂ v₅ ra₁ gl₁ Hso₂ v₅ ra₁ gl₁ Tp₁ra₁ gl₁ ij bdTp₁vp₉ gl₁; wxChromosome 8gl_gv₁₆ j₁v₁₆ j₁; l₁v₁₆ ms₈ j₁

"necrotic 6697" (seedling)

"sienna 7748" (seedling)

Primary trisomic 8

Chromosome 9Bf₁Bf₁ bm₄bm₄bp Wx; P^{RR}

C Ds wx

C sh₁ Wx; A₁ A₂ R

Chromosome 9 (continued)

C sh₁ wx; A₁ A₂ R
 c sh₁ wx; A₁ A₂ R
 c sh₁ wx g¹₁₅
 c sh₁ wx g¹₁₅ Bf₁
 c sh₁ wx bk₂
 C wx; A₁ A₂ R
 c Wx; A₁ A₂ R
 c wx; A₁ A₂ R
 c wx v₁
 c wx Bf₁
 Dt₁ (See chromosome 3 stocks)
 g¹₁₅
 g¹₁₅ Bf₁
 g¹₁₅ bm₄
 I Ds Wx
 I wx; A₁ A₂ R B pl
 K^L₉ C sh₁ wx; A₁ A₂ R
 l₆
 l₇
 ms₂ sh₁; A₁ A₂ C R
 sh₁ wx g¹₁₅
 sh₁ wx l₇
 sh₁ wx v₁
 wx Bf₁
 wx Bf₁ bm₄
 wx bk₂

Chromosome 9 (continued)

wx bk₂ bm₄
 wx d₃
 wx l₆
 Wx p_g₁₂; Y₁ p_g₁₁
 wx p_g₁₂; Y₁ p_g₁₁ pl
 wx p_g₁₂; Y₁ p_g₁₁
 wx^a
 y_g₂ c sh₁ wx; A₁ A₂ R
 y_g₂ c sh₁ bz wx; A₁ A₂ R
 y_g₂ c sh₁ wx g¹₁₅; A₁ A₂ R
 y_g₂ C sh₁ bz wx; A₁ A₂ R
 Primary trisomic 9

Chromosome 10

bf₂
 du₁
 g₁
 g₁ r^g; A₁ A₂ C
 g₁ r^{ch}
 g₁ r; A₁ A₂ C wx
 g₁ R sr₂
 g₁ r sr₂
 l₁
 l₁; seg w₁
 li g₁ R; A₁ A₂ C
 li g₁ r; A₁ A₂ C
 nl₁ g₁ R; A₁ A₂ C

Chromosome 10 (continued)Og R; A₁ A₂ C B Ploy "oil yellow"
(seedling and plant)r^r; A₁ A₂ Cr abnormal 10; A₁ A₂ CR^g sr₂; A₁ A₂ Cr^r sr₂; A₁ A₂ Cr^g wx; A₁ A₂ CR^r: Boone; A₁ A₂ CR^{mb}; A₁ A₂ CR^{nj}; A₁ A₂ CRst; A₁ A₂ Cv₁₈w₂w₂ l₁

zn

Primary trisomic 10

Unplaced genes

el

gl₁₂gl₁₄gl₁₆

h

l₃l₄ms₆Unplaced genes (continued)ms₉ms₁₂ms₁₃ms₁₄Rs₁v₁₃w₁₁ws₁ ws₂zb₁zb₂zb₃

"luteus 4923" (seedling)

"necrotic 8376" (seedling)

Multiple gene stocksA₁ A₂ C R^r Pr B PlA₁ A₂ C R^g Pr B PlA₁ A₂ C R PrA₁ A₂ C R Pr wxA₁ A₂ C R Pr wx gl₁A₁ A₂ C R Pr wx y₁A₁ A₂ C R prA₁ A₂ C R pr y₁ gl₁A₁ A₂ C R pr y₁ wxA₁ A₂ C R pr y₁ wx gl₁A₁ A₂ c R Pr y₁ wx

Multiple gene stocks (continued)A₁ A₂ C r Pr y₁ wxbm₂ lg₁ a₁ su₁ pr y₁ gl₁ j₁ wx g₁

colored scutellum

lg₁ su₁ bm₂ y₁ gl₁ j₁su₁ y₁ wx a₁ A₂ C R^G pry₁ wx gl₁Popcorns

Amber Pearl

Argentine

Black Beauty

Hulless

Ladyfinger

Ohio Yellow

Red

South American

Strawberry

Supergold

Tom Thumb

White Rice

Exotics and VarietiesBlack Mexican Sweet Corn
(with B-chromosomes)Black Mexican Sweet Corn
(without B-chromosomes)

Gourdseed

Maiz chapolote

Papago Flour Corn

Exotics and Varieties (continued)

Parker's Flint

Tama Flint

Zapaluta chica

Chromosome rearrangements

The following rearrangements are being maintained primarily for use in determining the chromosome locations of new traits. All are marked with closely-linked endosperm or seedling traits.

The cytological positions of Inv 2a were determined by Dr. Morgan; those of Inv 9a were determined by Dr. Li. The indicated interchange points of the reciprocal translocations are taken from published work of Dr. Longley.

Inversions

*gl₂ Inv 2a (also available with Ch) 2S.7; 2L.8
 *wx² Inv 9a 9S.7; 9L.9

Reciprocal translocations

*wx 1-9c	1S.48; 9L.22
*wx 1-9 4995	1L.19; 9S.20
*wx 1-9 8389	1L.74; 9L.13
*wx 2-9b	2S.18; 9L.22
*wx 3-9c	3L.09; 9L.12
wx 3-9 5775	3L.09; 9S.24
*wx 4-9b	4L.90; 9L.29
*wx 4-9 5657	4L.33; 9S.25
*wx 4-9g	4S.27; 9L.27
*wx 5-9a	5L.69; 9S.17
*wx 5-9c	5S.07; 9L.10
*wx 5-9d	5L.14; 9L.10
wx 5-9 4817	5L.06; 9S.07
*wx 6-9a	6S.79; 9L.40
*wx, y 6-9b	6L.10; 9S.37
wx 6-9 4505	6L.13; 9 cent
wx 6-9 4778	6S.80; 9L.30
*wx 7-9a	7L.63; 9S.07
*wx or gl ₁ 7-9 4363	7 cent; 9 cent
*wx 8-9d	8L.09; 9S.16
*wx 8-9 6673	8L.35; 9S.31
*wx 9-10b	9S.13; 10S.40

*These constitute a basic series of twenty rearrangements for use in locating unplaced genes.

Stocks of A-B chromosome translocations

B-1a	1L.2	Proximal to <u>Hm</u>
B-1b	1S.05	
B-3a	3L.1	
B-4a	4S.25	Proximal to <u>su</u> ₁
B-7b	7L.3	Proximal to <u>ra</u> ₁
B-9a	9L.5	Proximal to <u>Bf</u> ₁
B-9b	9S.4	Between <u>C</u> and <u>wx</u> ; close to <u>wx</u>
B-10a	10L.35	Proximal to <u>g</u> ₁

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