MAIZE GENETICS COOPERATION

NEWS LETTER

41

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I. FOREWORD

It is my pleasure to acknowledge again the efficient and dedicated service of Miss Ellen Dempsey in the editing, supervising and assembling of the Maize Genetics News Letter. She alone has had this responsibility; it is one of considerable magnitude and we are all grateful for the admirable way she has performed an arduous task. Grateful mention should be made of the voluntary assistance of Wayne Carlson, Earle Doerschug, William Laughner, Judith Miles, John Mottinger, Paul Nel, Reid Palmer and Karl Rinehart in proof reading.

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 major portion of 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.

M. M. Rhoades

II. REPORTS FROM COOPERATORS

UNIVERSITY OF AGRICULTURAL SCIENCES
Gödöllö, Hungary
Department of Plant Breeding

1. Studies on nucleic acid of maize hybrids and mutants.

In 1958 we first reported our tests on biologically active materials, among them the nucleic acid, found in seeds of maize hybrids and parental lines.

Data of similar character have been found in the investigations of Cherry et al (1961) as well. In his tests Semenenko (1964) has determined the base composition of RNA in the seeds of some single crosses and the parental lines.

Our tests were extended, in addition to the RNA, to the base composition and base proportion of the DNA as well.

In the course of our investigations the single steps of the preparation and determination of the matter were performed with the methods of Schneider-Schmith-Tannhauser 1945, La Page 1957, Brown 1962, and Martyn-Doby 1949.

In our tests a Radi Rac fraction collector was used. The data given are the average values of 3-6 investigations with $\pm$ 5 per cent of error possibility.

The results of the two crosses (Table 1) show a significant deviation. As early as 1958 our data showed that the total nucleic acid content of the combination C5 x O14 essentially surpassed the related values of the parental lines. This fact has been confirmed by the recent data. At the same time, in the other single cross the values for the F_1 were intermediate.

As for the proportion $\frac{A + C}{C + D}$, the value of the first single cross is nearer that of the mother; that of the second one essentially surpasses even the better paternal line.

The RNA values show a trend similar to that of DNA. The correlation of the $\frac{A + C}{C + D}$ is lower than that of the parents in the first combination; in the second one the value is higher than that of the parents.

Although we cannot draw any general conclusions from studies of two combinations, nevertheless it seems to be probable that the quantitative correlation of the single bases, without regard to the sequence of bases, cannot account for the substantial factors of the phenomenon of heterosis. However, these data can be considered reliable characteristics of the lines and their hybrids.

Table 1
The DNA and RNA base composition of corn lines, single crosses and mutants

Populations	DNA Mol %				$\frac{A+G}{C+T}$	RNA Mol %				$\frac{A+G}{C+U}$
	A	C	T	G		A	C	U	G	
Lines and single crosses										
C5	4.576	4.280	3.251	4.301	1.178	35.464	12.509	16.046	13.387	1.710
C5 x 014	16.133	13.801	10.736	11.352	1.120	36.166	15.867	32.258	21.274	1.123
014	5.824	7.097	4.583	5.698	0.986	23.426	9.680	14.903	14.300	1.534
T18	8.684	7.220	4.299	5.170	1.202	17.225	7.294	25.600	18.062	1.072
T18 x WF9	10.621	6.765	5.434	7.007	1.445	29.055	8.942	28.274	20.482	1.331
WF9	16.770	12.607	6.568	8.305	1.307	33.735	16.506	35.976	25.542	1.129
Mutants										
WF9K	16.770	12.607	6.568	8.305	1.307	33.735	16.506	35.976	25.542	1.129
WF9/1	17.095	18.352	14.129	16.962	1.048	30.836	23.124	50.998	46.387	1.041
WF9/2	16.250	9.188	5.481	5.357	1.472	50.661	16.568	36.761	37.774	1.658

WF9/1 - mutant with broad leaves

WF9/2 - dwarf mutant

In the formation of morphological changes we think the differences in base composition are characteristic in the case of the mutants. Some substantial differences were seen in the base composition of the morphologically deviating forms, derived from the WF9 line in 1961 with 7,000 r X-ray, and even in the formation of the base correlations as well. The trends were similar in both nucleic acids. Additional tests will yield data as to whether a quantitative change in the base composition appears in every case during a mutational change, or whether the values obtained must be considered as exceptional.

A. Bálint
Mrs. G. Kovács
J. Sutka

ANDHRA UNIVERSITY
Waltair, India
Department of Botany

1. Chromosome knobs in maize types from the North-Eastern Frontier Area (NEFA) of India.

Chromosome knobs in two maize types from Nefa were reported earlier (MNL 39: 185, 1965). More types from this area are now investigated and reported below.

M 34: 8 knobs were observed, one each on the long arm of chromosomes 2, 3, 5, 6 and 7, two on chromosome 8 and one on the short arm of chromosome 9. There is a chromomere on the short arm of chromosome 1 and three near the end of the long arm of chromosome 4.

M 35: 6 knobs were observed, one each on the long arm of chromosomes 2, 6 and 7, two on chromosome 8 and one on the short arm of chromosome 9. There is a chromomere on the short arm of chromosome 1.

M 37: 7 knobs were observed, one each on the long arm of chromosomes 2, 4, 5, 6, 7 and 8 and one on the short arm of chromosome 9. Two chromomeres are present on the short arm of chromosome 1 and on the long arm of chromosome 3 and one each on the long arm of chromosomes 4, 6 and 9.

M 38: 5 knobs were observed, one each on the long arm of chromosomes 2, 4, 6 and 8 and one on the short arm of chromosome 9. There is a chromomere on the short arm of chromosome 1, two on the long arm of chromosome 5 and one each on the long arm of chromosomes 6 and 8.

Except the knob on the short arm of chromosome 9 which is terminal, the rest are interstitial in all the above types.

J. Venkateswarlu
K. G. Raja Rao

2. Chromosome knobs in maize types from the Sikkim region.

During the course of a cytogenetic survey of maize types cultivated in the Sikkim region some types from that area have been analyzed and reported below.

M 306: 9 knobs were observed, one each on the long arm of chromosomes 2, 4, 6 and 7, two on chromosome 8 and one each on the short arm of chromosomes 2, 3 and 9. There is a chromomere on the short arm of chromosome 1.

M 308: 7 knobs were observed, one each on the long arm of chromosomes 4 and 6 and two on chromosome 8 and one each on the short arm of chromosomes 2, 3 and 9. There is a prominent chromomere on the short arm of chromosome 1.

M 303: 6 knobs were observed, one each on the long arm of chromosomes 2, 4, 5, 6 and 7 and one on the short arm of chromosome 9. Two chromomeres are present on the short arm of chromosome 1, one on the short arm of chromosome 3 and one on the long arm of chromosome 6.

Except the knob on the short arm of chromosome 9 which is terminal, the rest are interstitial in all the three types.

K. G. Raja Rao

3. Increase in the frequency of plants with chromosomal interchanges in a mixed population of Coix aquatica.

Cytological abnormalities in two populations of Coix aquatica (obtained from Orissa and Madhya Pradesh) were reported earlier (MNL 39:183-184, 1965). These two populations and a third population from Andhra Pradesh were grown side by side in an experimental garden. From a random seed lot taken from the total seed harvested from open pollinated (naturally outbreeding) plants of the three populations, a mixed population was raised the next year. The process of collection of random seed lot from the bulk seed and raising the progeny the following year was repeated four times. This year 72 random plants of the mixed population were scored cytologically to see whether all the categories of cytological variations reported earlier persist in the mixed population also and with the same frequency. The variations observed presently were mostly chromosomal interchanges, a few cases of accessory chromosomes and a single case of aneuploidy. Polyploidy, even as sectorial, was not observed. The categories of cytological variations, their frequency and percentage of occurrence in the mixed population are given below.

Category	Frequency	Per cent
1. Normal plants	43	58.33
2. Plants with interchanges	26*	36.05*
3. Plants with accessory chromosomes	4*	5.56*
4. Plants with aneuploid chromosome number	1	1.39

While there is a good increase in the percentage of individuals with interchanges in the mixed population from 20 - 25 (earlier report) to 36.05, there is either a total disappearance or a reduction in frequency of plants with other chromosomal abnormalities when compared to the observations recorded from the original populations. In Coix aquatica it may be assumed that when all the plants in the population are provided equal chance of survival and growth in the experimental garden, it appears likely that translocations float in the population with increased frequency rather than being lost like the other chromosomal variations.

J. Venkateswarlu
Panuganti N. Rao

4. Variation in pollen fertility in a population of Coix aquatica.

Pollen fertility in 40 plants of a mixed population of Coix aquatica was determined by counting stained pollen grains in acetocarmine. Pollen fertility varied between 28.12% and 95.88% in plants with normal meiosis and about three-fourths of these plants showed pollen fertility of over 50%. The plants with chromosomal interchanges, involving nucleolar and non-nucleolar chromosomes, forming higher associations of 3, 4, 5 and 6 chromosomes at meiosis have a pollen fertility ranging from 17.10% to 94.00%. Nearly one-half of these plants have more than 50% of pollen fertility. It may therefore be assumed that at least some of the interchanges do not seem to have any adverse effect on pollen fertility and the plants with interchanges show as high or as low a pollen fertility as normal plants. This is perhaps indicative that in C. aquatica pollen fertility is more a function of the genetic make-up of the individual than the effect of the chromosomal interchanges.

J. Venkateswarlu
Panuganti N. Rao

5. Meiosis in autotetraploid Chionachne koenigii.

Autotetraploidy was induced in Chionachne koenigii through colchicine treatment. Root portions of seedlings (raised from seed obtained from Maharashtra) with 2-3 leaves were dipped in 0.4% aqueous solution of

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*Two plants which showed the presence of interchanges as well as accessory chromosomes are included in both categories for the purpose of calculating the frequency and percentage.

colchicine for 12 hours. The seedlings were then thoroughly washed in running water and planted in pots under shade. Out of 30 plants treated, 10 survived to maturity. Of these three are complete autotetraploids (4n = 40). The rest of the seven plants showed sectorial autotetraploidy of varying degrees, either limited to a few panicles on the same plant or only to a few meiocytes in an anther. In one of the plants showing sectorial autotetraploidy, meiosis was studied. Chromosome associations were studied in 60 cells at diakinesis. The types of associations observed were quadrivalents, trivalents, bivalents and univalents. Quadrivalents varied in number from 0-6 per cell. Trivalents, not more than one per cell, were observed in four cells. A univalent was observed only when a trivalent was present in a cell. The average frequency of chromosome associations per cell was 2.73iv, 0.07iii, 14.5ii, 0.07i; 27.83% of the chromosomes have gone into the formation of multivalents. The average chiasma frequency per cell in tetraploid was 33.67, which is more than twice the 15.63 in the diploid (2n = 20) undoubled sectors of the same plant. Laggards 1-4 and irregular distribution of chromosomes at either pole (19:21) were observed in 18.75% of the cells each; bridges were found in 12.5%, and normal distribution of 20:20 chromosomes was observed in 50.00% of the cells at anaphase I. In the second division, laggards and/or micronuclei (1-4) and bridges were seen in 38.46% of the cells examined. Micronuclei (1-3) were found in 22.28% of the pollen tetrads.

J. Venkateswarlu
Panuganti N. Rao

6. Colchicine induced autotetraploids of Job's tears (Coix lachryma-jobi).

Induction of autopolyploidy in Job's tears was reported last year (MNL 40: 165, 1966). This year seedlings raised from seed collected from open pollinated plants of Coix lachryma-jobi (2n = 20) growing wild in the University campus were treated with colchicine in the manner described for Chionachne (item 5 above). Out of 28 plants so treated only three survived and lived to maturity. All three plants exhibited gigas characters and cytological examination of pollen mother cells showed that these are autotetraploids (4n = 40). The tetraploid plants, like the diploid ones, produce stem suckers from the basal nodes of the original tillers. Cytological check of the male florets borne on the stem suckers also showed a tetraploid chromosome number and behavior. Seed set is extremely poor in the tetraploids in spite of selfing, intercrossing among tetraploids or crossing tetraploid with diploid plants. For cytogenetical study, besides propagation through seed, it is expected that it should be possible to propagate the tetraploid stock vegetatively by carefully nursing the stem suckers.

J. Venkateswarlu
Panuganti N. Rao

7. Apomixis in Job's tears (Coix lachryma-jobi).

Two plants of Job's tears were found growing isolated in the Botany Experimental Farm. The plants were weak and produced a few female spikelets. The male spikelets were either absent or only poorly developed. The latter when opened and examined were found to be empty. The plants, therefore, were male sterile but they produced normal healthy seeds. Since there was no pollen source anywhere near, it was suspected that the seeds were formed apomictically. To make sure that there was no contamination by pollen from outside, the female spikelets were bagged before the styles showed. Normal healthy seeds were obtained from these also. Further, when the ovules were squashed and examined, they showed the presence of several five-nucleate embryo sacs in each indicating the occurrence of apomixis. It was reported earlier (MNL 39: 183, 1965) that Coix when crossed with maize (used as male) produced parthenogenetic diploid mother plants.

J. Venkateswarlu
Panuganti N. Rao

UNIVERSITY OF ARIZONA
Tucson, Arizona

1. Physiological studies of the stature mutant nana-1.

The stature mutant, nana-1, is a non-responder to gibberellins. Through the use of bioassays van Overbeek demonstrated a higher rate of auxin inactivation in the coleoptile of the mutant as compared to that of its normal sib.

Using extracts of both nana and normal coleoptiles, we have tested for inhibition of elongation in the Avena coleoptile section test, qualitatively analyzed the peroxidase isozymes by use of polyacrylamide gel disc electrophoresis, and tested peroxidase activity quantitatively by spectrophotometric assays. These studies have revealed no qualitative or quantitative differences in peroxidase activity between nana and its normal sib.

Inhibitors of peroxidase activity, such as ferulic and caffeic acids elicited no differential response between treated normal and nana intact coleoptiles or mesocotyls. However, incubated mutant coleoptile sections responded to lower concentrations of IAA and elongated more than incubated sections of normal coleoptiles as well as comparable sections on intact normal seedlings.

These initial studies suggest that differences in growth rates of the nana and normal coleoptiles and mesocotyls are due to differential auxin production and not to differences in peroxidase activity.

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R. M. Harris

BHABHA ATOMIC RESEARCH CENTRE
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Biology Division

1. Occurrence of two pollen-fertile plants following mitomycin treatment of maize seeds carrying male-sterilizing Texas cytoplasm.

Maize seeds (250) carrying male-sterilizing Texas cytoplasm when treated with 200 µg/ml of mitomycin (MC) for 16 hours, yielded 2 plants that were sectorially male-fertile. One of these had a rudimentary cob and was also out of phase with the testers and therefore could not be tested. The other plant was self-pollinated and also crossed to the male-sterile line. From the latter only pollen-sterile plants were obtained indicating that no change had occurred in any restorer gene. The selfed plant yielded a progeny of 40 male-sterile and 6 pollen fertile plants. Six fertile plants on selfing gave only fertile plants and the 40 sterile plants on being crossed by the sterility maintainer yielded only sterile plants. The observations and interpretations are consistent with the notion that the change had occurred at the cytoplasmic level.

P. S. Chourey
N. K. Notani

2. A sequel to note 1.

While this appeared to be an authentic case of induced restoration of fertility, in a recent and a much larger experiment (about 2000 plants treated with MC) no restored plants were observed. The conditions of treatment were similar except that the treatment was given for 24 hours so that the failure in the present experiment was unexpected.

D. Sen
N. K. Notani

3. Inhibition of DNA synthesis by MC in germinating maize embryos.

Mitomycin (MC) is a known inhibitor of DNA synthesis. Some success in male-fertility restoration by MC in our early experiments suggested that this might be due to an inhibition of DNA synthesis. We have found that DNA synthesis in germinating embryos is inhibited in the presence of MC. This inhibition is released when the seeds are transferred to MC-free medium (H_2O).

Maize seeds carrying male-sterile cytoplasm (T type) were germinated in the presence of 50 µg/ml, 100 µg/ml and 200 µg/ml concentration of MC and ^{3}H-thymidine (20 µc/ml) for 24 and 48 hours in dark at 32°C. under shaking. Appropriate controls were kept. After the treatment, seeds were washed in cold, embryos were excised, transferred to cold saline-EDTA (0.15M saline - 0.10M EDTA at pH 8.5), homogenized, lysed with lysozyme (100 µg/ml) and sodium lauryl sulphate (final conc. 1%). Samples of the lysates were spotted on filter paper discs, dried, washed

with cold 10% TCA and cold acetone, dried and counted in a Tricarb Scintillation spectrometer. The acid-insoluble counts give a measure of DNA synthesis. MC inhibited the incorporation of ^{3}H-thymidine into DNA, the maximum inhibition being about 70% at conc. of 200 µg/ml.

The seeds were not killed at this concentration and when returned to MC-free medium, the thymidine incorporation returned to normal in about 12 hours.

D. Sen
N. K. Notani

4. Rates of DNA synthesis in embryos of Black Mexican Sweet strains with and without B chromosomes.

Rates of DNA synthesis were measured by ^{3}H-thymidine and ^{32}P incorporation in DNA of germs of Black Mexican Sweet strains with and without B chromosomes. The strain with B chromosomes had an average of two B chromosomes. In both cases 10 seeds of each strain were treated with fungicide, washed thoroughly and then incubated in the isotope solutions at 35°C with shaking. Results might have been somewhat vitiated as the germination in the two strains was not uniform. DNAs were extracted by the procedure of Marmur (J. Mol. Biology 3:208-218) after the embryos from the two strains had been matched, homogenized and lysed. DNA solutions were treated with RNase (50 µg/ml) to degrade any RNA. Spots (0.1 ml) were made on filter paper discs, treated with cold 10% TCA and cold acetone, dried and counted in a Tricarb Scintillation spectrometer. The counts are given below:

	DNA Soln.	DNA Soln.
	^{3}H Cts./min/0.1 ml	^{32}P Cts./min/0.1 ml
Strain without B chromosomes	2051	1618
Strain with B chromosomes	1359	1633

While ^{32}P incorporation is equal in the two strains, ^{3}H incorporation is considerably lower in the strain with the B chromosomes. No unequivocal conclusions are possible.

D. Sen
N. K. Notani

5. Effect of change in chromosomal position of endosperm markers in maize on their radio-sensitivity.

It has been shown that the loss of endosperm markers following pollen irradiation in maize is dependent on the absolute (pachytene) length of the chromosome arm and on its position in that arm. Although, evidence

had been obtained from data collected for the loss of markers I Sh_1 Bz Wx, it nevertheless allowed a forecast of the relative loss of other endosperm markers. The forecast was verified for A_1, Pr, R, Su and Sh_1 (Faberge). An independent verification of the forecast may be obtained by comparing the calculated and observed loss rate of markers when they are situated on chromosome arms with changed pachytene lengths. This condition may be provided in stocks with suitable chromosomal translocations.

A number of such translocation stocks were selected and made homozygous. Four of such homozygous stocks with proper genetic background were used for experiments. Two of the stocks (I and II) had increased length of the 9th chromosome short arm, one stock (III) had increased length of the 10th chromosome long arm and one stock (IV) had decreased length of the 4th chromosome short arm. Fresh pollen from each stock was collected, cleaned and irradiated with 1 Kr of γ-rays and crossed on to appropriate testers. With each sample of pollen, a sample of pollen from an appropriate control stock was also irradiated and test-crossed.

Stocks I and II showed complete and partial loss of markers by formation of breakage-fusion-bridge cycles for the 9th chromosome short arm linked markers in 3.43% and 3.40% of kernels, respectively, whereas in the control only 0.57% kernels showed loss of markers. The figure for the control is comparable to figures found in the literature. It seems that changed arm length in stocks I and II did bring about change in the expected direction in the loss rate of markers on them. There was very little seed set from stock III and nothing could be scored. Stock IV showed whole or partial loss of kernel markers in 4.78% cases but no comparison could be made as the control had no seed set. The known loss rate in the normal type is 1.0%. It is difficult to compare these data with those from other sources because of different genetic backgrounds of the stocks and possible differences in the dosimetry. In our experiments, though the translocation and normal stocks were irradiated together, eliminating any difference in dose, the genetic background of the stocks was not the same. For a proper comparison of loss rate a reference marker in the normal position should be used as a control. We are preparing double tester stocks for that purpose.

D. Sen
N. K. Notani

6. Inhibitor of aleurone colour.

A comparison has been made of aleurone colour inhibiting capacities of inhibitors from different sources. While the inhibiting capacities of I^{Coe} and $I^{Coop.}$ are roughly similar, $I^{Trombay}$ inhibits pigmentation to a lesser extent when tested against a common tester (color indices 2.31, 2.16 and 3.54 respectively). $I^{Trombay}$ is apparently allelic to I^{Coe} as borne out by linkage tests and by segregation pattern of kernels from a testcross of $I^{Coe}/I^{Trombay}$ heterozygotes. With our ACR tester (originally obtained from Prof. R. A. Brink), all inhibitors effect complete inhibition only when transmitted through the female. When

transmitted through the male, inhibition is only partial. It might be considered that such a behavior of $\underline{I}^{Coe}$ and $\underline{I}^{Trombay}$ is due to a gene-dose effect. However, the isolation of a variant of $\underline{I}^{Trombay}$ which gives very little inhibition through the female would cast a doubt on such an explanation. Schwartz (Genetics Today 2:131-135, 1965) has discussed the inadequacies in explaining the action of several gene loci in terms of gene dose.

With a low frequency, the cross $\underline{I}^{Trombay}\ \underline{I}^{Trombay}$ x AACCRR yields colored or mottled kernels. To date 25 such kernels have been obtained from over 35,000 kernels scored (120 cobs). Out of these 25 kernels, only one was a genuine case of mutation from the dominant to the recessive colorless form. The remaining kernels did not indicate any germinal change.

The frequency of colored kernels is somewhat higher when $\underline{I}^{Trombay}/\underline{I}^{Coe}$ is pollinated by ACR. One hundred and twenty kernels have been obtained from 57,057 kernels scored (207 cobs). Thirty-four of the 120 kernels when crossed by ACR gave smoky kernels ranging in number from 5% - 60% of the total, suggesting a possible change in the expression of the inhibitors.

Chandra Mouli
N. K. Notani

7. Stability of Ds at the A_1 locus.

No change has been detected in the expression of Ds at the A_1 locus after pollen or seed of an A_1 Ds no Ac stock was subjected to the following treatments:

Treatment	No. Analyzed	Observations
1. U. V. irradiation of pollen	12,300	All kernels colorless
2. P^{32} injected i) 30 days after sowing ii) at the time of flowering near tassel node. Pollen used.	21,665	All kernels colorless
3. Desiccated A_1 Ds no Ac seeds heated to 90°C., 100°C. and 110°C. for 15, 30 and 45 minutes and then chilled rapidly. Plants grown.	406	All kernels colorless

Chandra Mouli
N. K. Notani

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1. Selection experiments on irradiated maize populations.

Ionizing radiations are known to induce new genetic variability which may be used to improve characters of economic value through selection.

On the other hand, ionizing radiations applied to seeds are known to produce deleterious effects that might reduce the practical utility of induced mutations.

Since Scossiroli (1965) has shown that at least in part, after seed treatment, these deleterious effects are of non-nuclear nature it appeared worthwhile to test the effectiveness of a selection applied to irradiated material in which the radiation treatment was applied to the male gamete.

With this goal, selection experiments were started from populations of maize derived from plants whose tassel was irradiated with different doses of X-rays. After the radiation treatment the plants were selfed for three successive generations so that at the start of the selection experiment the populations to be selected were made of many progenies derived by selfing from single plants and classified according to their pedigree.

Two selection experiments were performed, one using material which received doses of 0 r, 1500 r, 3000 r, the second using material obtained after treatments with doses of 0 r, 500 r, and 1000 r.

Three characters were considered:

a. number of branches on the tassel
b. number of internodes below the highest ear
c. total length of internodes below the highest ear.

Disruptive selection in plus and minus directions and stabilizing selection for the average value were performed with a 25% pressure in the first experiment and a 15% pressure in the second.

Reproduction of the selected plants was performed by intercrossing plants within progenies, in order to avoid as much as possible the consequences of inbreeding on the amount of genetic variability present in the selected progenies at the onset of the selection.

In the experiment started on populations treated with 0 r, 1500 r, 3000 r, selection was performed within groups of plants corresponding to the progeny of R_2 plant. In the other experiment, selection was performed disregarding any pedigree classification.

When considering the results shown in Tables 1 and 2, we must remember that we expect a more effective response to the selection applied in the irradiated populations in respect to the control population.

As may be seen from the results, for selection in the plus direction there seems to be an increase of the response with the dose, in the two experiments, only for the character "number of internodes below the highest ear," whereas the other characters considered show for the same direction of selection a decrease of the mean value with the increase of the dose of X-rays applied.

The mean values of the plants selected in the minus direction show a slight tendency toward increase with increase of dose in the three characters considered in the two experiments.

The stabilizing selection (for the mean value) seems to produce a slight decrease of the means of the characters considered, with the increase of dose applied.

Considering the results obtained with disruptive selection in both plus and minus directions, the results suggest the existence of a kind of "reverse response." The easiest explanation we can supply for these results is that the phenotypic expression we have selected for, did not correspond to the genotypic value of the plant chosen.

In other words we believe that the genetic variability of the population from which the selection experiments were started was mainly contributed by epistatic and dominance effects.

The results of our selections also suggest that these effects of dominance and epistasis manifest themselves in both directions, plus and minus.

The decrease of the mean value with the dose observed in selections for the population mean suggests that the deleterious effects of the ionizing radiations are produced also when the male gamete is treated.

A possible explanation for the deleterious effects may be found in the presence of "bad mutations" as a consequence of mutagenic treatment, which are transmitted and retained in later generations after radiation under inbreeding when showing heterotic effects.

Table 1
Mean values of the plants selected (1st experiment)
after three generations of selections

Characters	Direction of selection	Control = 0r	1500r	3000r
Number of branches on the tassel	plus	14.95	14.33	14.28
	average	13.49	13.11	13.03
	minus	13.38	13.70	13.04
Number of internodes below the highest ear	plus	13.02	13.06	13.23
	average	13.02	13.21	12.94
	minus	12.98	13.17	12.93
Total length of internodes below the highest ear	plus	59.56	57.55	58.56
	average	59.58	60.83	57.43
	minus	56.40	59.94	56.66

Table 2
Mean values of the plants selected (2nd experiment)
after three generations of selections

Characters	Direction of selection	Control = 0r	1500r	3000r
Number of branches on the tassel	plus	13.24	13.53	13.27
	average	13.40	13.23	13.11
	minus	13.28	13.36	13.53
Number of internodes below the highest ear	plus	13.24	13.42	13.44
	average	13.43	13.34	13.37
	minus	13.20	13.32	13.24
Total length of internodes below the highest ear	plus	48.12	47.20	45.77
	average	45.07	47.07	43.12
	minus	44.19	43.89	45.29

D. L. Palenzona

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Department of Biology

1. B-chromosomes in Mexican teosinte.

Seeds from open-pollinations of Guanajuato teosinte of Mexico were planted in the summer of 1966. Microsporocytes of five plants of this teosinte were examined cytologically. One to five B-chromosomes were present in all of these plants.

Morphologically these B-chromosomes are the same as those found in various maize strains. They were acrocentric. Next to the centromere, there was a heterochromatic region or a knob. It was followed by a euchromatic region equivalent to about one-fourth of the total length of the chromosome. Heterochromatin organized into four discrete segments occupied the distal portion of this chromosome. The senior author has reported finding evidence of teosinte introgression into maize (American Naturalist, 1967). The observation of common B-chromosomes in maize and teosinte constitutes one more proof of this introgression. Studies on the inheritance of B-chromosomes in teosinte and on the effects of these chromosomes on the plants are in progress.

Y. C. Ting
R. G. Pendola

2. Fine structure of maize bivalent chromosomes.*

Despite the rapid progress made in the studies of cytoplasmic organelles of both plants and animals with the application of electron microscopy, results of the studies on chromosomes with the same technique have been very disappointing. The reasons are two-fold: (1) The electron microscope fails to demonstrate the characteristic structure of the components of chromosomes as revealed with the light microscope and concluded by cytogenetic investigations. (2) Up to the present, there has been no fine structure model of the chromosome accepted by biologists. However, the discovery of the synaptinemal complex in the chromosomes of meiotic prophase in certain plant and animal species has made electron microscopy promising in chromosome research. With the observation of this complex it is safe to say that chromosomes at meiotic prophase are not structureless under the electron microscope. This is also the case for maize.

In the summer of 1966, maize anthers at meiotic prophase were studied under the electron microscope by following the standard method of fixation and staining. At the same time anthers at the same division stage were also examined with the light microscope in order to relate the observations to those of electron microscopy. The synaptinemal complex was consistently found from early prophase to the stage of diplonema. Each bivalent had only one such complex which consisted of three parallelly arranged elements. In clear micrographs these elements could easily be identified. Their average diameter measured about 400Å. However, between the central element and the two lateral ones in each complex there were clear zones along the whole length of the bivalent chromosome. The width of the clear zones was approximately 300Å. Even though not so conspicuous as the longitudinal sections, cross sections of the synaptinemal complex could be discerned with little difficulty. The three component elements were also clearly delimited.

Among a limited number of nuclei examined, this complex was not found to be attached at one end to the nuclear membrane even though this was observed in certain animal species by other investigators.

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*The experiment was done in the Department of Biology, Brookhaven National Laboratory, Long Island, New York. Credit should go to Dr. A. Underbrink of the Laboratory for his collaboration.

After diplonema, the synaptinemal complex was no longer observed. The polycomplex comprised of various synaptinemal complexes observed in the oocytes of mosquito after diplonema was certainly not demonstrated.

Since the synaptinemal complex has also been identified in the nerve cells of mosquito, the question as to what role this complex plays in chromosome synapsis and crossing over remains to be answered. It is likely, however, that with continued research, the function and the exact fine structure of this complex will soon be revealed.

Y. C. Ting

3. Further studies on haploid maize.

a. Fertility: In the summer of 1966, three haploid maize plants grew to maturity in the field. They were completely male sterile due to poorly developed pollen. Ears of these plants were pollinated with sib plants. Eighteen well-developed kernels were obtained from the three ears. When the ovules were counted, it was calculated that more than one per cent set seed. This is much higher than the expected less than 0.1 per cent seedset for haploid maize. It might be accounted for by the fact that some of the megasporocytes formed restitution nuclei instead of undergoing complete division at the first meiosis. Therefore, the two spores produced by any restitution nucleus might receive the regular 10 chromosomes. A study of the chromosome constitutions in the immediate generation of sib-crossings is in progress.

b. Production: During the last two years, experiments with the objective of obtaining a large number of haploid maize plants were carried out. Two strains of maize were employed as kernel parent: one was homozygous for $\underline{gl}_1$, the other homozygous for $\underline{C}$ (colored endosperm and scutellum). Plants of these strains were pollinated by Coe's stock No. 6, homozygous for $\underline{C}^I$ (colorless endosperm and scutellum). Over seven thousand kernels from the cross $\underline{gl}_1\ \underline{gl}_1 \times \underline{C}^I\ \underline{C}^I$ were obtained. After careful screening, 95 putative haploid seedlings were selected. As root tip chromosomes were counted, it was found that over 50 per cent of the plants were diploid. Therefore, this technique is inefficient in screening haploids. The inefficiency can probably be accounted for by the difficulty in discriminating glossy from normal seedlings. However, among about 300 selected kernels (colored scutella) in the cross $\underline{CC} \times \underline{C}^I\underline{C}^I$, almost all proved to be maternal haploids by root tip chromosome counts. Hence, the technique based on the use of the colored scutellum marker in selecting for maternal haploid embryos is highly efficient.

c. Radiosensitivity: In a preliminary test, it was found that haploid maize seedlings two weeks old were more susceptible to the damage of ionizing radiations than diploids of the same age. A detailed report of this experiment is in preparation.

Y. C. Ting
Carolmarie Smith

4. The effect of X-rays and fast neutrons on maize pollen.

An experiment was conducted in the last two years to compare the effects of X-rays and fast neutrons on maize pollen. Pollen from Plants 65-27 and 65-26 was irradiated with X-rays and fast neutrons of the same dose (1500 rads), and was used in self-pollinations. Five plants for each of these two types of radiation were employed. At harvesting, it was found that about 70 per cent of the ovules pollinated with X-rayed pollen set well-filled kernels, while about 95 per cent of the ovules pollinated with fast neutron treated pollen set well-filled kernels. Last summer, 345 bulked kernels from neutron treatment and 330 bulked kernels from X-ray treatment were planted in the field. Only four per cent of the former emerged while over 90 per cent of the latter gave viable seedlings.

It is postulated that acute irradiation with fast neutrons at a dose of 1500 rads on the pollen is adequate in inducing dominant embryo lethal mutation in maize of the immediate generation while X-rays of the same dose are less effective. However, at the same dose rate, X-rays are more effective than fast neutrons in reducing the percentage of seedset.

Y. C. Ting

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1. Chemical mutagens on maize: Ethyl methanesulfonate.

Many geneticists have been interested in the production of mutations, to be used mostly in fundamental studies. The chemical mutagen ethyl methanesulfonate (EMS) has been shown to produce a high frequency of mutations accompanied by a relatively low frequency of chromosomal aberrations in plants.

The following studies have been conducted to study the feasibility of modifying the effects of EMS on maize. If the effect of this chemical mutagen can be modified it may also be possible to alter its effectiveness and efficiency. These terms have been defined by Nilan et al (1965). They state that effectiveness of a mutagenic agent usually means the rate of "point" mutations as related to dose. Efficiency usually refers to the "point" mutation rate in relation to other biological effects induced, usually a measure of damage. Biological effectiveness is used in this paper as the amount of damage as related to dose.

Post-treatments being investigated to influence the effectiveness and efficiency of the mutagen treatment are soaking of the seeds in water and drying them. Other modifying factors being investigated are temperature and duration of treatment and concentration of mutagen. If seeds

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

Table 1

Plant height at 14 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment. This was compared to an EMS treatment (second column) that was not post-soaked and planted wet (wet compared to dry).

Molarity	Planted wet % of control	Post-soak time (hr.)													
		0		6		12		18		24		36		48	
		% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry
0.00125	100.0	100.0	100.0	104.8	104.8	99.1	99.1	112.7	112.7	97.1	97.1	87.0	86.9	104.0	104.0
0.0025	88.5	55.4	62.5	100.0	113.0	91.5	103.3	104.4	117.9	96.2	108.7	83.3	94.1	96.5	109.0
0.005	99.5	75.8	76.2	102.0	102.4	80.7	81.1	87.3	87.8	84.6	85.0	100.9	101.4	92.0	92.4
0.01	107.6	38.6	35.8	74.0	68.8	57.0	53.0	70.2	65.2	80.3	74.6	78.9	73.2	81.4	75.6
0.02	78.5	5.6	7.1	14.9	19.0	22.9	29.1	36.1	46.0	40.0	50.9	63.5	81.0	75.4	96.1
0.04	61.7	--	--	0.7	1.2	--	--	--	--	11.1	18.0	25.2	40.9	36.7	59.4
0.08	50.7	--	--	--	--	--	--	--	--	--	--	1.6	3.1	5.0	9.9

are dried immediately after being treated with EMS, many dose levels will prove fatal to the seeds. This is probably because EMS and its hydrolysis products, which are apparently detrimental, remain in the seed and accumulate or are more harmful when the seeds are dried. There is also the possibility that if seeds are post-soaked, some of the unhydrolyzed EMS may be removed from the seed, and the mutation frequency will be decreased.

If a satisfactory treatment regime can be established by post-soaking, drying and storing seeds after EMS treatment, it should give the investigator considerably more control over conditions at planting time such as inclement weather and will allow the shipment of treated seeds, that would not be possible if wet seeds are used. Of equal or greater importance is the possibility that the efficiency of the mutagen may be increased.

A genetic stock dominant for many endosperm and plant genes was used in this study. The seeds were treated with EMS (Eastman Organic Co.) for 10 hrs. at 25°C. $\pm$ 0.02°C. in a 0.1 M aqueous phosphate buffer (pH 7.5). The seeds were treated with 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04, 0.08 M EMS. After treatment the seeds were rinsed in distilled water and soaked at 3°C. $\pm$ 0.02°C. in distilled-deionized water for 0, 6, 12, 18, 24, 36, 48 hrs. and shaken at 75 cycles/min. After post-soaking, the seeds were dried for 72 hrs. after the last post-soaking collection in a room maintained at 22°C. and 60% relative humidity. A fan was used to circulate the air. A portion of the seeds were planted in flats containing soil, peat and sand in a growth chamber maintained at 22°C., 2600 foot candles light intensity and 18 hr. photoperiod. Seeds were also stored at 25°C. and -20°C. for 4 and 8 weeks and then planted. For the 4 and 8 week storage periods the seeds were stored in evacuated desiccators which had a relative humidity of approximately 35%. For a further comparison, when the 0, 4 and 8 week experiments were planted, seeds were treated as described above but were planted immediately after the EMS treatment, without being post-soaked or dried. Two replicates of 15 seeds each were used. The criteria used to evaluate biological effects of the treatments were plant height and survival at 14 days and survival at 30 days.

As indicated in Table 1 and Figure 1 there is an increase in plant height by post-soaking the EMS treated seeds in water before drying. This is also noted for survival at 14 and 30 days (Tables 2 and 3). The advantage of post-soaking is more pronounced with the higher concentrations of EMS. In fact there is no survival of the 0.08 M treatment unless the seed is post-soaked for at least 36 hrs. before drying. As indicated in Tables 1, 2 and 3 and Figure 1, 0.02 M is the heaviest dose that can be used and still obtain surviving plants, if the material is dried immediately after the EMS treatment.

The data in the second column in the tables were obtained from seed treated with EMS planted wet without being post-soaked. This material was planted with the material that had been treated with EMS, post-soaked and dried. This is displayed as per cent of control also. In the columns designated "wet compared to dry" the material planted wet

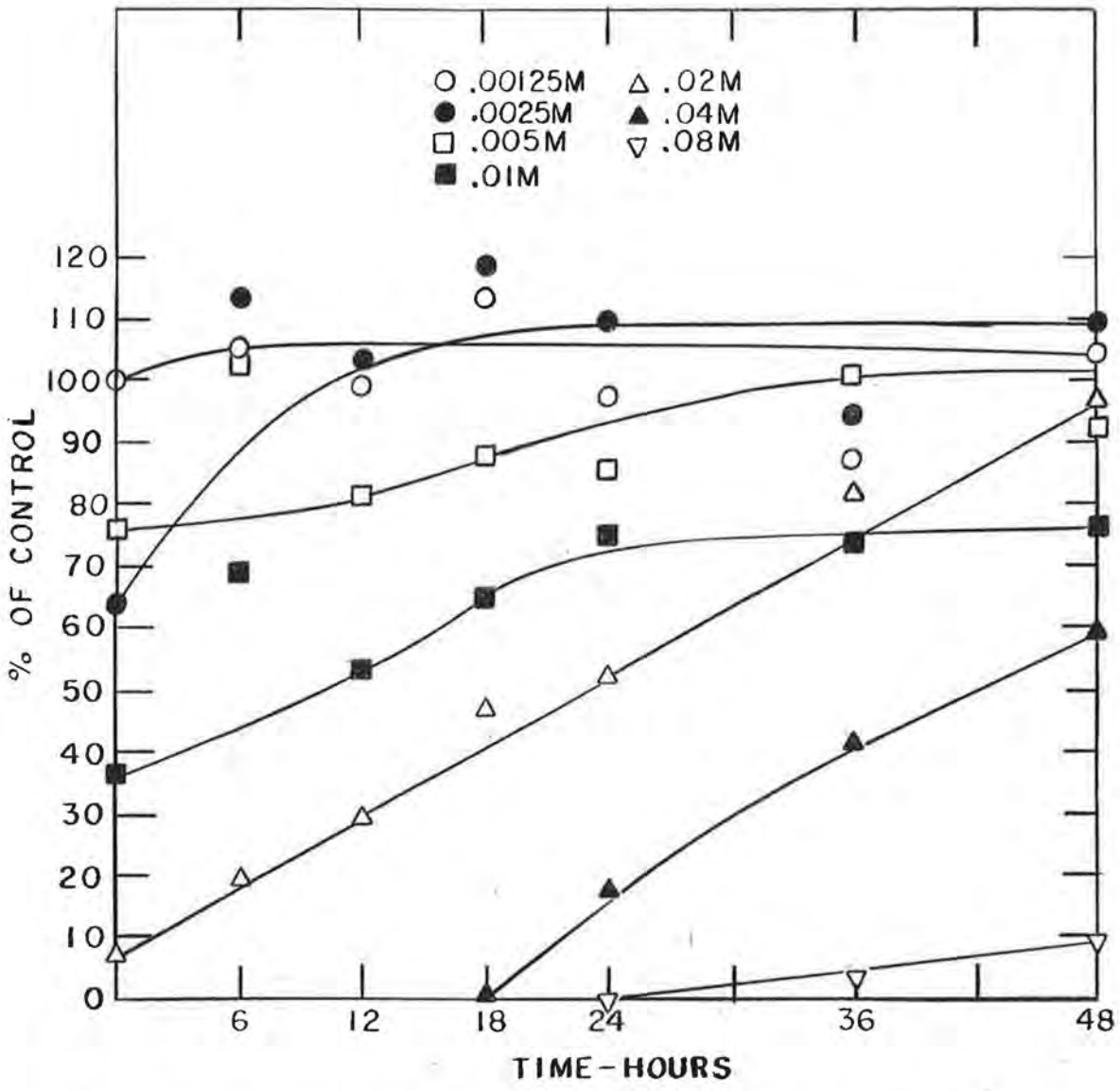

Figure 1. Plant height at 14 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment, compared to an EMS treatment that was not post-soaked and planted wet.

was compared to the material that had been treated with EMS, post-soaked, dried, then planted. In addition to this comparison, in which the dried seed treatment was compared to the material planted wet, the treatment post-soaked and dried was evaluated alone. Although a similar response was obtained from both comparisons, the one involving the drying of seed generally gave lower values, especially for survival.

Experiments using diethyl sulfate, post-soaking and drying barley seed, were done by Konzak et al (1961). Related studies by Froese-Gertzen et al (1964) and Gaul et al (in press) have also been done. Barley seed can be soaked in water for rather short periods of time and still maintain germination (Froese-Gertzen et al, 1964). Maize seed is apparently much more resistant than barley to soaking in water and drying (Briggs, in press and unpublished).

The biological effectiveness of the chemical is increased considerably if the seed is dried without post-soaking. That is, plant height and survival are considerably reduced. This can be seen if, for example, column 2 is compared to column 3 etc. in the tables.

The treatment levels chosen in this experiment range from those that produce very little to no effect compared to those that produce a severe effect and sometimes lethality. Based on observations made at this laboratory, an EMS treatment of 10 hrs. and 0.05 M is the heaviest treatment that will produce an adequate seed set if treated seeds are planted wet.

It is quite apparent that when the seeds were dried, post-soaking improved plant height and survival. However, the effects of drying the seeds after being treated with high concentrations of EMS were rather severe, as determined by reduced plant height and survival, compared to the EMS treatment in which the seeds were planted wet. Even the 48 hr. post-soaking conditions did not increase plant height and survival above the material that was treated with EMS and planted wet. When seeds treated with low concentrations of EMS were dried back they did not differ greatly from the material planted wet. However, post-soaking did improve this material somewhat.

From general observations, variability seems to be increased by the 4 and 8 week storage times at -20°C. and 25°C. Storing treated material at the higher temperature lowered plant height and survival more than the lower temperature. Also the -20°C. temperature permits survival whereas in many cases the 25°C. storage temperature does not, especially at the higher concentrations of EMS. The 8 week storage at both temperatures seemed to have reduced plant height and survival more than the 4 week storage condition. Therefore, if storage of treated material for any length of time before planting is contemplated a low temperature such as -20°C. should probably be used.

When seeds are treated with the above concentrations of EMS and post-soaked up to 48 hrs and not dried but planted immediately after collection little advantage is obtained. The values (actual data not reported) remain nearly the same, within concentrations, from one

Table 2

Survival at 14 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment. This was compared to an EMS treatment (second column) that was not post-soaked and planted wet (wet compared to dry).

Molarity	Planted wet % of control	Post-soak time (hr.)													
		0		6		12		18		24		36		48	
		% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry
0.00125	115.4	86.7	75.1	111.5	96.7	96.6	83.7	103.4	89.7	96.6	83.7	90.0	78.0	108.0	93.6
0.0025	107.7	96.7	89.8	115.4	107.1	89.7	83.3	96.6	89.7	100.0	92.9	90.0	83.6	112.0	104.0
0.005	100.0	100.0	100.0	115.4	115.4	96.6	96.6	89.7	89.7	100.0	100.0	93.3	93.3	116.0	116.0
0.01	111.5	90.0	80.7	115.4	103.4	103.4	92.8	82.8	74.2	89.7	80.4	96.7	86.7	116.0	104.0
0.02	103.8	40.0	38.5	100.0	96.3	93.1	89.6	93.1	89.6	100.0	96.3	90.0	86.7	120.0	115.6
0.04	92.3	--	--	7.7	8.3	--	--	10.3	11.2	48.3	52.3	93.3	101.1	100.0	108.3
0.08	84.6	--	--	--	--	--	--	--	--	--	--	3.3	3.9	24.0	28.4

Table 3

Survival at 30 days as "per cent of control" when seed post-soaked for indicated times and dried after EMS treatment. This was compared to an EMS treatment (second column) that was not post-soaked and planted wet (wet compared to dry).

Molarity	Planted wet % of control	Post-soak time (hr.)													
		0		6		12		18		24		36		48	
		% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry	% cont	wet cf dry
0.00125	115.4	100.0	86.7	103.6	89.8	100.0	86.7	103.4	89.7	100.0	86.7	93.3	81.0	103.8	90.0
0.0025	111.5	100.0	89.6	107.1	96.1	100.0	89.6	96.6	86.6	96.6	86.6	90.0	80.7	107.7	96.6
0.005	103.8	103.4	99.6	107.1	103.2	93.1	89.6	86.2	83.0	96.6	93.0	93.3	90.0	111.5	107.4
0.01	107.7	75.9	70.4	92.9	86.2	100.0	92.9	79.3	73.6	86.2	80.0	96.7	89.8	115.4	107.1
0.02	100.0	--	--	32.1	32.1	62.1	62.1	79.3	79.3	86.2	86.2	86.7	86.7	115.4	115.4
0.04	92.3	--	--	--	--	--	--	--	--	13.8	14.9	53.3	57.8	69.2	75.0
0.08	80.8	--	--	--	--	--	--	--	--	--	--	--	--	7.7	9.5

collection time to the next, i.e., there is very little if any increase in plant height or survival by the post-soaking. However, the heavier concentrations do give a greater effect.

The hydrolysis products and EMS are probably removed by post-soaking but little advantage is noted from their removal if seeds are planted wet. Apparently the effect produced by the EMS treatment is irreversible in this case. Therefore, it appears that post-soaking is of little value in influencing the biological effectiveness if the seeds are planted wet. Genetic studies are in progress to determine if any of these post-treatment conditions will increase the effectiveness of EMS.

Acknowledgement is made to Nan Jackson and Gary McGovern for assistance in performing these studies.

Robert W. Briggs

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CARGILL, INCORPORATED
Grinnell, Iowa

1. Survey of attempts to hybridize maize with sorghum.

Attempts to hybridize maize with sorghum may receive more attention now that the germplasm of another member of the Andropogoneae, Manisuris, has been implemented in the improvement of maize itself. Maize and the Andropogoneae have also been hybridized through the highly polyploid genus Saccharum. These points alone cannot strongly endorse a maize x sorghum effort, yet they do offer a certain element of encouragement. Certainly most efforts to date have been quite modest and this is stressed repeatedly by those investigators willing to report their work. Even a weakly fertile hybrid would seem worthy of considerably more effort, especially if it can serve as a bridge to the exchange of germplasm between these two important crop species.

Forty of the larger American crop research groups were contacted in this survey. Eighteen of these indicated an effort of some kind, and 16 furnished descriptions of the scope, techniques and success of their work. What portion of the total USA and world effort this represents is not known.

Cargill, Incorporated Grinnell, Iowa E. E. Gerrish

1964 - female 4 plants 2n maize - silks shortened - one week after pollination 10 " blisters" on each ear inoculated with 14-day old normal 3n maize endosperm fluid - no development

1966 - female 150 plants 2n maize (single crosses) - silks treated with 100 ppm Gibrel, later shortened and shucks removed - pollination with 2n sorghum (Reliance, Norghum) pollen followed at intervals of 4 to 48 hours by application of maize pollen carrying dominant endosperm and embryo color markers - all seed sets greatly reduced by combined shuck removal and silk shortening - seed set apparently unaffected by presence of Gibrel - no seed development where pollination limited to sorghum - seed development comparable to check (shortened silks, shuck removed, and pollination with maize pollen) where maize pollen followed sorghum pollen - no positive identification of hybrid (colorless) embryos in normal marked maize endosperm.

Texas A and M University College Station, Texas K. F. Scherty

Female 2n ms sorghum - male 2n and 4n maize - a few seed developed which produced typical sorghum plants.

University of Florida Gainesville, Florida J. R. Edwardson

1956 - female 24 plants 2n maize (F6) and 24 plants 2n sorghum - maize silks shortened - greenhouse - no seed development.

Funk Bros. Seed Company Bloomington, Ill. C. Laible

1964 - female 20 plants 4n maize - male 4n sorghum - silks shortened - pericarp stimulation only.

University of Illinois Urbana, Ill. D. E. Alexander (reported 1963 MNL)

1961 - female 4n maize (synthetic B and inbred N6) - male 4n sorghum - silks shortened - sparse pollination with maize prior to hybridization - 100 putative hybrid embryos excised - strongly resembled maize seedlings - no survivors through nutrient cultures.

1962 - female both 4n maize and 4n ms sorghum - silks shortened - total 53 putative hybrid embryos excised - growth aberrant and abortive in nutrient culture - developmental pattern of many suggestive of hybrid nature - no root tips available - germination and sluggish growth of maize pollen in sorghum styles - reciprocal obs. inconclusive - further effort planned.

Iowa State University Ames, Iowa P. A. Peterson

1963 - female 50 plants 2n ms sorghum - wide spectrum maize races and types male - nick poor - 30 small seed developed - all sorghum, perhaps halapense x vulgare.

Iowa State University Ames, Iowa G. F. Sprague (Beltsville)

1946 - female 30 plants 2n ms sorghum - isolated in maize nursery - several seeds developed - all sorghum - outcross source located.

Iowa State University Ames, Iowa M. L. Kinman

1943 - female 2n ms sorghum (Blackhawk Kofir) - isolated in maize nursery - massive amounts of maize pollen applied in addition - several seeds developed - all sorghum - outcross source located 2 miles downwind.

University of Kentucky Lexington, Kentucky J. F. Shane

1965 - female 6 plants 2n ms sorghum (A385) - male 2n maize (Wf9) - no seed development.

1966 - female 25 plants 2n ms sorghum (A385 and A Martin) and 96 plants 2n maize (T8 and CI21E) - maize silks shortened in some cases - males 16 different varieties of 2n sorghum and 5 inbreds of 2n maize - 5 seeds developed on maize females and 5 seeds on sorghum females - all suspected of outcross origin - further effort planned.

University of Massachusetts Waltham, Mass. W. C. Galinat

1958 - female 24 plants 2n ms combine Kafir - 14 embryonic-type developments excised at 17th day - no further growth or differentiation.

1959 - female 12 plants of 4n sugary maize - silks shortened - male a mixture 4n maize and 4n sorghum - 9 putative hybrid embryos excised - no hybrids developed.

Michigan State University East Lansing, Mich. E. C. Rossman

1951 - female 100 plants 2n maize - silks not shortened - 12 seed developed - all maize.

Missouri Farmers Association Marshall, Mo. C. O. Grogan

1948 - female 10 plants 2n maize - silks shortened - 3 seeds developed - failed to germinate.

University of Missouri Columbia, Mo. E. R. Sears

1938 - female 10 plants 2n antherless sorghum - no seed development.

Ohio Agricultural Research and Development Center, Wooster, Ohio, W.R. Findley

1963-64 - female 1000 plants 2n ms white sorghum - isolated in maize nursery - nick poor - 25 yellowish seed developed - all sorghum - further effort planned.

Paymaster Seed Farms Plainview, Texas N. W. Kramer

1950's - female 500 plants 2n ms sorghum - isolated in maize nursery - no seed developed.

Pioneer Hi-Bred Corn Company Johnston, Iowa W. L. Brown

1957-59 - female several hundred 2n ms sorghum - isolated in maize nursery - several poorly developed seed - most germinated and survived one month - died at approximately one inch height.

Purdue University Lafayette, Indiana R. Pickett

1959-60 - female several dozen 2n ms sorghum - hand pollinated - several seeds developed - no germination.

South Dakota State University Brookings, S. D. C. J. Franzke

1932-64 - reciprocal crossing involving total 20,000 plants, mostly 2n, wide divergence of types in both parents - maize as female mostly shortened silks - sorghum as female hand emasculated - all hybridizing greenhouse - no success maize as female - on sorghum shiveled seed yielding maize-like plants maturing without terminal or lateral inflorescences.

E. E. Gerrish

CENTRAL MAIZE RESEARCH STATION
Yousafwala (Sahiwal), West Pakistan

1. Further studies on sorghoid maize.

The sorghoid plants reported in the Maize Genetics News Letter (1966) were selfed as well as sib pollinated during the spring season of 1966. Some of these plants were also crossed to normal maize. The F_1, F_2 and backcross generations resulting from such crosses were studied during the regular season, i.e. late summer.

Plants in the first filial generation had normal tassels and ears except that in several plants the ears were branched. In almost all cases, there was only one branch and that arose from near the bottom of the ear. Number of kernel rows on the branches varied from 6 to 8. These branched ears were no different than those commonly observed in some of the modern races of maize. The main ear and the branch had normal cob (pith). In the second filial generation some plants were observed that had relatively more branching of the ear than the F_1. The number of kernel rows on the branches was decreased and the amount of pith was also reduced. Plants with three to four branches were also observed in this generation. Backcrossing to the normal parent gave progenies which had normal ears. Progenies of the plants backcrossed to the sorghoid type parent had a relatively higher percentage of branched ears.

Study of the selfed progenies of the sorghoid plants revealed that the tassel character, especially the condensed branching, attained a relatively high degree of uniformity while the typical sorghoid branching of the ear could only be observed in a few plants in some of the progenies. The most interesting feature in the progenies of self-pollinated sorghoid plants was the tendency towards hermaphrodite florets in the tassel. The carpels were, however, nonfunctional and the stigmas were unbranched. Study of this character in further generations will be continued.

A. Ghafoor Bhatti

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1. Notes on Tripsacum cytotaxonomy.

Cytological studies of plants at the Fairchild Tropical Garden during the past year have contributed information that may be useful in clarifying certain species relationships. Most of the observations were made on plants from our 1963-1965 collecting trips to Mexico, Guatemala and Colombia, S. A., additional collections from Guatemala and Honduras made in December, 1966, and a recent collection by Dr. John Popenoe, director of the Fairchild Garden, from Great Inagua Island in the Bahamas.

Chromosome counts of $2n = 36$ were obtained from three populations of T. latifolium Hitch. at Cubilguitz, Alta Verapaz in the tropical rain-forest area of central Guatemala, from two additional populations of this species from the north shore of Lake Izabal in southeastern Guatemala,

and from a cultivated field of Guatemala Grass near El Zamorano, Honduras consisting of plants in the rosette stage having the foliage characteristics of this species. Since there are elsewhere in Guatemala tetraploid populations having many of the characteristics of T. latifolium described by Hitchcock it is possible that the chromosome counts by Reeves and Mangelsdorf, on the basis of which this species was reported in 1935 to be tetraploid, were obtained from such populations.

Our chromosome studies of T. laxum Nash, a species very similar to T. latifolium and also grown for forage in various Latin American countries and the Caribbean as Zacaton Maizar or Guatemala Grass, has confirmed that it is a tetraploid as originally reported by Reeves and Mangelsdorf. Plants collected in the general neighborhood of Vera Cruz, Mexico, where the type locality is believed to have been located, had approximately 72 chromosomes and the very irregular meiotic chromosome behavior considered by Dodds and Simmons to explain the very low fertility of this species. The presence at meiosis of multivalents and univalents in addition to bivalents and the fact that laxum and latifolium have many taxonomic features in common suggest that the former species originated as an amphidiploid having the latter species as one parent.

Specimens of Tripsacum collected recently on Great Inagua Island in the Bahamas by Dr. John Popenoe, identified in the Bahama Flora of Britton and Millspaugh as T. dactyloides, are now growing at the Fairchild Garden, and chromosome counts from root-tip smears of plants from three different clones of this collection showed them to be diploids with 36 chromosomes.

This may be quite significant since these plants do resemble the tetraploid dactyloides of Florida in foliage characteristics such as width of rosette leaves, absence of pubescence on leaf sheaths and blades, and varying amounts of sun-red plant color. And being located geographically between Florida and South America, where there is an essentially glabrous form of T. australe very similar to the Florida dactyloides widely distributed on the western slopes of the Andes in Venezuela and Colombia, the Inagua plants might be a missing link between these two taxonomically similar, widely distributed species of North and South America. But more critical evaluation of these taxa is needed; difference in ploidy should not be overlooked; and determinations of chromosome knob frequencies and size differences incidental to tests of crossability between indigenous types of maize and Tripsacum at Medellin, Colombia in 1964 and much earlier at Ithaca, New York indicate that their pachytene karyotypes are very different and that superficial resemblances may not be a reliable indicator of natural relationships among these taxa.

When Hitchcock described T. dactyloides ssp. hispidum from northern Mexico (Bot. Gazette 41: 295, 1906), he emphasized that it connects the dactyloides of the central United States with the T. lanceolatum Rupr. of Mexico but he did not know of the difference in chromosome number between the diploid dactyloides of the central United States and the tetraploid form of this species from the eastern United States. In this connection it is perhaps noteworthy that we have examined cytologically many populations from central and southern Mexico and also from

Guatemala that are more similar to T. lanceolatum than to other species thus far described from these countries, and all have been tetraploid. However, a recent collection by Garrison Wilkes from Chihuahua in north-central Mexico, now growing at the Fairchild Garden under accession number FG65-1253, is listed as a diploid, and appears to have the differentiating characters of Hitchcock's T. dactyloides ssp. hispidum.

If the natural affinities of T. latifolium and laxum; australe and the dactyloides of Inagua, and of the eastern United States; the dactyloides of the central United States and dactyloides ssp. hispidum and lanceolatum of northern Mexico; suggested by similarities in traits of taxonomic significance and numerical chromosome relationships, are substantiated by tests of crossability, pachytene karyotype analyses, and other cytogenetic evidence, there will remain for evaluation the many dissimilar populations of Mexico, Guatemala and elsewhere in Latin America that appear to be diverse genetic recombinants at the tetraploid level of natural alloploid derivatives of the very dissimilar T. maizar and zopilotense.

L. F. Randolph

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1. Perennial maize.

The perennial habit in maize appears to be conditioned by the recessive condition at three loci, pe, gt, and id.

A cross was made in 1964 between homzygous pe/pe gt/gt maize and normal segregates in selfed progenies segregating for id. The cross was selfed in the winter of 1964-1965, and the progeny grown out in the summer of 1965 in a pilot experiment to determine if any segregate had a perennial phenotype. From a progeny of 940 in the Salinas Valley nursery, 15 possibly perennial plants were selected, propagated, and observed critically in the Florida winter nursery for perennialism. Only 3 proved to be perennial, and continued to make totipotent growth through the end of the winter season. One of these was grown as a houseplant during the same winter season, and was reset into the 1966 summer nursery at Greenfield, where it continuously made totipotent growth all summer, and still survives as of January 1967, at the age of 21 months. The clone has produced seed twice, and flowered many successive times without loss of vegetative vigor.

According to the mechanics of the cross made, the expected frequency of perennial segregates is 1/2 x 2/3 x 1/64, or 5 triply recessive (perennial) plants from the progeny of 940. Only 3 were realized. However, cultural conditions may be held suspect since any plant that died a cultural death would have been classified as nonperennial. In fact, it has been found that perennial maize is difficult to clone unless one waits until the propagules have formed adventitious roots before they are removed from the

vegetative parent. In the present background, at least, perennial maize is also susceptible to infections admitted through wounds occasioned by removing propagules prematurely.

A more comprehensive experiment was conducted in the 1966 summer nursery from remnant seed of families previously determined to be segregating for id. From a total progeny of 2091, 527 were identified as id/id. Of the latter, 36 were classified as good perennials at the end of the growing season in late December 1966. Criteria for classification were (1) ability to regrow after being cut down near the ground, (2) the continued production of totipotent growth through at least three successive vegetative generations with no loss of vigor, (3) ability to undergo cloning, (4) production of numerous axillary plantlets from culms which have been induced into a prostrate attitude (see Figures 7 and 22, Genetics 50:395, 400).

The extremely long growing season at Greenfield, in the opinion of the author, gives sufficient time to critically assess perennialism before the end of the growth period. However, a further check is being made by taking several of the 36 clones into the greenhouse.

The expectation for frequency of perennial segregates in the 1966 experiment is 1/64 x 2091, or 33. The realization of 36 is in good agreement with expectation, and appears to confirm the hypothesis that perennialism is simply conditioned, by the pe/pe gt/gt id/id genotype. However, the author believes that this interpretation must be tempered with caution. The background-environment dependency of pe has been well established in many previous experiments. It is not hard to imagine that the apparently good segregations may relate to more complex background effects which could quantitatively mimic unitary genetic segregation. It may be, and indeed one needs to assume that gt/gt id/id is the background which is critical for the identification of pe. Most experienced maize geneticists will not find it difficult to concur with the writer that the simple interpretation of data in this case ought to be held suspect for a while.

Because of the difficulty in trying to obtain good genetic data involving an incompletely penetrant gene like pe, other experiments were performed to provide additional, inferential information as to the nature of perennialism. The two-gene synthesis, gt/gt id/id was made and observed in the summer of 1966. Although the gt-id phenotype could easily be identified, it was not perennial. It resembled somewhat the pe-gt phenotype described previously by the author in its ability to produce more than one successive generation of tillers. However, these "ran out" more quickly than in pe-gt maize, through rapidly progressing preinduction and the attendant vegetative suppression.

In another experiment, an attempt was made to derive perennials from the progeny of a cross between id/id maize and a stock which has ability to produce fully indeterminant tillers (McClintock's c tester). The id/id segregates in this freely tillering background succeeded in producing only one generation of tillers, and thus had fewer perennial attributes than either gt-id or pe-gt phenotypes.

Observations and data strongly support the three gene hypothesis. At worst, the basis of perennialism cannot be very complex.

D. L. Shaver

2. Modification of the id/id phenotype.

The id phenotype is commonly thought of as being photoperiod-dependent for floral initiation, and as being almost invariably earless. In a previous report the author has shown that in his stocks, id/id segregates have not been photoperiod-controlled in outdoor experiments since floral induction can occur during the summer solstice and, in winter nurseries, may fail to occur during the winter solstice. In no case has any factor other than simple age of the culm (as a function of the inherent earliness of the specific stock involved) appeared to affect time of flowering in outdoor culture. However, when the author cultured one of his perennial plants as a houseplant during the winter of 1965-1966, by spring it had become highly induced, to the extent that new basal branches were prematurely flowering at a very small size, producing mixed, seed-bearing terminal inflorescences. To all appearances, the plant was "running out" in the manner previously described for the pe/pe gt/gt phenotype. Upon the return of summer weather, however, the plant was reset out-of-doors April 1966 whereupon it immediately resumed producing indeterminant and totipotent growth and continues to do so at the present time, January 1967. It is to be remembered that another propagule of this same clone was grown in the winter nursery concurrently, and showed no signs whatever of premature induction at any time. Since most of the reported work with id deals with greenhouse culture, it is possible that light quality, perhaps UV content, is critical in the expression of id.

Earlessness of id/id maize is obviously of great concern, since all perennial maize is homozygous id. Accordingly perennial maize has never produced ears. The several instances of seed production have been cases of tassel seed formation. However, a homozygous id synthetic has been established by the simple procedure of recombination among rare segregates which were successful in producing ears. The main segment of this population is only in its S_2 generation, but was successful in producing ears on 243 plants in a population of 313, a proportion of nearly 78%. Moreover, the ear fertility of this population was nearly doubled between the S_2 and the S_3 generation. Ear conformation is normal and ear size occasionally exceeds what one might expect from normal plants of this background. It is certain that earlessness of the id/id phenotype is another example of effects that are completely under the control of modifying genes. By inference, the perennial phenotype would produce ears normally if it were transferred to this background.

D. L. Shaver

3. Decussate phyllotaxy in maize.

Among the id/id segregates of a progeny grown in 1965, several plants had irregular patterns of leaf placement. In one case, a plant had a completely regular decussate phyllotaxis, beginning with the 5th node.

Leaves were borne oppositely, 2 per node, those of one node being rotated 90° to those of the preceding node, in perfect symmetry. Leaves were of normal size and conformation, with the nodal pair being of equal rank to each other. Being id/id, the plant was not ear fertile, but it produced pollen which was used to outcross. Selfed progeny is available for observation in the summer of 1967.

D. L. Shaver

4. When is hybrid vigor? (a)

Maize workers are accustomed to the fact that the F_1 between two inbred parents is not only much larger because of hybrid vigor, but flowers 10 to 14 days earlier than its parents.

In two summers of work in the unique Salinas Valley climate, it has been found that earliness is not an aspect of hybrid vigor. While F_1 progenies show the typical increase in plant size, they flower at the same time as their inbred parents. If a cross is made between an early and a late line, the F_1 flowers at a time intermediate to its two parents. Rather than condition of vigor, the number of plant parts to be cut off, in other words, leaf number, is the more reliable index to maturity.

A typical midsummer day in the Salinas Valley has a high of 82° which occurs as a rather sharp temperature peak soon after midday. Nightly temperature invariably drops into the low 50's. It would appear that this regime imposes a limitation upon the growth cycle which is not relieved by heterosis.

Only one important exception to this generalization has been found. F6, an apparently heat-loving Florida line, grows more slowly than its leaf number would predict. It spends much of its juvenile period in a condition of nearly stagnant terminal growth while tillers are freely produced. These grow out rhizome-like, showing little or no geotropism. Finally both the primary and secondary culms become geotropic, elongate rapidly, and flower, the tillers being equal in size to the first culm, each bearing two normal ears. The production of 10 large ears per seed planted was not unusual.

D. L. Shaver

5. When is hybrid vigor? (b)

Crosses between Florida teosinte and maize are typically freely tillering. The Salinas Valley environment accentuates this tendency both in maize and in its hybrids with Florida teosinte. In one case, however, the F_1 of a cross between Mangelsdorf tester and Florida teosinte produced no tillers at all among a progeny of 15 plants. Each leaf produced by this hybrid had limited viability. As judged by the formation of anthocyanin, sugar translocation was so impeded that each leaf died in turn, and only 4 or 5 functioning leaves were present at one time. Both parents were growing in the nursery in adjacent locations. The teosinte parent (Shaver's Florida teosinte inbred 2) grew normally, though slowly, and produced a typical

teosinte plant with many branches and a high degree of vegetative luxuriance. The Mangelsdorf tester likewise grew normally and was ear-fertile. Moreover, the cross between Wf9(T)MS and Florida teosinte grew normally into huge, highly tillered plants.

There is no question as to the identity of the unusual F_1 progeny described here, as the progeny was grown from a composite of two Mangelsdorf tester ears pollinated by teosinte. Also, the "ears" produced by the hybrid were distichous, the seeds were borne enclosed in a bony rachis as is typical of this cross, and the plants otherwise perfectly resembled maize-teosinte hybrids.

D. L. Shaver

6. Agronomic effects of the cytoplasm.

Early 20th century literature records undoubted cases where cytoplasmic inheritance affects a multitude of plant characters which would be considered agronomic in a crop plant. In maize, however, results have been contradictory, except in the case of male sterility, particularly that involving the "S" cyto steriles. In this case, early workers in the hybrid corn "sterile revolution" often noted that Wf9(S)MS showed chlorotic striping and plant dwarfing, a condition greatly accentuated, in the writer's experience, in the cooler winter Florida environment.

In keeping with the suggestion implicit in this experience, the Wf9 nucleus has been inserted into the cytoplasm of several exotics, in a search for other agronomic effects. Two extractions of cytoplasm were made from perennial teosinte, and BC_7 progenies were grown out in 1966. In one extraction, the recovered Wf9 is considerably dwarfed and is male sterile. This dwarfing was greatly increased during the long, unremitting cold of Florida's 1965-1966 winter season, so that the cyto-altered version of Wf9 made less than 1/3 the dry weight growth of normal. In the other extraction, the recovered Wf9 is male fertile and appears to be a superior seed producer by comparison with the original Wf9. Since both extractions were made from the one original clone of perennial teosinte (E16515), one must conclude that the process of extraction was accompanied by (or preceded by) cytoplasmic mutation. A careful check of records and remnant seeds reveals no error of identification which could provide an alternative explanation.

A conflict exists in the literature over the question of whether maize bearing annual teosinte cytoplasm exhibits agronomic modification. The work reported here pointing to cytoplasmic mutation during extraction provides a workable protem resolution. However, the author has not discovered any evidence of cytoplasmic modification of Wf9 in two separate extractions from annual teosinte.

It is interesting that apparent mutation in the cytoplasm reported here provides new evidence on the nature of cytoplasmic male sterility.

D. L. Shaver

7. Use of Hawaii as a site for winter generations of maize.

Our second experience in growing mainland maize in Hawaii has shown that the 50th state can be successfully utilized for the outdoor greenhouse function. It has many advantages over traditional areas: (1) Losses of grain to birds does not occur, because of faunal discontinuities with the mainland. (2) Leaf blight and the necessity of frequent prophylactic sprayings are obviated in the warm, dry, leeward locations. (3) Insect problems are minimal even though earworm and leafhopper control should probably be practiced. (4) Proof of the temperance of the Hawaiian climate is best illustrated by the fact that there is no Hawaiian word for "weather." All-time record lows are above 50° to leeward. Extreme heat is absent as well as oppressive humidity and heavy dews. (5) Hawaii is out of the path of typhoons, and while winter Konas do occur, they are mild by comparison with mainland summer storms or the winter storms of other locations. (6) The best corn-growing season in Hawaii is actually the summer, making Hawaii a true year-round growing area. Simple conversion projects, e.g., Opaque-2 or Rf-MS could be "cranked out" in situ, and then brought home, completed. The much greater speed made in Hawaii in winter generations make possible more than 3 generations per year. (7) While volcanic soils do tie up phosphates, they are immeasurably better than coral-derived soils in regard to pH, tilth, water and ion holding capacity. It is not unusual for natives to grow small plantings of table corn with no fertilizer or irrigation or insect sprays. (8) Hawaii is south of the Tropic of Cancer, and has longer winter days with stronger insolation. (9) A much less obvious advantage is that those long, soul-testing periods of unremittingly cold, non-growing weather cannot and do not, occur in Hawaii. Salaried people will be able to conduct their work on an orderly, predictable schedule with shorter, more productive stays on site. Embarrassments will be avoidable in being able to accurately count on harvesting times. (10) Hawaii has truly spectacular scenery, unbelievably clean, blue waters and pristine beaches and an extravagance of tropic isle weather for the enjoyment of fishing, sailboating, diving, and relaxation.

It is expected that saving of time and expenses by salaried people will offset the greater travel distance, even in programs that may not be left in Hawaii the year around. Certainty of avoiding losses from freezes and birds will likewise offset travel costs. All farming costs in terms of equipment, fuel, fertilizer, labor and irrigation water are higher, but it is expected that the saving of most of the spraying expense and a large part of the fertilizer application will offset.

A less obvious disadvantage is the lack of going operations to plug into. Proper exploitation of Hawaii will be even more a matter of transplanting mainland personnel, equipment and methods to the islands. Equipment, parts, and supplies for Hawaiian operations are obtained from California on a day-to-day basis, and are thus to a large degree, extensions of the California Agricultural Establishment. It is believed that the closed insularity of tiny Hawaii could make competition among maize people on site prohibitively expensive in terms of driving up prices on highly limited land opportunities. It is believed that Hawaii is now

obviously economic, but that its potential can best be unlocked by one large, unified, completely mechanized effort among maize people. Since the entire winter maize enterprise lies well within the scope of a modern one-man management and equipment corn growing unit, and since we have already established the roots of such an organism, we are seeking to make our own operation economic by inviting outside interest.

D. L. Shaver

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1. An effect of the o_2 gene in maize.

Preliminary experiments with opaque-2 material showed a lower test weight and a very high susceptibility in a cold test. The results of the tests with two selfed flint/opaque-2 crosses are given in the table.

	Phenotype	Test wt.	Average germination	
			10 days at 10°C. 4 days at 27°C.	7 days at 27°C.
CR37/o_2 ⊗	flint	67	84.0	100.0
CR37/o_2 ⊗	opaque	57	20.0	77.5
CR39/o_2 ⊗	flint	66	83.0	99.5
CR39/o_2 ⊗	opaque	59	25.0	95.5

Eventually selection for high specific weight through sucrose or sodium thiosulfate solutions can be useful as it has been observed in other preliminary tests whose results are not shown.

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1. A second R aleurone color factor on chromosome 2.*

The first R gene found to assort independently of the regular R locus on chromosome 10 was discovered by Styles in the strain Peru 1497 (News Letter 38:134; 39:172). His results showed that this duplicate R locus, designated R^g-2, is located at or near the B locus on chromosome 2.

The second R factor on chromosome 2 was found in the acquisition Bolivia 706. This factor conditions pale aleurone, green anthers, and a B-like plant color effect, and is neither paramutable nor paramutagenic. Attempts to separate the aleurone and plant color effects by crossing over were as yet unsuccessful. The data obtained are as follows:

I. Assortment with standard R^{st}: 'R'/r; R^{st}/r^g X $r^g r^g$.

Aleurone phenotypes from five plants

	pale	pale, stippled	stippled	colorless
Observed	166	186	189	193
Expected	183.5	183.5	183.5	183.5
X^2	1.67	0.03	0.16	1.49 = 2.35

.70> P > .50

II. Chromosomal location: r^g/r^g; Wx 'R'/wx T2-9b X r^g/r^g; wx/wx

Kernel phenotypes from three plants

	colored, waxy	colored, non-waxy	colorless, waxy	colorless, non-waxy
Observed	51	144	121	72
Expected	97	97	97	97
X^2	21.8	22.7	5.9	6.4 = 56.8

P < .01

Frequency of crossing over between 'R' and wx = 32%.

*This investigation was carried out under the direction of Dr. R. A. Brink at the University of Wisconsin as a part of Ph.D. studies. The author wishes to express his gratitude to Dr. Brink for guidance and counsel.

III. Assortment with gl_2: C/c; r^r/r^g; b Gl_2 'R'/B gl_2 Inv 2a ♀

Seedling phenotypes from eight plants

red, glossy (colored seed)	green, glossy (colored seed)	non-glossy (colored seed)
18	4	535

Frequency of crossing over between 'R' and gl_2 (based on green, glossy seedlings only) = 17%.

Frequency of crossing over between 'R' and gl_2 (based on all glossy seedlings) = 8%.

IV. Assortment with Peru 1497 R^g-2: (R^g-2 Bolivia 706 X R^g-2 Peru 1497) ♀.

No colorless seeds were observed in the F_2 progeny; hence, these two R genes on chromosome 2 are closely associated and could be alleles.

V. Interaction with Pl: 'R'/r; pl/pl X r^g/r^g; Pl/Pl.

Plants derived from this cross were purple in color, resembling a B Pl phenotype.

The experiments reported by Styles and those described above show the existence of a duplicate R color factor (or factors) on chromosome 2, at or near the B locus. The R and B loci both condition anthocyanin formation. Furthermore, both loci are known to undergo heritable changes in expression (paramutation). These indications of homology between R and B were further supported by the finding that the Bolivia 706 R^g-2 gene, like B, boosts the expression of Pl.

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1. Cytoplasmic effect of the male gamete.

Results of our work with a double-cross maize hybrid show that the cytoplasm of a male gamete in maize can influence the hereditary expression of characters in the progeny. Furthermore, the expression of the male cytoplasm can be influenced by the female cytoplasm. These experiments open a new frontier in cytoplasmic inheritance.

A. A. Fleming
J. B. Campbell

2. Chlorophyll content in maize.

In a study on the chlorophyll content of 17 inbred lines and 24 single-crosses, highly significant differences occurred for total chlorophyll, chlorophyll a, chlorophyll b, visual chlorophyll ratings, $C_a:C_b$ ratios, and yield in pounds per plant both among inbreds and among hybrids. Highly significant correlations of r = -.76** for inbreds and r = -.69** for hybrids occurred for total chlorophyll and visual rating.

Total chlorophyll of the hybrid was in all instances characteristically greater than the mean total chlorophyll of the inbred parents. Thus, heterosis for chlorophyll was present. In fact, a high correlation (r = .93**) was found between hybrids that exhibited high heterosis for yield and those that showed a high heterosis for total chlorophyll.

James H. Palmer
A. A. Fleming

3. A combined source of resistance to maize dwarf mosaic virus and corn stunt virus.

In the 1966 Maize Genetics Cooperation News Letter, GA 209, a white inbred line, was reported as giving excellent ratings of resistance to dwarf mosaic virus in tests in Tennessee and Ohio. Since then, tests in Louisiana and at the Corn Virus Laboratory at Mississippi State University reveal that this inbred also has resistance to the corn stunt virus. Thus, a single inbred is available for use in inheritance studies and in breeding programs as a source of resistance to both virus diseases.

A. A. Fleming

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1. Effect of kernel position on fatty acid composition of corn oil.

Considerable interest has recently been shown in genetic studies of the fatty acids of corn oil. Fatty acid analysis of the oil from individual kernels will be required to obtain the appropriate genetic information. Therefore, the question arises whether single kernels can be selected at random for analysis or whether the location of the kernel on the ear may influence oil composition. If kernels are selected at random and a significant kernel position effect on oil composition exists, then the environmental effect (kernel position) cannot be separated from the genetic effect.

The effect of kernel position on oil composition was studied on 12 inbred lines and 2 single crosses grown in 1965 and on 4 of these inbreds grown again in 1966. Five oil samples (4 single kernels and a bulk oil sample

from 6 kernels) from each of 3 positions (base, middle, and tip of ear) for 5 ears of each inbred and single cross were analyzed by gas-liquid chromatography. Only a brief summary of the results and conclusions will be given in this report and representative results from several of the inbred lines. Stearic and linolenic acids make up a small proportion of the total oil. Out of 12 inbred lines, only 2 inbreds (GE84 and P121) showed a significant effect of kernel position for stearic and 1 inbred (GE80) for linolenic. Kernel position influenced oleic and linoleic more consistently and to a greater degree than palmitic acid. However, all inbred lines did not show a significant effect of kernel position on oil composition. Mp428 and R196 showed no effect of kernel position on any of the 5 fatty acids. The combined analysis of variance for 12 inbred lines showed an increase in palmitic and linoleic and a decrease in oleic progressing from the base of the ear toward the tip. Results of 4 inbred lines and the average of all 12 are given in Table 1. Although not always significant, the oil from the tip kernels was lowest in oleic and highest in linoleic for 11 of the 12 inbred lines. Data for linoleic acid are shown in Table 2 for individual ears of R196 and Tx39-16. R196 shows no consistent effect of kernel position on linoleic acid. However, every ear (5 in 1965 and 3 in 1966) of Tx39-16 showed an increase in linoleic from base to tip of the ear.

Three oil samples from each of 3 kernel positions of 5 ears each of 2 single crosses (Va42 x GE129 and Va42 x GE281) were analyzed for oil composition. Kernel position did not significantly influence oil composition of these 2 single crosses. The data were too limited to conclude whether hybrid ears (F_1 and F_2 ears) are not as subject to kernel position effects as are certain inbred lines. Further analyses on hybrid ears are required to answer this question.

Estimates of variance components were also determined for individual inbred lines and for the combined analysis. The relative magnitude of the different variance components showed the importance of kernel position in certain inbred lines, but not in others. The ear variance component was relatively large for certain inbred lines and would indicate that segregation for oil composition was occurring. Single ear selection may be effective in changing oil composition in some of these inbred lines.

In general, the composition of the oil of kernels from the middle portion of the ear was intermediate in the majority of inbred lines. Therefore, sampling of kernels should be restricted to the middle of the ear in certain studies where kernel position effect on oil composition is not desired. No explanation is apparent why certain inbreds show a kernel position effect and others do not. Also, a reason has not been determined why the oil of tip kernels is different from oil of base kernels. Several questions remain unanswered and will require further research.

Table 1
Average fatty acid composition of oil from 3 positions on the ear of 4 inbreds and the combined average of 12 inbred lines

Inbred	Kernel position	Fatty acid composition (%) Palmitic	Stearic	Oleic	Linoleic	Linolenic
GE84	Base	11.25 a	2.63 a	32.65 ab	52.07 ab	1.42 a
	Middle	10.76 b	2.33 b	34.78 a	50.71 a	1.38 a
	Tip	11.18 a	2.55 ab	31.26 b	53.61 b	1.36 a
R196	Base	13.65 a	1.66 a	16.01 a	67.55 a	1.11 a
	Middle	13.26 a	1.64 a	16.24 a	67.66 a	1.21 a
	Tip	13.28 a	1.59 a	15.90 a	68.06 a	1.17 a
T202	Base	14.33 a	2.04 a	44.07 a	38.12 a	1.43 a
	Middle	14.56 ab	2.12 a	41.08 b	40.88 b	1.35 a
	Tip	14.86 b	2.09 a	39.31 b	42.38 b	1.34 a
Tx39-16	Base	15.27 a	2.37 a	35.24 a	45.71 a	1.34 a
	Middle	15.54 ab	2.51 a	33.27 b	47.39 b	1.27 a
	Tip	15.73 b	2.53 a	32.01 c	48.40 c	1.29 a
Combined Analysis	Base	13.89 a	2.31 a	33.30 a	49.26 a	1.20 a
	Middle	13.97 ab	2.36 b	33.00 a	49.47 a	1.17 a
	Tip	14.11 b	2.36 b	31.47 b	50.85 b	1.17 a

Averages followed by the same letter are not significantly different -- Duncan's Multiple Range Test (5% level).

Table 2
Kernel position effect on linoleic acid of individual ears of R196 and Tx39-16 inbreds

Kernel position	Ear 1	2	3	4	5
			R196 - Linoleic - 1965		
Base	67.58	66.99	68.51	67.97	66.69
Middle	68.51	66.80	67.96	68.52	66.49
Tip	67.73	67.08	67.98	70.05	67.48
			Tx39-16 - Linoleic - 1965		
Base	45.26	45.32	45.11	45.50	47.36
Middle	47.58	47.29	47.02	47.62	47.44
Tip	48.30	47.54	49.38	49.00	47.78
			Tx39-16 - Linoleic - 1966		
Base	48.16	45.94	46.48		
Middle	48.88	46.51	48.02		
Tip	50.63	49.47	50.43		

M. D. Jellum

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1. Cryptic genes for tripsacoid characteristics in Latin-American maize varieties.

One of the most common effects of the experimental introgression of teosinte or Tripsacum into maize is the induration of the tissues of the rachis and lower glumes. These characteristics are found in many Latin-American varieties and can be transferred to U.S. inbred strains by repeated backcrossing accompanied by selection. We have assumed that they are the product of previous introgression of teosinte or Tripsacum in these varieties but have never had direct proof of this.

The experiments reported here are concerned with an attempt to determine whether chromosomes extracted from modern Latin-American varieties, which affect induration of the tissues of the rachis and lower glumes, also carry genes for other characteristics such as distichous spikes and solitary pistillate spikelets which were derived from teosinte or Tripsacum introgression but whose effects are ordinarily concealed because they

Table 1
Characteristics of F_1 hybrids of Guerrero teosinte with inbred A158 (Control) and strains of A158 modified by substituting chromosomes from teosinte and from Latin-American varieties

Pistillate parents of crosses	Length cm.	Per cent staminate	Grades tripsacoid influence			
			Rank	Pairing	Fragility	Total
A158 (control)	18.4	47	2.2	0.6	0.6	3.4
Florida teosinte chromosome 3	18.4	52	4.0	2.2	1.2	7.4
Florida teosinte chromosome 1, 3, 9	21.6	56	2.2	1.4	0.2	3.8
Florida teosinte chromosome 4	20.4	64	4.0	2.6	3.0	9.6
Durango teosinte chromosome 1, 7, 9	18.8	68	3.0	2.4	3.2	8.6
Nobogame teosinte chromosome 4A	12.0	37	2.8	1.8	3.6	8.2
Nobogame teosinte chromosome 4B	3.8	68	2.4	2.8	3.8	9.0
Averages, teosinte derivatives	15.8	58	3.1	2.2	2.5	7.8
Mexico 1077	19.2	59	1.6	1.2	2.0	4.8
Honduras 1639	28.2	72	4.0	3.0	2.0	9.0
Nicaragua 501	19.8	67	2.2	3.6	2.0	7.8
Cuba 394	18.6	54	2.8	2.4	2.0	7.2
Averages, middle American varieties	21.4	63	2.7	2.6	2.0	7.2
Brazil 1691	13.4	83	4.0	3.0	3.0	10.0
Paraguay 333	18.4	67	4.0	2.6	2.4	9.0
Argentina 1807C	22.0	64	3.0	3.0	2.0	8.0
Bolivia 1157	18.8	49	2.6	2.0	3.0	7.6
Averages, South American varieties	18.2	66	3.4	2.7	2.6	8.6
Mexico 1077 X Venezuela 1536	16.0	42	2.0	1.8	2.2	6.0
Honduras 1639 X Nicaragua 501	26.6	72	4.0	3.2	2.4	9.6
Nicaragua 501 X Brazil 1691	17.2	58	3.6	3.0	3.8	10.4
Bolivia 1157 X Argentina 1807C	22.4	68	4.0	3.2	2.4	9.6
Averages, derivatives of two varieties	18.0	60	3.4	2.8	2.7	8.9

cannot be phenotypically expressed in genotypes which consist predominantly of maize germplasm.

The experiments involve crossing the inbred A158 and its various chromosome-substitution derivatives with a teosinte from Guerrero, Mexico, on the assumption that cryptic genes of this nature might express themselves in F_1 hybrids of maize and teosinte although unable to do so in a genetic background which is predominantly maize. Earlier observations on maize-teosinte hybrids had suggested that this may be true.

The lateral inflorescences of the F_1 hybrids were scored for prominence of a terminal staminate spike, distichous vs polystichous spikes, solitary vs paired spiklets, fragile vs solid rachis. The data on the first mentioned characteristics are based on actual measurements; the remaining three, which are arbitrary grades, were combined in a final total "tripsacoid" grade. The results are shown in Table 1.

The data show that (1) every hybrid involving a modified strain of A158 is more tripsacoid in one or more characteristics than the control; (2) substitution chromosomes from Latin-American varieties are on the whole about as strong in their effects as the chromosomes introduced into A158 directly from teosinte; (3) the substitution chromosomes from varieties of the South American countries, Bolivia, Argentina, Brazil, and Paraguay, where the introgression presumably came from Tripsacum, since teosinte is unknown in South America, are as strong in their tripsacoid effects as those from Middle America where the introgression may have come from either teosinte or Tripsacum; (4) the strongest average effect occurred in the hybrids involving strains which had been modified by substituting chromosomes from two different Latin-American varieties.

Paul C. Mangelsdorf

2. Additional prehistoric maize from Bat Cave, New Mexico.

In a paper published in 1949 we described the prehistoric maize turned up in excavations made in 1948 by Herbert W. Dick in Bat Cave, a once-inhabited rock shelter, in New Mexico. We have recently completed an analysis of additional material from other parts of the cave turned up in a second excavation made in 1950. This analysis has produced the following conclusions:

1. The earliest maize from the second (1950) Bat Cave expedition is more primitive than any of the specimens turned up in the first (1948) expedition.

2. Maize from the lower levels of the cave is definitely a popcorn. There are several popped kernels among the prehistoric remains and other prehistoric kernels proved to be still capable of popping after having their moisture content raised.

3. The earliest maize is probably a form of pod corn. At least it has relatively long soft glumes partly enclosing the kernels which are borne on long rachillae. These are characteristics of pod corn.

4. The maize from the lowest levels has brown pericarp color and is related to the Mexican race, Chapalote. Brown pericarp color is presumably the primitive or "wild" color. Brown pericarp is replaced by red and colorless pericarp in the upper levels of the cave.

5. The occurrence of variegated pericarp in the 36-48" level shows that a mutation system similar to that involving the "modulator" factor was in existence at an early stage in corn's domestication.

6. Clear-cut evidence of the introgression of teosinte or Tripsacum or both appears in the 36-48" level of the cave and there is some evidence of introgression in the earlier 48-60" level.

7. There is an increase in diameter of the rachis of the cobs from the lower to the upper levels; this is accompanied by an increase in kernel-row number and in the length and width of the kernels. There is a slight decrease in kernel thickness.

8. The remains of the husks and other parts of the husk systems suggest that the long husks found in one of the levels of the 1948 excavations enclosed not a single ear but a cluster of ears each enclosed in its own husk.

9. Correlation of cultural characteristics with other sites and with the prehistoric maize of other sites suggests that the earliest maize from Bat Cave should be dated at not earlier than 2300 B.C. and perhaps several centuries later.

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H. W. Dick
Julián Cámara-Hernández

3. Prehistoric maize from a site near Huarmey, Peru.

We have recently completed the analysis of remains of prehistoric maize turned up in a site near Huarmey on the northern coast of Peru in excavations made by Dr. David Kelley, now of the University of Nebraska, and Dr. Duccio Bonavia of Lima, Peru.

The earliest maize from this site was thought to be preceramic. Radiocarbon determinations made of the cobs at different levels are quite inconsistent and we assume that there has been contamination from organic sources. The earliest specimens are at least as primitive with respect to size and other characteristics as the earliest prehistoric corn from Huaca Prieta.

The collection of prehistoric specimens includes all parts of the maize plant. Brief descriptions of these parts follow.
Roots. Lower internodes of the stalk with roots show both seminal and permanent root systems. One specimen shows the scar of an attached tiller; others show no evidence of tillering.
Stalks. These are more slender than those of most races of modern maize and one specimen shows the stump of a peduncle of an ear arising at the fourth node from the base. The internode pattern is similar to that of the modern Peruvian race, Confite Morocho, described by Grobman et al., 1961.
Leaf Sheaths. All specimens are completely glabrous like those from the early levels in the prehistoric maize from Tehuacán, Mexico.
Leaves. Many specimens of leaves and midribs show them to be similar to those of modern corn. The structure of the lower epidermis is quite similar to that described by Prat for modern maize.
Prophyll. Virtually identical in its characteristics to prophylls of modern maize.
Husks. The venation is more strongly parallel and the anastomosing venation between the ridges less conspicuous than in modern maize. Also the differences between the outer and inner husks are not as marked as in modern maize. Two more or less complete husk systems suggest that the husks are much longer than the ears which they once enclosed. Extending beyond the point of attachment of the uppermost husk is a long internode subtending the ear which probably became exposed at maturity.
Cobs. The variation in the characteristics of the cobs, especially in the shape and hairiness of the cupules, indicates that the earliest corn is not a wild corn. Most of the earlier cobs are similar, including the presence of stumps and staminate tips, to the cobs of the early cultivated maize from the caves in Tehuacán and could be assigned to the Mexican race, Chapalote. Several of the cobs, however, are quite different from any prehistoric specimens found in Mexico and may be derived from a different race of wild maize, probably one of the Peruvian highland races.
Silks. These are somewhat more flattened in cross section than those of modern maize, are quite hairy, and are not bifurcated at the tips.
Kernels. There are few intact kernels but one well preserved specimen is round with a slight indication of pointing and has a brown pericarp color as does the earliest corn of Bat Cave as well as the corn from the Los Cerillos site on the southern coast of Peru.

Tassels. Well preserved tassel branches and entire tassels show the typical arrangement of paired spikelets, one member sessile and the other pedicelled. The spikelets are smaller than those of modern maize. Tassel diagrams of two almost intact tassels are similar to those of the Peruvian race, Confite Morocho.
Anthers. Several tassel branches contained well preserved anthers filled with pollen.
Pollen. The prehistoric pollen when mounted in lactic acid and iodine assumes the shape of modern pollen but is somewhat smaller in size than the pollen of most modern varieties. Pollen from four different tassels measured 78.1, 80.6, 82.5, and 86.6 microns respectively. This is well within the range of pollen size of some of the modern races regarded as ancient such as Chapalote and Nal-Tel.
Conclusions. The majority of the specimens from this site could be assigned to the Mexican race, Chapalote, but they are also related in some respects to the Peruvian race, Confite Morocho. A few specimens differ in some characteristics from either of these races and are more closely related to the Peruvian highland popcorns. In all of its basic botanical characteristics the earliest maize from Huarmey is identical with modern maize.

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4. Prehistoric maize from Huaca Prieta, Peru.

The specimens from this site excavated some years ago by Dr. Junius Bird consist almost entirely of cobs. These are quite different from those of the Huarmey site. The majority are globular in shape having eight-kernels rows at their butts and tips and 10, 12, or 14 rows in the middle regions. Because of this change in row number most of the cobs do not show distinct rows but many exhibit a spiral arrangement of the spikelets similar to the spiral of a pine cone. Ears of this type rarely have stumps of staminate tips.

These cobs differ also from those of Huarmey and of the early prehistoric cobs from the Tehuacán caves in their cupules. The differences are best illustrated by photographs and drawings to be published soon but may be briefly described here. The cupules of the typical Huarmey maize are similar to flat saucers almost square in outline attached to a four-sided central stem. The typical cupules of the majority of Huaca Prieta specimens are structures similar in shape to the toe halves of pointed shoes inserted into an egg-shaped central stem at an angle with the toe pointing toward the tip. Since we have not encountered cobs of this type in the prehistoric corn from the caves in Mexico or southwestern United States and since they are similar to the cobs of the living races in the Peruvian highlands, especially Confite Puneño, we conclude that they have stemmed from a different wild race than the prehistoric wild maize of Mexico. However, in the Huaca Prieta collection there are a few cobs similar to the majority of those from the Huarmey site in Peru and the Tehuacán caves in Mexico as well as some intermediates which may have resulted from hybridization of these two distinct types.

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5. Prehistoric maize from the Ica Valley, Peru.

These specimens come from the Los Cerillos site in the Ica Valley on the south coast of Peru which was excavated by Dr. Dwight Wallace, then of the University of California. The maize is estimated to be about 2300-2500 years old. The ears, which are among the oldest and best preserved of any prehistoric corn yet found, have been briefly described by Grobman and Mangelsdorf in MNL, 1959. At that time we thought this maize, because of its predominantly brown pericarp color might be related to the Chapalote race of Mexico. Additional studies recently made suggest that this is probably not true. The majority of the cobs do have kernels with brown pericarp color but in other respects they are quite different from Chapalote. The ears are globular in shape and bear irregular rows of kernels. The cupules are similar to those of the predominating type in the Huaca Prieta site. Staminate tips or the stumps of such tips are rare. Except for its brown pericarp color, which may be the universal "wild" color, this maize is quite different in several respects from any prehistoric maize found in Mexico or the southwestern United States. We conclude that the Los Cerillos maize, like the Huaca Prieta maize, has descended from a different race of wild corn than that found in the Tehuacán caves in Mexico and probably stems from a wild Peruvian maize which also gave rise to the Peruvian highland race, Confite Puneño. The Los Cerillos maize considered with the Huaca Prieta maize suggest that there may once have been at least two wild races of maize, one Mexican and the other Peruvian. There may have been still a third wild race, the ancestor of the pointed-seeded popcorn of the Toluca Valley of Mexico, Polomero Toluqueño.

Paul C. Mangelsdorf
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6. Meiosis in diploid interspecific hybrids of Tripsacum.

Three interspecific hybrids involving T. floridanum, T. dactyloides and T. zopilotense have been studied to obtain data on chromosome synapsis and fertility relationships.

The hybrids of T. dactyloides and T. floridanum showed an intimate synapsis at pachytene. A minute duplication has been observed in this hybrid by Chaganti (1965). Post pachytene stages were found to be normal. Eighteen bivalents were regularly observed both at diakinesis and metaphase I. Chiasma frequencies obtained at diakinesis showed no significant differences from the parents. The mean chiasma frequency is approximately the same as in the parents (Table 2). The hybrids are vigorous and highly fertile.

The hybrids of dactyloides-zopilotense are partially sterile and showed various meiotic abnormalities. The most common phenomenon is intense pycnosis of the nuclei observed at different stages of meiosis. At the pachytene stage in some nuclei, all the chromosomes formed a confluent mass of chromatin material. In others, only a part of the nucleus was affected and the other part showed normal bivalents. In addition to pycnosis, the pachytene chromosomes showed a loose association or more

Table 2

Table showing the frequency distribution of bivalents with different numbers of chiasmata in three diploid (2n = 36) species of Tripsacum and their inter-specific hybrids (stage of analysis-Diakinesis)

Parents and hybrids	Number of cells analyzed	Bivalents with				Total xta	Average xta per cell	Percentage of stainable pollen
		3 xta	2 xta	1 xma	0 xma			
T. floridanum (Collier Co., Fla.)	30	--	452	88	--	992	33.06	96.6
T. dactyloides (Bussey clone, Manhattan, Kansas)	30	36	425	79	--	1037	34.55	95.3
T. dactyloides x (F_1) T. floridanum	15	--	210	60	--	480	32.00	94.8
T. zopilotense (25101; 65-1218)	30	--	409	131	--	949	31.63	91.3
T. floridanum x (F_1) T. zopilotense	30	--	270	174	96	714	23.8	58.1
T. dactyloides x (F_1) T. zopilotense	30	--	368	109	63	845	28.2	63.2

often a pairing failure. Approximately 30 per cent of the cells suffered from pycnosis and degeneration. At diakinesis all the 18 bivalents were seldom observed. The mean chiasma frequency was found to be lower than in the parents (Table 2).

The hybrids of floridanum-zopilotense are also partially sterile, in fact more sterile than the preceding hybrids (some of the plants never exserted the anthers). At pachytene a minute duplication, a deletion, interlocking and terminal pairing failure have been observed. In none of the diakinetic nuclei were all the 18 bivalents observed. The data on the various types of associations classified as those with two, one and zero chiasmata are given in Table 2. A maximum of 12 univalents was observed. The most frequent type of association is 14 bivalents and 8 univalents. Lagging chromosomes were observed in both the hybrids.

R. V. Tantravahi
W. C. Galinat

7. Morphology and behavior of B chromosomes in Tripsacum.

The presence of B chromosomes which has been established in maize (Randolph, 1928; McClintock, 1933) and teosinte (Longley, 1937) is now known to occur also in Tripsacum. During the course of study of pachytene chromosome morphology in Tripsacum species, a single B chromosome was observed in T. floridanum, T. maizar and T. zopilotense. The B chromosomes of T. maizar and T. floridanum are as long as the shortest member of the A complement while that in T. zopilotense appeared as a small fragment. In their morphology the B chromosomes of T. floridanum and T. maizar resembled the B chromosomes of maize at the pachytene stage and like those often showed 'fold back' pairing.

The B chromosomes of T. maizar and T. zopilotense were found to be scattered in the nucleus while the B chromosome of T. floridanum often showed a nonhomologous 'association' with the centromere of one of the chromosomes of the regular complement. In all three species the B chromosomes showed a precocious movement to one of the poles while the bivalents of the regular complement were still on the metaphase plate.

One of the characteristic features of the B chromosomes is their nondisjunction at mitosis (Randolph, 1941). In the present study, a variation in the number of B chromosomes was observed in the pollen mother cells of the same plant of T. zopilotense. This variation could have come about by nondisjunction of the B chromosomes at the premeiotic mitosis.

R. V. Tantravahi

8. Pachytene chromosome morphology and meiosis in Tripsacum maizar.

The pachytene chromosomes of T. maizar are differentiated into proximal heterochromatic and distal euchromatic regions. The heterochromatic regions gradually merge into the distal euchromatic regions. The pachytene chromosomes are characterized by constant and reproducible quantitative features such as their relative lengths, position of the centromere and the arm ratios. Data obtained on these characters showed that the longest chromosome in the complement measures on an average 73.10 microns while the shortest is 13.20 microns long. Chromosomes 2, 5, 6, 11, 16, 17 and 18 have nearly median centromeres while those of chromosomes 4 and 7 are more nearly subterminal and the values for the arm ratios are large. The nucleolus organizing chromosome is assigned the 16th position in the idiogram. The nucleolus organizing body itself is in the long arm near the centromere. Only three knobs have been observed in the complement. Detailed data on the relative lengths, arm ratios and the position of knobs are give in Table 3.

At diakinesis 18 bivalents are regularly observed. Both ring and rod types are found, the former in greater proportion (71.12%). At metaphase I, occasionally a bivalent was replaced by two univalents. These cases were considered to represent precocious terminalization of a chiasma in one of the shorter bivalents since no meiotic abnormalities were found. The rest of the stages of first and second division are normal.

R. V. Tantravahi

Table 3

Table showing the lengths and arm ratios of the 18 chromosomes of T. maizar. (All measurements in microns)

Chromosome number	Total length	Length of the long arm	Length of the short arm	Arm ratio
1	73.10	52.08	20.83	2.5:1.0
2	62.49	36.46	23.96	1.5:1.0
3	58.33	42.71	14.58	2.9:1.0
4	58.08	44.79	10.94	4.1:1.0
5	46.35	27.08	18.23	1.5:1.0
6	41.66	25.52	15.10	1.7:1.0
7	39.06	30.21	7.81	3.9:1.0
8	36.46	24.28	10.42	2.3:1.0
9	35.25	23.96	9.89	2.4:1.0
10	31.25	21.87	7.81	2.8:1.0
11	30.62	15.62	13.02	1.2:1.0
12	26.04	16.67	7.81	2.1:1.0
13	26.04	16.14(K)	7.82	2.0:1.0
14	23.96	16.14(K)	6.25	2.6:1.0
15	23.91	16.67	5.73	2.9:1.0
16	23.44	9.37	7.81	1.2:1.0
17	21.87	11.62	10.09(K)	1.3:1.0
18	13.20	7.92	4.16	1.9:1.0

(K) indicates the presence of a terminal knob in the arm.

R. V. Tantravahi

9. Pachytene chromosome morphology and meiosis in tetraploid species of Tripsacum.

In a program aimed at studying the chromosome morphology and meiosis in tetraploid species of Tripsacum, it has become possible to make a detailed study of the chromosome morphology and the behavior of the 'associations of four' chromosomes in T. laxum. The pachytene chromosomes of T. laxum are differentiated into eu- and heterochromatic regions much the same way as in the diploid species. The longest chromosome in the complement measures on an average 68.04 microns while the shortest is 15.08 microns long. Altogether seven knob positions have been observed on the 18 sets of chromosomes. Some of the knobs were found to be in a heterozygous condition. The details on the relative lengths of the chromosomes, their arm ratios and the knob positions are presented in Table 4. Chromosome 10 is the nucleolus organizing chromosome.

A total of 218 pachytene associations have been studied in T. laxum to obtain data on the frequency and the position of exchange of partners, the relative distribution of exchanges in the eu- and heterochromatic regions and the mean number and mean length of pairing blocks. Such a study showed the maximum and the minimum numbers of exchanges ranged from three to none. There was observed a positive correlation between the physical length of the arm and the distribution of exchanges. Besides the 'two by two' pairing, 16.4 per cent of the associations showed an association of the four centromeres with or without partner exchanges elsewhere on the chromosome. In the long chromosomes, chromosomes 4 and 8 showed a low frequency of 'two by two' pairing while chromosome 2 almost always showed exchange of partners.

The initial points of pairing, as inferred by the position of exchange of partners, seem to lie at random along the length of the chromosomes. However, in the case of chromosomes 5 and 14 a clustering of exchanges was observed in a particular region of the long arms.

The frequency distribution of exchanges in the eu- and heterochromatic regions showed that such exchanges are rare in the heterochromatic regions. The mean number of pairing blocks increased with increase in length of the chromosome up to a certain limit beyond which it decreased. Thus, the mean number of pairing blocks for chromosome 1 is lower than for chromosome 2. The mean length of the pairing block increased with increase in length of the chromosome. These observations are in general agreement with the inferences drawn by Darlington and Mather (1932) and Stone and Mather (1932) on the basis of a diakinesis study of triploid tulips and hyacinths.

Detailed studies have been made at diakinesis in all five tetraploid species of Tripsacum to obtain data on the types and frequencies of multivalents so that the data can be used to compare the meiotic behavior among the five species. This study showed that all the tetraploids are characterized by few multivalents and many bivalents. The frequencies (percentages) of various types of associations are presented in Table 5. The highest quadrivalent frequency is found in T. dactyloides and the lowest in T. laxum. Quadrivalent types 11 (chain of four) and 17 (ring

Table 4

Table showing the relative lengths and arm ratios of the haploid complement of 18 pachytene chromosomes of tetraploid T. laxum. (All measurements are in microns.)

Chromosome number	Total length	Length of the long arm	Length of the short arm	Arm ratio
1	68.04	45.24	18.20(k)	2.7:1.0
2	54.60	41.60	13.00(K)	3.1:1.0
3	52.00	35.88	13.00(k)	2.7:1.0
4	50.96	34.32	15.08	2.3:1.0
5	44.20	27.56	15.08	1.8:1.0
6	41.60	28.60	11.44	2.5:1.0
7	37.44	23.40	13.00	1.8:1.0
8	33.80	28.60	5.20	5.3:1.0
9	33.80	21.84	10.40	2.5:1.0
10	32.24	16.12	15.60	1.0:1.0
11	28.60	16.64(k)	10.40	1.6:1.0
12	27.56	20.80	5.20(K)	4.0:1.0
13	27.04	17.68	7.80	2.2:1.0
14	22.36	16.12	5.20	3.1:1.0
15	21.32	13.00	5.72	2.3:1.0
16	18.20	12.48(K)	4.68	2.6:1.0
17	17.68	13.00(K)	2.60	4.5:1.0
18	15.08	8.84	5.72	1.5:1.0

(K) indicates the presence of a terminal knob in the arm.
(k) indicates the presence of a heterozygous terminal knob in the arm.

Table 5

Table showing the relative frequency distribution of various types of associations (quadrivalents, trivalents, bivalents and univalents) observed at diakinesis in the tetraploid (2n = 72) species of Tripsacum

Species	Types of associations					
	Quadrivalents	Trivalents	Bivalents			Univalents
			Ring	Rod	Total	
T. dactyloides (Florida)	22.8	--	39.1	36.9	76.0	1.2
T. lanceolatum (27294; 65-1251) Mexico	17.6	3.2	58.2	17.7	75.9	3.3
T. pilosum (26488; 65-1240) Mexico	19.3	2.8	51.6	21.7	73.3	4.6
T. laxum (44179; 65-1245) Guatemala	15.7	--	52.5	29.6	82.1	2.2
T. latifolium (44116; 65-1269) Guatemala	21.3	--	40.0	36.9	76.9	1.8

of four) are the two most frequent types observed in all the species. T. lanceolatum and T. pilosum had a low trivalent frequency.

On the basis of the meiotic behavior, distribution patterns and segregation of morphological characters, it is suggested that all the polyploid species of Tripsacum are segmental allopolyploids. T. laxum, T. latifolium and T. dactyloides are stabilized segmental allopolyploids; T. lanceolatum and T. pilosum are relatively young and are at an active stage of segregation.

R. V. Tantravahi

10. Heterosis: Kernel weight, ovules per ear row, and rows per ear.

The following data, gathered at DeKalb several seasons ago, may be of interest in regard to heterosis of 'yield components' of the maize ear, i.e., kernel weight, ovules per ear row and row number. The HD prefix indicates doubled haploid lines. L indicates a low value of the attribute in the parental line, M indicates a median value, and H a high value. Line averages are indicated in the columns to the right of each 2 x 2 table. Central values in these tables are those of the various single cross hybrids measured. Crosses were made one way only.

a. Kernel weight (grams per 100 kernels):

	L HD907	L HD82	L HD159	H HD2380	H HD1464	H HD1344	Lines per se
L HD907	----	25.8	21.9	27.6	28.5	29.9	17.1
L HD82	25.8	----	26.6	31.2	34.0	30.3	17.5
L HD159	21.9	26.6	----	30.3	31.7	29.2	18.5
H HD2380	27.6	31.2	30.3	----	37.9	35.2	30.0
H HD1464	28.5	34.0	31.7	37.9	----	36.7	34.5
H HD1344	29.9	30.3	29.2	35.2	36.7	----	37.0
Averages:	26.7	29.6	27.9	32.4	33.8	32.3	25.7

Summary:	L	H	Lines
L	24.8	30.3	17.7
H	30.3	36.6	33.8
Averages:	27.6	33.5	25.7

*Heterosis:
L x L = 40.9%
L x H = 17.9%
H x H = 8.3%

*Heterosis given as % increase of hybrids over average of parents.

b. Ovules per ear row:

		L HD1344	L HD73	L HD1937	H HD1801	H HD82	H HD1951	Lines per se
L	HD1344	--	50	50	49	59	58	25
L	HD73	50	--	54	56	61	59	34
L	HD1937	50	54	--	48	57	61	37
H	HD1801	49	56	48	--	62	54	42
H	HD82	59	61	57	62	--	65	45
H	HD1951	58	59	61	59	65	--	49
Averages:		53.2	56.0	54.0	53.8	60.8	59.4	38.6

Summary:	L	H	Lines
L	51.3	56.4	32.0
H	56.4	60.3	45.3
Averages:	53.8	58.4	38.6

Heterosis:
L x L = 60.3%
L x H = 45.7%
H x H = 33.1%

c. Rows per ear:

		L HD1092	L HD1464	LM HD212	LM HD1432	M HD73	M HD1668	MH HD920	MH Hy	H HD1689	H HD910	Lines per se
L	HD1092	----	12.8	13.5	13.6	15.1	15.4	15.2	16.4	14.5	14.5	11.2
L	HD1464	12.8	----	14.7	14.8	15.5	16.0	14.7	15.1	15.6	16.5	12.0
LM	HD212	13.5	14.7	----	15.3	15.8	17.9	16.2	15.8	18.7	16.4	13.3
LM	HD1432	13.6	14.8	15.3	----	16.0	17.3	15.7	15.6	17.1	17.3	14.4
M	HD73	15.1	15.5	15.8	16.0	----	18.7	17.2	16.5	19.2	19.0	16.1
M	HD1668	15.4	16.0	17.8	17.3	18.7	----	19.3	18.2	19.2	20.5	16.1
MH	HD920	15.2	14.7	16.2	15.7	17.2	19.3	----	17.5	20.2	19.4	17.1
MH	Hy	16.4	15.1	15.8	15.6	16.5	18.2	17.5	----	20.0	18.5	17.5
H	HD1689	14.5	15.6	18.7	17.1	19.2	19.2	20.2	20.0	----	20.7	20.6
H	HD910	14.5	16.5	16.4	17.3	19.0	20.5	19.4	18.5	20.7	----	21.3
Averages:		14.6	15.1	16.0	15.9	17.0	18.0	17.2	17.1	18.3	18.1	15.96

Summary:	L	IM	M	MH	H	Lines
L	12.8	14.1	15.5	15.3	15.4	11.60
IM	14.1	15.3	16.7	15.8	17.4	13.85
M	15.5	16.7	18.7	17.8	19.5	16.10
MH	15.3	15.8	17.8	17.5	19.5	17.30
H	15.4	17.4	19.5	19.5	20.7	20.95
Averages:	14.6	15.9	17.6	17.2	18.5	15.96

Heterosis:	L	LM	M	MH	H
L	10.3%	10.2%	11.5%	5.5%	-3.7%
LM	10.2%	10.1%	11.3%	1.3%	-0.6%
M	11.5%	11.3%	16.2%	6.6%	4.8%
MH	5.5%	1.3%	6.6%	1.2%	1.6%
H	-3.7%	-0.6%	4.8%	1.6%	-1.4%
Averages:	6.76%	6.46%	10.08%	3.24%	0.14%

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1. Winter breeding nurseries on the island of Molokai, Hawaii.

Commercial winter corn breeding nurseries were instituted in 1966 on the island of Molokai, Hawaii, in an area chosen for its uniquely dry temperate climate. Corn Belt and tropical maize varieties produced excellent seed yields in these nurseries and future development of the area by the seed industry appears certain. Some characteristics of this area and of corn grown there will be cited; detailed performance data can be obtained upon request.

The area chosen for nursery development is in the vicinity of Kaunakakai (sea level), on the southern, leeward coast of Molokai, 25 mi. by air from Honolulu (4 flights/day). The area is sunny, dry, and cooled by tradewinds that often blanket the island's mile-high hills with clouds. (Details on the 260 sq. mi. of Molokai may be found in "Molokai; Present and Potential Land Use" by Harold Baker, U. Hawaii Land Study Bur. Bull. 1, 1960).

Rainfall near Kaunakakai averaged 13.5"/yr. over a 25 yr. period (range, 2.8" to 29.2"), with monthly medians as follows:

Month:	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Rainfall:	0.2	0.6	1.1	2.2	0.8	1.0	0.1	0.2	0.0	0.0	0.0	0.0

Temperatures at Kaunakakai exceed by about 2° the following 10-yr. averages computed at the Molokai airport (elev. 443'):

Month:	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Mean Temp:	76°	74°	71°	70°	70°	71°	72°	74°	75°	76°	77°	76°

Max-Min temperatures in the winter of 1966-67 (Oct. to Feb.) were 86 and 65, resp., at the Molokai airport; it is doubtful whether temperatures below 55 or above 95 have ever occurred in this area.

Winter daylengths in Hawaii (19° N) minimize at 10 hr. 50 min., and the Kaunakakai area is rarely overcast. Winds are mild on the Kaunakakai

plain, compared with the airport at which a 15 mph tradewind (ENE) is commonplace; while sporadic winds exceed 45 mph at the airport, hurricane velocities do not occur.

Preliminary winter nursery studies have been conducted since 1961 at the University of Hawaii's 13 experiment stations, and were continued in 1965-66 under a cooperative project with Cornnuts, Inc., including lines from Illinois Foundations Seeds, Inc. Results of the 1965-66 tests led to the search for a dry leeward location for these nurseries, and the firm of Cornnuts, Inc., negotiated with the Molokai Ranch of Kaunakakai for the first commercial nursery in 1966.

The area chosen is evidently too dry for Helminthosporium turcicum blight, which fares best only in Hawaii's cool, wet highlands. The sweet corn mosaic-stunt (transmitted by a leafhopper, Peregrinus maidis) was virtually absent from 1966-67 nurseries, and future build-up should be checked easily with insecticide. Earworms and aphids were sporadic, while leaf-feeding insects were of no consequence. Other major pests of corn (e.g., cutworms, borers, rusts, mildews, smuts) are rare or absent in Hawaii.

Most Corn Belt inbreds set silk in 60-65 days at Kaunakakai (planted Nov. 21). Days to silking of temperate corns are reduced about 15% in Hawaii as indicated by the following days to silk on Molokai for the major seasonal types of sweet corn hybrids.

Season	U.S. Mainland	Molokai	Class
Very early	45 days	44 days	Spancross
Early	55 days	48 days	Carmelcross
Mid-season	65 days	52 days	Golden Cross
Late	75 days	57 days	Country Gentleman

Plant heights and ear lengths are affected proportionately by this telescoping. Seed production in 1966-67 trials was excellent, with ear lengths and seed sets estimated to exceed 80% of corn belt averages. Most major corn belt inbreds were included in these trials; performance data on these and other lines will be provided upon request.

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(In collaboration with D. L. Shaver, Cornnuts, Inc.)

2. Pollen esterase isozymes.

Starch gel electrophoresis of pollen extracts from two sweet corn inbreds from Hawaiian Sugar demonstrated 7 anode-wandering esterase isozymes. The bands, labeled 1-7, had Rf values at pH 8.6 of 86, 80, 77, 74, 42, 37 and 31 respectively. Genetic studies reported here involve bands 2 (Rf 80) and 4 (Rf 74).

Band 2 was present in pollen of inbred AA1, but absent in inbred AA7, while the reverse was true of band 4. In an F_2 population of 61 plants, band 2 was present in 49 plants (presence: absence ratio of 4.08 to 1). Band 4 was present in 50 of the 61 F_2 plants (ratio 4.54 to 1).

The F_2 distribution of bands 2 and 4 is presented in Table 1. The 9:3:3:1 ratio expected on the basis of non-linked loci was approximated satisfactorily by the observed data (X^2=4.26, P=0.27). The null type was not observed.

Table 1
Distribution of esterase bands 2 and 4 in pollen of an F_2 population of 61 plants

	(bands 2 and 4)	(band 2)	(band 4)	(null)
Observed frequency	38	11	12	0
Expected frequency (based on 9:3:3:1 ratio)	34.29	11.43	11.43	3.81

Bands 2 and 4 could be classified in segregating populations into 2 classes, on the basis of the intensity of the esterase stain reaction. These were referred to as 'strong' and 'weak' reactions, or 2^S and 4^S versus 2^W and 4^W respectively. Classification of the F_2 population on this basis is presented in Table 2. The data were best interpreted by the assumption that the strong bands represented homozygotes, while the weak bands represented heterozygotes, a conclusion warranted by observation of the inbred and F_1 lines. Following this assumption the expected F_2 phenotypic ratios were computed (Table 2) and they compared favorably with the observed ratio (X^2=6.29, P=0.29).

Table 2
Distribution of esterase bands 2^S, 2^W, 4^S and 4^W in pollen of an F_2 population of 61 plants

	(2^S4^S&2^W4^W)	(2^S4^W)	(2^W4^S)	(2^S&2^W)	(4^S&4^W)	null
Observed frequency	24	9	5	11	12	0
Expected frequency (based on 5:2:2:3:3:1 ratio)	19.05	7.62	7.62	11.43	11.43	3.81

It has not been possible to determine whether the pollen esterase isozymes are synthesized under gametophytic or under sporophytic control. Using the gametophytic model, expected F_2 phenotypes of pollen are presented in Table 3. The table assumes that in heterozygotes the different types of pollen are produced in equal numbers; e.g., pollen of the heterozygote

AaBb consists of ¼ having both bands 2 and 4, ¼ having only band 2, ¼ having only band 4 and ¼ having neither band. Theoretically, this results in 9 phenotypes (2^S4^S, 2^W4^W, 2^S4^W, 2^W4^S, 2^S, 2^W, 4^S, 4^W and a null type). Of these 9 phenotypes, only 6 would be apparent in the gel (since it is most difficult to differentiate between 2^S4^S and 2^W4^W, between 2^S and 2^W, and between 4^S and 4^W, owing to the effect of minor differences in concentration of extracts applied to the gel).

Table 3
Expected F_2 ratios and phenotypes for esterase bands 2 and 4. (A, band 2; B, band 4; a and b, null alleles)

	per cent of pollen producing bands:				
F_2 genotype	(2 & 4)	(2)	(4)	null	F_2 phenotype
AABB	100	0	0	0	2^S4^S*
AABb	50	50	0	0	2^S4^W
AaBB	50	0	50	0	2^W4^S
AaBb	25	25	25	25	2^W4^W
AAbB	50	50	0	0	2^S4^W
AAbb	0	100	0	0	2^S -
AabB	25	25	25	25	2^W4^W
Aabb	0	50	0	50	2^W -
aABB	50	0	50	0	2^W4^S
aABb	25	25	25	25	2^W4^W
aaBB	0	0	100	0	- 4^S
aaBb	0	0	50	50	- 4^W
aAbB	25	25	25	25	2^W4^W
aAbb	0	50	0	50	2^W -
aabB	0	0	50	50	- 4^W
aabb	0	0	0	100	- -

* s, strong; w, weak

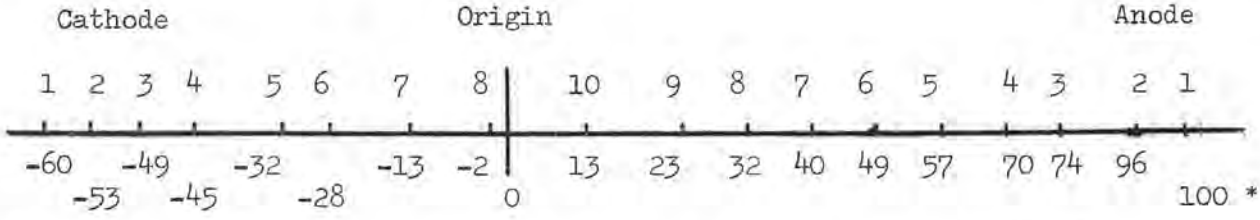
At the present time there appears to be no immediate method to distinguish whether pollen esterase isozymes 2 and 4 are synthesized under gametophytic or sporophytic control.

Timothy Macdonald

3. Peroxidase isozymes in maize.

Electrophoretic separation of maize extracts on starch gel reveals several distinct zones or bands with peroxidatic activity. Different strains of maize are being used in genetic studies of these peroxidase polymorphisms. Maize tissues studied intensively have been the seedling root and shoot, and 14-day old endosperm. Seedlings are germinated in petri dishes, and saline extracts used for electrophoresis. Starch gels are stained with o-dianisidine, a stain superior to benzidine for permanent gels.

Roots provide the clearest isozyme patterns, while endosperm produces weakly staining bands. Cathode-migrating bands stain more distinctly than anode-migrating isozymes. A total of at least 18 bands have been distinguished in different gels, 10 moving to the anode (A1 to A10) and 8 to the cathode (C1 to C8; see diagram).



*Approximate Rf values in terms of the fastest anode wandering band.

Table 1 presents the distribution of the 18 peroxidase isozymes. Bands A1 and A2 (Table 1) have been observed only in roots; they stain best with benzidine and have not been observed separately in about 20 inbreds. Both were absent in Connecticut inbred C53, but present in hybrids involving this inbred. Bands A3 and A4 were also present in roots but absent in shoots and endosperm, and showed genetic polymorphism. Although both bands stain intensely, they often appear blurred on starch gels. A5, A6, and A7 have been observed only in shoots; no genetic polymorphisms have been observed. A8 is a strong clear band in all 3 tissues studied, and shows no polymorphism, making it useful as a reference band. Isozymes A9 and A10 are strong bands near the origin which are often blurred. They are best seen in roots and are absent from endosperm. Diffuse staining near the origin is common on gels from shoot and root tissues.

In general, the bands moving to the cathode are clearer and more sharply defined than those going to the anode. C1 and C2 were found in roots and endosperm, absent from young shoots, but present in leaves from mature plants. Genetic polymorphisms were common for both bands among inbreds and within tropical races of maize. C3 is very distinct and useful as a reference band. C4 appears always to be present in roots, shoots and endosperm, although it is very weak. C5 and C6 are clear bands, difficult to separate; while C7 and C8, like A9 and A10, are close to the origin and often blurred.

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Table 1
Peroxidase Isozymes in Maize (Rt = root, Sh = shoot, End = 14 d. endosperm)

	Presence in:			In Roots:	
	Rt	Sh	End	Stainability	Polymorphism
A1*	+	0	0	weak, clear	+
A2	+	0	0	weak, clear	+
A3	+	0	0	strong, blurred	+
A4	+	0	0	strong, blurred	+
A5	0	+	0	weak, clear	-
A6	0	+	?	medium, clear	-
A7	0	+	?	medium, clear	-
A8*	+	+	+	strong, clear	0
A9	+	?	0	strong, blurred	+
A10	+	?	0	strong, blurred	?
C1	+	0	+	strong, clear	+
C2	+	0	+	strong, clear	+
C3*	+	?	+	strong, clear	+
C4	+	+	+	weak, clear	0
C5	+	?	+	strong, clear	?
C6*	+	+	+	strong, clear	?
C7	+	+	+	variable, blurred	?
C8	+	+	+	variable, blurred	?

*Useful reference bands.

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1. Protein differences in reciprocal crosses.

Reciprocal crosses were made between three inbred lines, 38-11, CI21E, and Oh7A, and Illinois High Protein (IHP) in the summer of 1966. The per cent protein was determined using the Kjeldahl method.

The mean per cent protein for each cross and the per cent difference between reciprocal crosses are listed in Table 1. Ten reciprocal crosses were made involving 38-11 while seven each were made with the other two lines. Using individual reciprocal crosses as a basis for pairing, each line crossed with IHP showed a highly significant difference in per cent protein using the paired t test.

Table 1

Cross (1)	Protein Content	Difference between reciprocal crosses
38-11 x IHP	13.77%	13.52%
IHP x 38-11	27.29	
CI21E x IHP	11.07	15.10
IHP x CI21E	26.17	
Oh7A x IHP	10.71	16.37
IHP x Oh7A	27.08	

(1) Female listed first.

Gene dosage effects associated with the endosperm, effects of cytoplasm contributed by the female parent, and maternal effects attributable to the female sporophyte could possibly explain the above differences. Related experiments are planned to study the cause of these differences.

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1. Recombination studies of alleles at the Rp_1 locus for resistance to P. sorghi.

Thirteen dominant alleles, Rp_1^a, Rp_1^b, ... , Rp_1^m, at the locus Rp_1 in corn that condition resistance to specific biotypes of the corn rust, Puccinia sorghi Schw., are known. In the 1964 Maize Genetics News Letter, data were reported suggesting crossing over between alleles Rp_1^a and Rp_1^k identified, respectively, in corn inbreds GG208R and Mex212. In that study, however, the screening scheme was such that only one recombinant, the susceptible type, was identified.

By using an appropriate mixture of P. sorghi biotypes both recombinant phenotypes have now been identified. Data from the testcross (B14[5] - BYD x Mex212) x R168 are reported here.

Parents:		Biotypes of P. sorghi			Number of seedlings observed
		901aba	936c	941bR	
B14[5] -BYD	Rp_1^c / Rp_1^c	R*	R	S**	
Mex212	Rp_1^k / Rp_1^k	R	S	R	
R168	rp_1 / rp_1	S	S	S	
Test Cross Progeny:					
Parentals	Rp_1^c / rp_1	R	R	S	19607
	Rp_1^k / rp_1	R	S	R	
Recombinants	Rp_1^c-Rp_1^k/rp_1	R	R	R	19
	rp_1^c-rp_1^k/rp_1	S	S	S	15

*R - resistant **S - susceptible

The crossing over value between Rp_1^c and Rp_1^k is 0.17%. However, resistant plants can also arise from accidental selfing and susceptibles can also arise from mutation or deletion of Rp_1. Recombinants have been saved for a progeny test.

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1. Elimination of knobbed chromosome arms in maize induced by supernumerary B chromosomes.

In the 1966 Maize News Letter we reported that a genetic stock homozygous for the closely linked $\underline{A}_1$ and $\underline{Sh}_2$ alleles in chromosome 3 and for the Dt gene in 9 gave high frequencies of $\underline{a}_1$ $\underline{sh}_2$ kernels, where none should occur, when used as the pollen parent in crosses with $\underline{a}_1$ $\underline{sh}_2$ dt testers. This strain was designated as the high loss strain. Although self or sib contamination resulting in kernels with the recessive maternal $\underline{a}_1$ $\underline{sh}_2$ phenotype could occur, the exceptional $\underline{a}_1$ $\underline{sh}_2$ kernels clearly did not so arise since they were dotted as a consequence of possessing the Dt gene present in the pollen parent and absent in the female.

When different plants of the high loss strain were used as the pollen parent in crosses with $\underline{a}_1$ testers, marked differences were found in the percentages of exceptional kernels. A good example is given in the 1966 News Letter where plant 27342-19 produced 7.8% of wholly colorless kernels while a full sib plant 27342-27 gave 0.1% of colorless kernels. There was no difference in the percentage of fractional (mosaic) kernels in the progeny from the two sib plants. It was concluded that sister plants differed in their ability to induce loss of the A allele in the sperm but the basis of this difference remained conjectural. It was known from previous studies that family 27342 carried B chromosomes, known to undergo nondisjunction at the second microspore division. The loss of chromosome 3 markers in sperm cells appeared to be somewhat like the behavior of the B chromosomes or the B^A translocation chromosomes of Roman and it seemed possible that the aberrant behavior of a member of the A set of chromosomes might be causally related to the presence of B chromosomes. Therefore, a duplicate planting of family 27342 was made in the summer of 1966 as family 28032. Plants from a sib ear comprise family 28033. Individual plants of these two families, which were homozygous for the A allele, were sporocyted and tested as the pollen parent for loss of chromosome 3 markers and to a more limited extent for chromosome 9 markers. The number of B chromosomes carried by individual plants and the rate of loss of the dominant A allele in the pollen grains are given below:

28032			28033		
No. of B's	% Loss of A in endosperm	Population	No. of B's	% Loss of A in endosperm	Population
3	0.9	1832	1	0.7	304
7	7.7	878	2	1.1	530
7	9.0	1528	4	14.9	1037
7	13.8	1031	4	10.2	1519
7	6.5	480	6	19.9	201
8	8.4	107			
8	8.8	1503			
11	7.4	243			

In both families, those plants with fewest B chromosomes had the lowest rate of loss of the A allele. The correlation between number of B chromosomes and marker loss is consistent and can hardly be fortuitous. Tests of loss rate for the A locus in chromosome 3 and for the C and Wx loci in chromosome 9 were made for several plants. Plant 28032-7 with 7 B chromosomes had 7.7% loss of A and 0.6% loss of C and Wx (which were coincident); plant 28032-20 with 7 B's gave 13.8% A loss and 2.1% C Wx loss; plant 28032-23 with 3 B's had 0.9% A loss and 0.0% loss of C and Wx. The exceptional c wx kernels have not been checked for contamination. Silks on the c wx testers were difficult to bag and some of the c wx kernels doubtless came from self or sib contamination, so the percentage of c wx kernels due to marker loss in the sperm may well be lower than the observed percentage of c wx kernels. A possible clue to the higher rate of loss for chromosome 3 markers and the lower rate for chromosome 9 markers came from the cytological observations at pachynema. In all examined plants, chromosome 3 was homozygous for a large knob in the long arm at position 0.6 while chromosome 9 had a small terminal knob on the short arms. No other conspicuous knobs were present on the remaining A chromosomes of family 28032 or 28033 except for one medium sized knob found in a heterozygous condition on either chromosome 2 or 5. The interaction of knobs and loss will be developed later.

Plant 27240-27, of A Sh/A Sh, Dt Dt, Pr Pr constitution, was used as the pollen parent in crosses with two a sh dt pr individuals. The resulting kernels on both ears consisted of the expected A Sh class and of those which were wholly colorless and shrunken. The presence of dots on the colorless kernels proved that they had not come from self contamination. One ear had 170 A Sh and 36 a sh Dt kernels. All kernels were planted in the field in the summer of 1966 and the ensuing plants testcrossed. In the family coming from the 170 A Sh kernels there were 115 normal appearing plants with no pollen abortion. When used in testcrosses as the

female parent, they gave 1:1 ratios for A and Sh. In addition to the 115 normal F_1 plants there were 25 individuals of reduced height and vigor. All 25 had high pollen abortion (approximately 50%). Thirteen of these 25 partially sterile plants were successfully testcrossed as female parents. The progeny of 12 consisted entirely of a sh kernels borne on semi-sterile ears. They were monosomic for all or part of chromosome 3. One plant with semi-sterile pollen and ovules segregated 1:1 for A Sh and a sh. This individual was presumed to be deficient for a chromosome other than 3. Two plants of normal stature arising from colored seed had pollen with a great range in size. These were triploids of A Sh/A Sh/a sh genotype. The occurrence of two triploids in a small population is somewhat unusual but more surprising is the fact that the pollen parent contributed the diploid number of chromosomes. Triploidy is believed in general to result from the union of an unreduced egg with a haploid sperm. The finding of two triploids where either a diploid sperm or two haploid sperm fertilized the egg suggests that the mechanism producing chromosome loss is also responsible for these unexpected triploids.

Fifteen mature plants came from the 36 a sh Dt kernels. Thirteen were vigorous plants with normal pollen but two were shorter in height and had ca. 50% pollen abortion. Ten testcrossed ears on plants with no aborted pollen had no ovule abortion and gave 1:1 segregations for A and Sh--i.e., they were disomic for chromosome 3. The embryos of these ten plants coming from the exceptional a sh kernels arose by fertilization of an egg with a sperm cell having one chromosome 3, while the sperm cell fertilizing the polar nuclei to form the exceptional a sh endosperms lacked the A and Sh markers on chromosome 3. One testcrossed plant with no pollen abortion gave an ear with 233 A Sh: 1 a Sh: 63 a sh kernels. These are the proportions expected from a trisomic plant with two of the three chromosomes carrying the dominant alleles. Evidently the sperm cell fertilizing the egg possessed two chromosomes 3, each with the A and Sh alleles, to produce a trisomic for the A Sh segment while the other sperm uniting with the polars was deficient for these markers. This is the consequence of nondisjunction at the second microspore mitosis.

The two plants from a sh Dt kernels which had semi-sterile pollen segregated 1:1 for A Sh and a sh. On one ear all of the A kernels were homozygous for the recessive pr allele on chromosome 5 although all other F_1 plants were heterozygous for Pr and pr. The a sh stock used in the testcross was pr pr. It appears that the plant giving only pr kernels in the colored class was hemizygous for the long arm of chromosome 5. This is one of the chromosomes that might carry a medium sized knob. Inasmuch as sporocytes were not taken from the two semi-sterile plants it is not possible to identify the deficient chromosome in the second plant mentioned above. However, it was neither chromosome 3 nor 5.

Thirty-six or 17.5% of the 206 kernels on the ear from plant 27240-27 had endosperms of a sh Dt phenotype produced by union with a sperm deficient for the dominant A and Sh alleles. This percentage represents the frequency of loss of the A and Sh loci from the sperm cell which united with the polars to form the endosperm. The frequency with which the

deficient sperm fertilized the egg to give a sporophyte deficient for chromosome 3 can be calculated from the above data; it comes to 14.6%, a value similar to the frequency of deficient endosperms. Judging by this small sample, selective fertilization of the egg by the non-deficient sperm does not occur as it does for the B and B^A chromosomes.

If we disregard for the moment the possibility of heterofertilization, the constitution of the second sperm cell in those pollen grains with one deficient sperm can be determined from the genotype of the embryo derived from the exceptional a sh kernels. There is in our material no way of ascertaining the genotype of the endosperm of those kernels with a deficient embryo; it can only be inferred. Non-identical sperm are produced by some mishap at the second microspore division as Roman showed for the translocated B^A chromosomes. Non-correspondence of the male contributions to embryo and to endosperm has been found in every exceptional kernel in the high loss studies that has been analyzed. The dissimilarity between the two sperm clearly arises from some event at the second microspore mitosis. To ascribe this dissimilarity to heterofertilization would require that it occurs in kernels which had lost the A allele 100% of the time and tests to date give no indication of an unusually high frequency of heterofertilization in related A/a male parents. In any event, heterofertilization does not appear to be of major importance and its occasional occurrence does not vitiate the conclusion that there is some unusual behavior at the second spore division and that unlike sperm are the consequence. If nondisjunction is responsible for dissimilar sperm, those kernels with colorless endosperms coming from the cross of a a x A A should have embryos with two chromosomes 3 contributed by the male parent; they would be A A a and, upon testcrossing, typical trisomic ratios should result. Embryo genotypes were determined by testcrosses of the ensuing sporophytes from 64 kernels with deficient (colorless) endosperms produced in the cross of a a ♀ x A A ♂ plants of the high loss line. Twelve individuals gave a ratio of dominant to recessive phenotypes approximating that expected from duplex trisomics while the remaining 52 gave the 1:1 ratio characteristic of the disomic condition. At this stage in the analysis it could be concluded that in about 20% of the mitoses where a sperm deficient for the marker gene A is produced, the sister sperm acquired two chromosomes 3 by nondisjunction. Much more frequently, however, a deficiency for the A gene in one sperm is not accompanied by a disomic condition for A in the sister.

Since A and Sh are near the end of the long arm of chromosome 3, their loss from either embryo or endosperm could mean no more than that the distal portion of the long arm is missing. Crosses were therefore made in which Gl_6 Lg_2 A_1 pollen from high loss plants was placed on silks of gl lg a plants. Since the Gl_6 locus is close to the centromere these three mutant loci afford excellent markers for nearly all of the long arm of 3. Loss of all three markers proved to be coincident in hypoploid plants which came from colored kernels. The colorless kernels gave only Gl Lg A seedlings. Cytological studies of somatic prophases have been made of a number of the exceptional gl lg a plants. Twenty-seven had 19 A chromosomes plus a telocentric A fragment in addition to varying numbers of B chromosomes. The size of this fragment,

which was apparently the same in all 27 plants, suggested that it might consist of the short arm of chromosome 3. Indeed, meiotic studies at diakinesis made from one greenhouse-grown exceptional gl lg a plant with 19 A and one fragment chromosomes disclosed a heteromorphic pair consisting of one normal chromosome and a telocentric short arm. Pachytene figures were poor so positive identification of chromosome 3 as the heteromorphic pair could not be made. However, the arm ratio and length of the heteromorphic pair strongly suggest the involvement of chromosome 3. Five of the exceptional gl lg a plants had 19 A chromosomes and no A fragment. In these instances only one chromosome 3 was present. Five of the gl lg a seedlings arising from kernels with colored aleurone apparently had 20 A chromosomes. The origin and chromosomal constitution of this unanticipated class remain to be elucidated. Meiotic studies should be revealing. An unequivocal distinction between A and B chromosomes can be made in somatic prophases when the number of B's is low but it proved more difficult when the B's were increased in number.

The cytological studies are admittedly incomplete and will be extended but the evidence at hand suggests that sperm may be deficient for all of chromosome 3 but more often is deficient for only the long arm. It is uncertain whether or not those plants giving trisomic ratios have three entire chromosomes 3 or if they possess only two and the third is a telocentric consisting of the long arm. The expected genetic ratio from a primary trisomic with two dominant and one recessive alleles might not differ significantly from that expected where the extra chromosome is a telocentric.

Analysis of the progeny of plant 27240-27 as the male parent disclosed that endosperms and embryos deficient for the A Sh alleles occurred with approximately equal frequencies. Furthermore, the great majority of F_1 sporophytes with pollen and ovule semi-sterility appear to be hypoploid for all or part of chromosome 3. If the other nine chromosomes of the haploid complement underwent the same rate of loss as did chromosome 3, there would be few if any F_1 sporophytes with the normal complement of 20 A chromosomes. However, more than 80% were euploids. The argument is advanced that chromosome 3 is subject to loss at the second microspore mitosis in plants with high numbers of B chromosomes because it carries a large knob in the long arm. If, so the argument runs, it were knobless there would be little or no loss. There is a marked reduction in the frequency of loss for chromosome 3 when the number of B's is below a certain level even though it is knob-bearing. Knobless chromosomes should undergo little loss irrespective of the number of B's. The available evidence is in accord with this hypothesis. As stated earlier, cytological examination at pachynema of high loss plants reveals that only chromosome 3 is homozygous for a large knob, that both chromosomes 9 have a small terminal knob on the short arm, and that a medium sized knob is present in a heterozygous condition on either chromosome 2 or 5. All other chromosomes were knobless and stable. Marker genes on chromosome 9 with its small knob are lost much less frequently than are markers on chromosome 3 with a much larger knob. Apparently knob size plays a significant role in determining rate of loss in plants with supernumerary B chromosomes.

Data from the following experiment are readily interpretable on the above hypothesis. A high loss plant of K A Sh consititution (K = knob; k = knobless) was used as the male parent in a cross with a k a Sh individual. The F_1 plants of K A Sh/k a Sh genotype were used as the pollen parents in testcrosses with a sh testers. The resulting progeny consisted of 416 A Sh: 448 a Sh: 35 a sh kernels. Sperm deficient for either the K A Sh or k a Sh segment would account for the exceptional a sh kernels. Elimination of all or part of the K A Sh chromosome from one pole at the second spore division results in one deficient sperm and one with the K A Sh chromosome. Fertilization of the two polar nuclei by the deficient sperm gives an endosperm that is colorless and shrunken while fusion of the non-deficient sperm with the egg produces an embryo with the K A Sh chromosome. The scutella of these embryos are colored since the necessary complementary factors for scutellum color were present. On the other hand, elimination of the k a Sh loci would yield kernels with a sh endosperms and embryos with the k a Sh chromosome. Such embryos would have colorless scutella. Twenty-seven of the 35 kernels with a sh endosperms had colored and eight, or 23%, had colorless scutella. If there was no recombination between K and the A locus, the data could be interpreted to indicate that the K A Sh segment is lost three times as frequently as is the k a Sh segment. However, a more likely explanation of the origin of the eight a sh kernels with colorless scutella is loss of all or a portion of a K a Sh chromosome derived by crossing over between K and the A locus. The 25% recombination between K and A expected to give K a Sh strands is very close to the 23% of a sh kernels with colorless scutella. That it is the knobbed chromosome 3 which undergoes loss and not the knobless one can and will be determined by cytological examination of the chromosomes 3 at pachynema in plants coming from kernels with a sh endosperms and colorless scutella. If they possess a knob on chromosome 3, then it follows that only the knobbed homologue is subject to elimination.

The above account is believed to shed light on a number of established facts which hitherto have had no rational explanation. Longley in his survey of knob number and location in diverse strains of maize found a negative correlation between knob number and the frequency of B chromosomes. Further, Randolph reported that plants with high numbers of B's were of reduced stature and highly sterile. If, as our data indicate, frequent loss of knobbed chromosome arms with consequent sterility takes place in plants where the number of B chromosomes is above a critical level, it follows that there would be strong selection against any strain of maize having an appreciable number of both knobbed A chromosomes and supernumerary B chromosomes.

Obviously many tests remain to be done. A low loss strain can be converted to a high loss by selecting for increased numbers of B's, providing that some A chromosomes are knobbed. Conversely, in the selfed progeny of a high loss plant individuals with lowered numbers of B's should occur; these should be in the low loss category. Crosses which combine a high knobbed strain with a knobless one carrying many B's should produce a high loss strain, etc. The cytological mechanism leading to the partial loss and elimination of knobbed A chromosomes remains

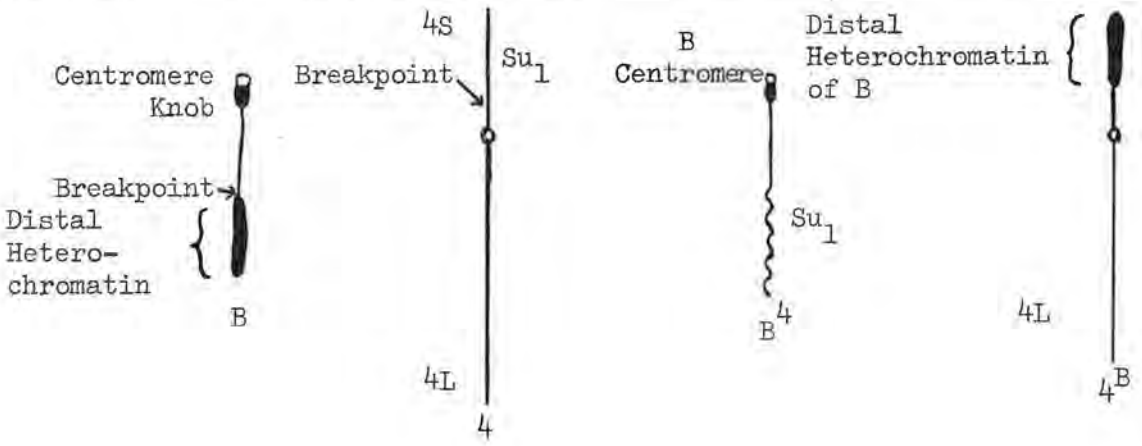
to be determined. Some kind of interaction between the heterochromatin of knobs and that of B chromosomes which leads to loss of all or part of the knob-bearing A chromosome would appear to be likely. Unfortunately, the second spore division occurs at a stage when the cytoplasm is full of starch grains and it is not a favorable stage to observe. It may also be difficult to distinguish between B chromosomes which undergo nondisjunction at this time and aberrantly behaving A chromosomes.

M. M. Rhoades
Ellen Dempsey
Achille Ghidoni

2. An interaction of B chromosomes and abnormal 10.

Y. C. Ting has proposed that abnormal chromosome 10 was derived from a normal 10 and a B chromosome. (Chromosoma: 9:286). Since then, attempts have been made to determine whether homology exists between B chromosomes and the extra chromatin of abnormal 10. Rhoades and Dempsey looked for pairing in pachytene between a single B and abnormal 10, as an indication of homology. Little if any pairing was found. (MNL:33:58). Ting found some association between B's and abnormal 10, which, however, also occurred between abnormal 10 and other heterochromatic knobs. (MNL:33:37). Even if we assume that pairing between abnormal 10 and B's is a rare event, some homology between the two cannot be ruled out. It is possible that some rearrangement in abnormal 10 has occurred since its hypothetical origin from a B chromosome. So a different approach to the problem was used.

It was first determined by Roman, and since confirmed by other workers, that the distal heterochromatin of the B chromosome is responsible for the nondisjunction of the B centromere region at the 2nd microspore division. Roman used the B-4a translocation.



Since the B^4 chromosome undergoes nondisjunction and the 4^B chromosome does not, the B centromere (or an adjacent region) is the site of nondisjunction. However, nondisjunction of the B^4 chromosome occurs only in the presence of the 4^B chromosome. Microspores that contain a normal chromosome 4 plus a B^4 show little, if any, nondisjunction of the B^4 chromosome. (The 4 + B^4 sperm, while unbalanced, does function often enough to be tested.) The same result has been found in other B translocations. Apparently the 4^B chromosome is required for nondisjunction because it contains the distal heterochromatin of the B chromosome.

If abnormal 10 arose from an interchange between a B chromosome and a normal 10, it may contain some or all of the distal heterochromatin of the B chromosome, including the region responsible for nondisjunction of the B centromere. As previously mentioned, microsporocytes that contain a normal chromosome 4 plus a B^4 do not show nondisjunction of the B^4 chromosome. The addition of abnormal 10 to such a microsporocyte will test whether or not abnormal 10 contains the region responsible for nondisjunction. This was done, but, for reasons of convenience, the translocation used was B-9b rather than B-4a. The break point in chromosome 9 is in the short arm, between sh and wx. The break point in the B chromosome is a short distance into the distal heterochromatin, but apparently does not include the region controlling nondisjunction. Plants were produced that contained two normal chromosomes 9 plus a B^9. One half of the plants were expected to be heterozygous for abnormal 10 and one half should have normal chromosomes 10. The presence of abnormal 10 was determined by examination of sporocytes. The plants were crossed as males onto a c sh wx tester:

$$\underline{c\ sh\ wx} \times \frac{\underline{c\ sh\ wx}}{9} \quad \frac{\underline{c\ sh\ wx}}{9} \quad \frac{\underline{C\ Sh}}{B^9}$$

The B^9 chromosome carries the dominants for C and Sh. Colored, full seeds produced in the cross indicate the presence of the B^9 chromosome in the endosperm. If nondisjunction has occurred, there should be two B^9's in the endosperm and none in the embryo. If it has not occurred, there should be one B^9 in each. The C Sh seeds were grown and the resulting plants used as female parents in backcrosses to a c sh wx tester. The occurrence of C Sh seeds in the backcross progeny at a rate of about 30% demonstrates the presence of one B^9 in the female parent. (The rate does not reach 50%, apparently because of poor pairing with the normal chromosomes 9 and lagging at anaphase. Some of the plants from C Sh kernels do give 50% C Sh when backcrossed. These plants contain a cross-over chromosome 9 carrying C Sh wx.) The absence of C Sh on the backcrossed ear indicates that nondisjunction has occurred. (This could also result occasionally from heterofertilization, rather than nondisjunction.) Results of the backcrosses are given below:

Male Parent		Constitution of Plants from C Sh Kernels		
$\frac{c\ sh\ wx}{9}$	$\frac{c\ sh\ wx}{9}\ B^{9}\frac{C\ Sh}{}$	No B^9	One B^9	Crossover
Normal 10				
202-3		1	14	1
202-28		1	30	2
203-2		1	9	3
203-3		3	17	2
203-17		0	32	3
	Totals	6	102	11

% Nondisjunction = 6/108 = 5.6%

Abnormal 10	No B^9	One B^9	Crossover
202-23	0	19	2
202-27	2	66	8
203-1	0	18	5
203-8	2	10	6
203-9	1	51	12
203-16	0	9	5
Totals	5	173	38

% Nondisjunction = 5/178 = 2.8%

The results show that abnormal 10 cannot replace the distal heterochromatin of the B in producing large scale nondisjunction.

However, it was still considered possible that abnormal 10 might increase the amount of nondisjunction occurring in the presence of the distal heterochromatin. For this reason, the effect of abnormal 10 on nondisjunction of B^9 was tested in the presence of the 9^B chromosome. In the presence of 9^B, nondisjunction should occur at a relatively high rate. The plants used were heterozygous for the translocation, and segregated abnormal 10 in a 1:1 ratio. Markers present are shown below:

$$9\frac{c\ sh\ wx}{} + 9^{B}\frac{Wx}{} + B^{9}\frac{c\ Sh}{}$$

The following classes of spores will be found after meiosis:

1. $9\frac{c\ sh\ wx}{}$
2. $9\frac{c\ sh\ wx}{} + B^{9}\frac{c\ Sh}{}$
3. $9^{B}\frac{Wx}{}$
4. $9^{B}\frac{Wx}{} + B^{9}\frac{c\ Sh}{}$

The 9^B class will abort. The two classes containing a normal 9 will be waxy. If $\frac{Wx}{}$ seeds are selected following the cross onto a $\frac{wx}{}$ tester, only the $9^B + B^9$ class will be represented. Crossing over between $\frac{Wx}{}$

and the break point is negligible. (Bianchi estimates it at 0.2% in MNL 40:75). The nondisjunction of the B^9 can, therefore, be measured in the presence of 9^B, without the need for a homozygous translocation. In this experiment, sporocytes from 22 plants heterozygous for TB-9b were examined for the presence or absence of abnormal 10. Each plant was used as a male parent onto 4-6 ears of a sh bz wx B Pl tester. Wx seeds were selected from the ears. Wx kernels which are also bronze result from nondisjunction of the B^9, with the endosperm being deficient for B^9 and the embryo carrying two doses. Purple kernels which give rise to bronze plants also indicate that nondisjunction has taken place, with the embryo, in this case, being deficient. Classification of seedlings has not yet been attempted, however, and only the one type of nondisjunction (bronze seeds) has been scored. The following table gives the frequency of this type of nondisjunction. There is good reason to believe that, when the total rate of nondisjunction is determined, the relative differences between the normal 10 and abnormal 10 groups will remain the same (see Catcheside Heredity 10:345).

Normal 10 Group			Abnormal 10 Group		
Male parent	Total Wx	% bronze	Male parent	Total Wx	% bronze
771-1	509	46.6%	771-7	710	23.5%
-3	825	43.0	-8	524	30.0
-4	935	56.6	-10	679	20.0
-15	791	52.9	-11	1176	3.4
-17	779	38.5	-14	623	49.0
-21	634	35.2	-20	1134	32.5
-22	579	55.0	-36	1174	25.5
-23	761	63.3	-39	827	33.0
-24	758	49.0	-41	928	54.9
-25	610	66.0		Av.	30.2%
-32	605	66.0			
-33	507	63.0			
-34	647	47.0			
	Av.	52.5%			

It is obvious from these results that abnormal 10 does not increase the rate of nondisjunction of the B^9. In fact, the rate is decreased in plants with abnormal 10. While the lowest member of the normal 10 group shows 35.2% nondisjunction, 7 of the 9 members of the abnormal 10 group fall below this level of nondisjunction, with one abnormal 10 plant having a rate of only 3.4%. It is possible that the difference here is not due to abnormal 10, but to a factor linked to it on chromosome 10. It is also possible that the presence of abnormal 10 changed the time of flowering of these plants slightly, and thereby changed the environmental condition at the time of nondisjunction. Bianchi has reported an apparent environmental effect on nondisjunction (Z. f. Vererbungslehre 92:213).

If abnormal 10 has the ability to reduce the rate of nondisjunction of a B chromosome, an explanation may be advanced for Longley's data on distribution of B chromosomes in different maize stocks. (J. Agric. Res. 56:177). Longley found that B chromosomes were seldom present in stocks with large numbers of heterochromatic knobs. If knobs in general, and not just abnormal 10, are capable of reducing the rate of nondisjunction among B chromosomes, they would tend to eliminate B chromosomes from the stock. Nondisjunction of B chromosomes together with preferential fertilization of the egg by the hyperploid sperm increases the B chromosome number in a population. In the absence of nondisjunction, it seems likely that a lack of selective advantage and irregularities in segregation at meiosis would result in the elimination of B's. The effect of other knobs on the rate of nondisjunction in TB-9b will be tested this summer. There is no evidence yet that knobs other than abnormal 10 can affect nondisjunction.

Wayne Carlson

3. Further study of the transmission of B^4 derived from the TB-4a standard stock.

In last year's News Letter (1966) preliminary data on the transmission of the supernumerary B^4 in normal genotypes were reported. A more intensive study of its genetic behavior is underway. Crosses were made using hyperploid plants ($4,4,B^4$) homozygous for Su as the male parent on a su_1 tester in order to investigate the transmission of B^4 together with chromosome 4 through the male gametophyte. In addition, the ability of such hyperploid gametes to fertilize the egg, as well as the nondisjunction rate of B^4 in the absence of 4^B at the 2nd microspore division, was studied.

The F_1, heterozygous Su/su and expected occasionally to be hyperploid for B^4, was backcrossed as female parent to the su_1 tester in order to recover the hyperploid plants. Three hundred thirty ears were scored for the ratio Su:su. The presence of B^4 would be indicated by an excess of ears showing a significant deviation from the expected 1:1 ratio (with a majority of Su kernels) over those deviating by chance. Also, 19 ears obtained by selfing were scored for the ratio 3:1. A total of 108,273 kernels were classified.

In Table 1 the P value corresponds to the X^2 deviation calculated on each of the ears examined. These results show a considerable excess of ears deviating from the expected ratios. The 6 ears deviating from the 1:1 ratio in the opposite direction (towards su) give an idea of the deviation by pure chance. In the backcrosses, among the significantly deviating ears, variations of ratios from 1.88:1 to 1.30:1 are presumably due to different rates of loss of the single B^4 in female meiosis or in the later stages of embryo sac development. These variations could be partially ascribed to the different backgrounds originally involved. The few data available from the ears obtained by selfing confirm those available from backcrosses. Here pollen transmission of B^4 occurs in addition to the main source of transmission through the female gametogenesis, but at a low rate since the B^4-hyperploid pollen is competitively selected

Table 1

P values corresponding to the X^2 values calculated	No. of ears scored for the following segregations: 1 : 1	>1Su: 1su	<1Su: 1su	Total	Average ratio Su : su (weighted values)	Average excess % of Su kernels
Backcross:						
P<0.001 (***)		28	0	28	1.88 : 1	46.7
P<0.01 (**)		11	2	13	1.46 : 1	31.5
P<0.05 (*)		9	4	13	1.30 : 1	23.0
P<0.05 (n.s.)	276			276	1.00 : 1	0.0
Total	276	48	6	330		
Self:	3 : 1	>3Su: 1su	<3Su: 1su	Total		
P<0.001 (***)		2	0	2	5.68 : 1	42.3
P<0.01 (**)		1	0	1	6.20 : 1	51.6
P<0.05 (*)		0	0	0		
P<0.05 (n.s.)	16			16	3.00 : 1	0.0
Total	16	3	0	19		

against in the presence of normal pollen. Nondisjunction of B^4 in the 2nd microspore division, when hyperploid plants (4, 4, B^4) were crossed as male parent to $\underline{su}_1$, would have resulted sometimes in tetrasomic plants heterozygous for $\underline{su}$ ($4\underline{Su}$, $4\underline{su}$, $B^4\underline{Su}$, $B^4\underline{Su}$). These should segregate $\underline{su}$ at a very low rate in backcrosses, since in this double condition B^4 should be lost only at a low frequency. None of the 330 ears examined showed such a ratio. This does not mean absence of nondisjunction(according to Roman, 1949), since such tetrasomic plants, obtained from selfing hyperploid (4, 4, B^4) genotypes, have a very low vigor and could have been lost.

In most of the backcrossed ears the excess of $\underline{Su}$ kernels was expected to be due to the presence of B^4 carrying the dominant $\underline{Su}$. Root tips from $\underline{Su}$ kernels were squashed with the standard Feulgen technique in order to score for the presence of B^4. This was found at a frequency closely corresponding to the excess of $\underline{Su}$ kernels:

Sampled progeny from a back cross of $4\underline{Su}$, $4\underline{su}$, $B^4\underline{Su}$ plants

	20 chromosomes	21 chromosomes	Total
Plants from $\underline{Su}$ kernels	13	8	21

The supernumerary chromosome observed in metaphase plates was a short telocentric chromosome, as expected for the B^4.

After selfing hyperploid plants (4, 4, B^4) the following three genotypes were expected:

1. = 20 chromosomes
2. = 20 chromosomes + B^4
3. = 20 chromosomes + B^4 + B^4

} The relative ratio of these three genotypes should give a preliminary idea about the transmissibility of B^4.

Microsporocyte samples were taken from 88 plants of such a progeny: 67 plants had 20 chromosomes and 21 plants had, in addition, one B^4; no plant was found having two B^4's (see 1966 M.N.L.)

After crossing the hyperploid plants found to $\underline{su}_1$ as mentioned above, 19 F_1 plants were selfed. Those heterozygous $\underline{Su}/\underline{su}_1$ and having in addition $B^4\underline{Su}_1$ gave rise to ratios near a 6:1. A sample of $\underline{Su}$ kernels has been planted, and root tips were scored from each of them.

Sampled progeny from self pollination of 4^{Su}, 4^{su}, B^{4Su} plants

	20 chromosomes	21 chromosomes	22 chromosomes	Total
Plants from Su kernels	67	47	8	121

Most attention was drawn to the few plants with two B^4's. Nevertheless, because of their very low vigor (presumably due to the unbalanced genotype) it was not possible to make either self or sib pollinations. However, there was good pollen shedding, and a testcross was made:

(4^{su}, 4^{su}, B^{4Su}, B^{4Su}) ♂ x su_1 tester ♀

Two ears obtained gave rise to these data:

Ear no.	Su kernels	su kernels	Total	% su
1	113	19	132	14.4
2	161	15	176	8.5

The few su kernels are due to loss of B^{4Su} when present in double condition in the plant. Scoring root tips from such kernels for the presence of B^4 will establish the rate of nondisjunction at the 2nd microspore division.

Most of the plants with one B^4 were selfed or backcrossed to su_1. Since the plants could be either homo- or heterozygous at the su locus, different ratios were expected:

genotypes:	Progeny after selfing
4^{Su}, 4^{Su}, + B^{4Su}	true breeding Su
4^{Su}, 4^{su}, + B^{4Su}	segregating >3 Su : 1 su
4^{su}, 4^{su}, + B^{4Su}	showing a low % of Su

The type true breeding for Su was actually found, and its recovery did not need a special attention.

The heterozygote expected to segregate in its progeny more than 3 Su to 1 su was examined in the following terms:

Ear no.	Su kernels	su kernels	Total	Ratio
1	164	23	187	7.14:1
2	136	19	155	7.16:1
3	305	88	393	3.47:1
4	352	66	418	5.34:1
5	211	33	244	6.43:1
6	226	36	262	6.28:1
7	398	62	460	6.38:1
Total:				
7	1792	327	2,119	6.00:1 (aver. ratio)

The genotype expected to show a low percentage of Su kernels among the progeny was examined in these terms:

Ear no.	Su kernels	su kernels	Total	% Su	Ratio
1	130	183	313	41.6	0.71:1
2	157	221	378	41.6	0.71:1
3	153	201	354	43.2	0.76:1
4	112	215	327	34.3	0.50:1
5	115	238	353	32.6	0.49:1
6	90	157	247	36.5	0.57:1
7	115	202	317	36.4	0.56:1
8	107	269	376	28.5	0.40:1
Total:					
8	979	1,686	2,665	36.8 (aver. %)	0.60:1 (aver. ratio)

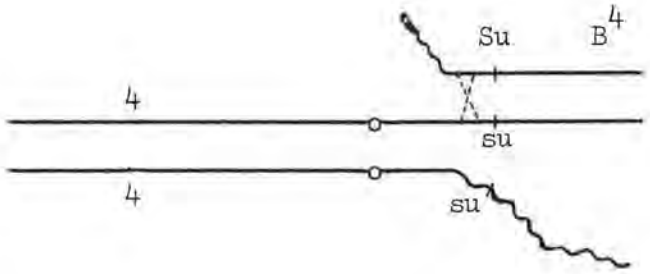
Data on backcrosses are not yet available.
Su kernels taken from ears of the class segregating on the average 0.60:1 should have one of these genotypes:

su su + B^{4Su}

su su + B^{4Su} + B^{4Su}

Su su (+ B^{4Su})

The third class with B^4 not necessarily present, should be rare and due to occasional crossing over involving the segment of the B^4 from the su_1 locus to the breakage point and the homologous region of the chromosome 4 as indicated below:



Fifty-six Su kernels were scored: 54 were found to have 21 chromosomes (presence of B^4) and 1 was found to have 20 chromosomes (no B^4). This could be the product of a rare crossover since in B^4 the breakage point is very close to su_1 (no more precise specifications have been reported to the author's knowledge); moreover, the pairing between specific segments of 4 and B^4, required for a crossover, occurs infrequently. However, the plant with 20 chromosomes could be a contaminant that was not detected since the stock is not provided with other markers. Another product of crossing over is a B^4Su; this can only be detected cytologically among the larger class of su kernels. One plant in this sample was found to have 22 chromosomes (two B^4's); this indicates very low transmission of B^4 through the pollen.

Testcrosses were made of these genotypes:

(su su B^4Su) ♀ x su_1 tester ♂ and reciprocals

in order to establish the transmissibility of B^4 through the meiotic barrier (the B^4 being single) and through the male gametophyte barrier (the B^4-hyperploid pollen grains being selectively disadvantaged when in competition with the normal ones). Data are not yet available, the material being in storage. Endeavors are being made to identify as many plants as possible with two B^4's in order to compare the kernel weight of classes having in the endosperm 0 B^4, one B^4, two B^4's, three B^4's and four B^4's. In the F_1 derived from pollinating inbred lines and hybrids with the TB-4a stock, the hyperploid endosperms were proven to be heavier (Bianchi, Genetics, 1962; Bianchi, Bellini and Ottaviano, Z. Vererb., 1962; Bellini, Ottaviano and Ghidoni, M.N.L., 1961).

Some conclusions can be drawn from the study of B^4 as a supernumerary in normal genotypes:

1. When B^4 is a single supernumerary it suffers severe losses during meiosis; and, when it escapes the meiotic barrier, such hyperploid

pollen is selected against because of competition with normal pollen (Ghidoni, Atti A.G.I., 1965; and M.N.L., 1966).

2. Hyperploidy for the B^4 results in poor growth of the plant which becomes more pronounced with increased doses.

3. When two homologues B^4 are present the transmission is still not ensured to all gametes; some meiotic losses are possible as well as a low rate of non-disjunction either during meiosis or in the microspore divisions. This seems to preclude the possibility of fixing lines with such specific hyperploidy, since this attempt of altering permanently the genotype results in a deleterious unbalance of genetic factors.

Achille Ghidoni

4. An improved method for detecting monoploids from different inbred lines.

A considerable number of methods have been used to screen for maternal monoploids. Most of them were suggested by S. S. Chase (PNAS, 1947 and Agr. J., 1952) and successfully carried out by E. H. Coe, Jr. (J. Her., 1964).

Recently A. Ghidoni and E. Ottaviano (Genetica Agraria, Proc. 1966) suggested (as did R. W. Briggs independently in the M.N.L. 1966) that the colored scutellum stocks, i.e., homozygous for $A_1 A_2 C R$ and the scutellum color markers (S_1, either two of the $S_2 S_3 S_4$ series, and the recessive s_5), can be used as male parent to pollinate several colorless inbred lines used as female parents. Monoploids can obviously be detected in those lines not carrying C^I, and carrying the recessive s_5. It should be easy to recognize in which lines these two conditions are satisfied, since:

1. the lines carrying C^I, pollinated as mentioned above, should have the F_1 fully colorless (endosperm and embryo);

2. the lines carrying S_5 (dominant) should have, in the absence of C^I, F_1 seeds with colorless scutellum only.

These two categories can be easily recognized and therefore discarded. Those showing both endosperm and scutellum color are to be scored for putative monoploids, which would have colored endosperm and colorless embryo. Crosses were made on 25 colorless inbred lines, but the colored scutellum stock used as the male parent was not homozygous for all factors and therefore no data were available. However, Briggs' data showed the effectiveness of the method.

This method of testing different lines as potential producers of monoploids is similar to those involving in the male parent either the dominant colored plumule factors ($Pu_1 Pu_2$) or the dominant "Purple Embryo Marker" (which is R^{nj} in a proper background) as they were developed by Chase and I. Greenblatt (M.N.L. 1965, 1966 and Crop Sci., 1966). However, it has the advantage that colored scutellum classification is normally less

ambiguous than that of the plumule in dormant seeds; thus, the recognition of monoploids would be made easier since a more restricted class of seeds would need to be certified cytologically or by other means.

The improved method described here consists of combining a stock with both the Purple Embryo Marker and the scutellum color factors. This will be used as male parent to test several inbred lines. The introduction of $\underline{R^{nj}}$ into a colored scutellum stock is easy and would overcome the limitation imposed by $\underline{s}$ which is required by the method of colored scutellum used as the male parent. Also, the presence of scutellum color factors will be of considerable help in detecting monoploids where the Purple Embryo Marker alone could be sometimes ineffective.

As Chase designated the Purple Embryo Marker PEM, it is proposed to call such a combined stock PEMS, with addition of an "S" to indicate the presence of scutellum color factors.

Achille Ghidoni

5. The "diffuse stage" in meiotic prophase I of maize PMC.

A microsporocyte sample taken from a plant of an Indian race of maize from Ecuador was found to be knobless. A more intensive study of this sample showed, after a normal pachytene, a stage in which the chromosomes lost their visible individuality, assuming an interphase-like appearance. A brief diplotene stage was also observed, and a perfectly normal diakinesis followed.

This feature, unusual in maize, seems to be comparable to the so-called "diffuse stage" which is common in female meiosis of many species of animals, mainly insects and mammals. However, it has been reported in a few cases in the Plant Kingdom, namely in Hyacinthus by C. D. Darlington (J. Gen., 1929) and more recently in some species of mosses by F. J. Dill under the name of "dictyotene" (Science, 1964). The "diffuse stage" was also reported in tomato by P. B. Moens (Chromosoma, 1964). According to Moens this stage should precede diplotene rather than follow it.

The phenomenon is still not understood. Poor stainability of the chromosomes (the so-called "achromatic stage") which is sometimes observed, but less intensively, in diplotene of maize PMC, does not seem to account for the phenomenon described.

(The sample, kindly furnished by Dr. Bianchi, was obtained from the personal collection of Dr. A. Brandolini, Como, Italy).

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1. Endosperm mutants induced by a chemical mutagen.

Endosperm mutants have been induced in an inbred line by NES (Neutral Ethyl Sulphate). The treatment was as follows:

1.5 gr/l for 24 hrs. at 24°C.

The mutants were detected and isolated in the second and third generations after treatment.

These mutants were classified in three phenotypic groups: floury, shrunken and sugary. Each group may include different origins, but this is not to be directly related to mutations within the observed individuals. One might have to incriminate the cultivation techniques of the M_1 plants. Because of the induced sterility not all these plants were selfed; however, they were isolated to prevent outcrosses. The occurrence of the same aberrant type in different lines could be explained either by natural crosses between M_1 plants, or, but not necessarily, by an increased mutation rate.

In order to identify the nature of these mutants, a set of crosses were performed: (i) all mutants were intercrossed within each group (diallel) and (ii) all mutants were crossed with a set of 16 U.S. marked lines. Furthermore, a liguleless mutant was crossed with a "liguleless leaf" marker.

Results

Starchy endosperm: Diallel crosses between mutated lines revealed three different mutations in a group of 12 lines tested. Crosses with the "floury endosperm" (fl_1) and "opaque endosperm" (o_2, o_1) marked lines were negative.

Half starchy endosperm: Diallel crosses revealed four different mutations in a group of 7 lines tested. Crosses with the "dull endosperm" (du_1) marker were negative.

Shriveled endosperm: The 3 lines tested have the same mutation, "etched endosperm" (et).

Shrunken endosperm: Two mutants out of the three lines tested were found to be "shrunken endosperm" (sh_2).

Sugary endosperm: The three mutants have the same mutated factor, "sugary endosperm" (su_1).

The liguleless leaf mutant is due to a mutation at the lg_1 site.

The above results are summarized in the following table:

Table 1

Mutant types	Tester	Results
Starchy endosperm (3 tested mutants)	fl_1	-
	o_1	-
	o_2	-
Half starchy endosperm (4 tested mutants)	du_1	-
Shriveled endosperm	et	+
Shrunken endosperm	sh_1	-
	sh_2	+
Sugary endosperm	su_1	+
	su_2	-
Liguleless	lg_1	+

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1. The geographic distribution of the fl^a gene (floury recessive in two doses) in the Paraguay Republic.

The fl^a gene is an allele of fl_1 but differes from fl_1 in that it is recessive in two doses (Mazoti, 1940, Anales del Instituto Fit. de St. Catalina 2:17-26). This fl^a gene has a wide geographic distribution in Paraguay, integrating with the genotype of the floury variety of corn with yellow aleurone designated "Blanco."

The experimental results are as follows:

Floury $\frac{fl}{fl}$ x floury "Blanco" = all floury

(Floury fl/fl x floury "Blanco") F_2 = all floury
(Floury fl/fl x floury "Blanco") x Flint = Table 1

Table 1
fl/fl^a (female) x Flint

Progeny	Floury	Flint
1	111	137
2	90	84
3	99	96
4	95	102
Total	395	419

It would be interesting to compare the percentage of lysine in fl and fl^a in order to be able to establish a possible case of genic action by intrachromosomal duplication. (fl^a duplicate = fl)?

Luis B. Mazoti

2. Further studies on the effects of the paramutagenic gene c^{IP}.

In 1966 (MNL 40:62) I described a new paramutagenic gene which is very stable, has normal viability and is localized at the locus C. This new paramutagenic gene c^{IP} produces in its alleles the mutational sequences: $C^i \rightarrow c^i$ and $c^i \rightarrow c^I$.

Further studies show that:

a. The mutation rate of C^i to c^{im} (m = mutation) due to the paramutagenic gene c^{IP} is 33%. This mutation rate is homogeneous in various progenies, and does not produce mosaicism phenomena (Table 1).

Table 1

Results of the cross: $\underline{c}^{IP}/\underline{C}^{i}$ x $\underline{c}^{i}/\underline{c}^{i}$, with $\underline{C}^{i}$ to $\underline{c}^{i}$ mutation

Progeny	Colorless aleurone	Colored aleurone	Ratio
1	219	116	1.88 : 1
2	98	47	2.08 : 1
3	234	117	2.01 : 1
4	276	123	2.19 : 1
5	266	116	2.29 : 1
6	128	54	2.37 : 1
7	256	123	2.08 : 1
8	213	100	2.13 : 1
9	228	101	2.28 : 1
10	189	117	1.61 : 1
11	266	109	2.44 : 1
Total	2,373	1,123	

b. The mutation rate of $\underline{c}^{i}$ to $\underline{c}^{Im}$ is heterogeneous in the various progenies and shows mosaicism phenomena on the ears (Table 2).

Table 2

Results of the cross: $\underline{c}^{IP}/\underline{c}^{i}$ x $\underline{C}^{i}/\underline{C}^{i}$, with $\underline{c}^{i}$ to $\underline{c}^{I}$ mutation

Progeny	Colorless aleurone	Colored aleurone	Ratio
1	143	117	1.2 : 1
2	140	110	1.2 : 1
3	219	161	1.3 : 1
4	176	97	1.8 : 1
5	224	86	2.6 : 1
6	136	29	4.6 : 1
Total	1, 038	600	

c. For initiation of instability in the genes $\underline{c}^{i}$ and $\underline{C}^{i}$, the presence of the paramutagenic gene $\underline{c}^{IP}$ is required. Nevertheless, this instability, once acquired, continues at least for two generations without the presence of the paramutagenic gene. This instability is the product of mutations and reversion(s). The reversion of the mutant gene $\underline{c}^{Im}$ to its original standard form $\underline{C}^{iR}$ (R = reversion) in the absence of the paramutagenic gene was detected with the following method (crossing-over is ignored due to its low frequency in relation to the mutation rate):

$$\frac{c^{i}\ sh}{c^{IP}\ Sh} \times \frac{C^{i}\ Sh}{C^{i}\ Sh}$$

$$\downarrow$$

$$\frac{c^{Im}\ sh}{C^{i}\ Sh} \times \frac{C^{i}\ sh}{C^{i}\ sh} \longrightarrow$$

100 C Sh

56 C sh (reversion)

0 c Sh

51 c sh

$$\frac{c^{i}\ sh}{C^{i}\ Sh}$$

$$\frac{c^{IP}\ Sh}{C^{i}\ Sh}$$

The ears with large sectors of aleurone color (containing $\underline{C}$ $\underline{sh}$ kernels) could be due to a reversion of $\underline{c}^{Im}$ to $\underline{C}^{iR}$.

d. Mutation of $\underline{c}^{i}$ to $\underline{c}^{Im}$ in the absence of the paramutagenic gene $\underline{c}^{IP}$ was detected by means of the following method:

$$\frac{c^{i}\ sh}{c^{IP}\ Sh} \times \frac{C^{i}\ Sh}{C^{i}\ Sh}$$

$$\downarrow$$

$$\frac{c^{i}\ sh}{C^{i}\ Sh} \times \frac{C^{i}\ sh}{C^{i}\ sh} \longrightarrow$$

216 C Sh

14 C Sh dilute

75 C sh

102 C sh dilute

51 c sh (delayed effect)

0 c Sh

$$\frac{c^{IP}\ Sh}{C^{i}\ Sh}$$

$$\frac{c^{Im}\ sh}{C^{i}\ Sh}$$

The colorless (c sh) kernels could be interpreted as a delayed mutation of c^{i} to c^{Im}, induced by the paramutagenic gene c^{IP}.

e. The reversion and mutation of the same C^{i} allele in the absence of c^{IP} (which is responsible for initiation of gene instability) was detected by means of the following method:

$$\frac{c^{IP}\ Sh}{C^{i}\ Sh} \times \frac{c^{i}\ sh}{c^{i}\ sh} \downarrow$$

$$\frac{c^{im}\ Sh}{c^{i}\ sh} \times \frac{c^{i}\ sh}{c^{i}\ sh} \longrightarrow \begin{array}{l} 44\ C^{i}\ Sh \text{ (reversion)} \\ 102\ c^{i}\ Sh \\ 139\ c^{i}\ sh \\ 1\ C^{i}\ sh \end{array}$$

$$\frac{c^{IP}\ Sh}{c^{i}\ sh}$$

$$\frac{C^{i}\ Sh}{c^{i}\ sh}$$

The colored (C Sh) kernels could be the reversion product of the mutant c^{im} to its original C^{i} (designated as C^{iR}). The C^{iR} kernels were saved and crossed again with c^{i} sh; the results are indicated in Table 3.

Table 3

Results of the cross: $\frac{C^{iR}\ Sh}{c^{i}\ sh} \times \frac{c^{i}\ sh}{c^{i}\ sh}$

Progeny	C Sh	C Sh dilute	c Sh*	C sh	c sh
1	50	47	35	1	134
2	53	28	53	1	104
3	68	20	48	1	78
Total	171	95	136	3	316

*The c Sh class of colorless kernels could be due to a second mutation of C^{iR} to c^{im2}.

Summary:

In the presence of the paramutagenic gene c^{IP}, the following mutational sequences have been obtained: $C^i \longrightarrow c^{im}$ and $c^i \longrightarrow c^{Im}$.

In the absence of the paramutagenic c^{IP} gene, the following mutational sequences have been obtained: $c^{im} \longrightarrow c^{iR}$, $c^i \longrightarrow c^{Im}$, and $c^{im} \longrightarrow C^{iR} \longrightarrow c^{im2}$. Symbols: C^i, c^i, c^I = standard alleles; m = first mutation; m2 = second mutation; R = reversion.

The present results may be interpreted in accordance with the hypothesis already described (MNL 40:62, 1966) as an excessive replication of DNA segment(s) of the paramutagenic gene c^{IP}. This segment(s) could have the power of self duplication and interaction with the C locus either in an attached or in a free state, not necessarily released into the cytoplasm. These findings suggest the possibility that a gene of a higher organism may originate episome-like particles.

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1. Pale phenotypes at the A_2 locus.

A number of distinct pale phenotypes, representing a wide spectrum of qualitative differences in anthocyanin coloration, have been isolated at the A_2 locus. These arose from a newly induced unstable a_2 mutant, $a_2^{m(1\ 1511)}$, but are themselves stable. They fall into a sequential series of pigment types from very light pales to darker shades. (Other phenotypes representing unrelated forms of phenotypic expression have also been isolated.)

Differences in pale phenotypes may be due to one of two alternatives: (1) differential placement of the $I^{(nr)*}$ element (Peterson, 1966) within the A_2 locus - the position hypothesis or (2) qualitative differences in the composition of the $I^{(nr)}$ element - the composition hypothesis. The position hypothesis may be tested by subjecting pales of different origin to crossover tests. Differential placement would be expected to yield full color types.

It is interesting to note that in a study of the a_1 - Dt system, Professor Rhoades found novel types at the a_1 locus that had not previously been recorded in natural populations. Similar types of variants have arisen at the A_2 and Wx loci following their exposure to the Ac-Ds system (McClintock, 1951). It is evident that systems such as a_1 - Dt, Ac-Ds and En-I can significantly influence types of variation originating at a locus.

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*$I^{(nr)}$ = suppresses gene action but does not respond to En.

2. En control of an a_2 mutable.

A number of unstable genes have been isolated in En- containing stocks. The new mutables originated from dominant alleles (MGCNL 40:64). One of them, $a_2^{m(1\ 1511)}$ has been shown to be under the control of Enhancer (En). A wide assortment of pattern types (differences in time and frequency of mutation events) has been identified. The relationship to En is based on a correlation between the presence of a_2^m and concomitant mutability in a tester a_1 allele, either $a_1^{m(r)}$ or a_1^{m-1}, both known to respond to En. The following tests are employed to demonstrate this relationship.

Cross #1 $a_2^m/-$, A_1/A_1 x A_2/A_2, $a_1^{m(r)}$ $Sh/a_1^{m(r)}$ Sh

Cross #2 A_2/a_2^m, $A_1/a_1^{m(r)}$ x A_2/A_2, $a_1^{m(r)}Sh/a_1^{ml}sh$ (En tester)

selected phenotypes: mutable types
non-mutable types

(The F_1 type, A_2/a_2^m $A_1/a_1^{m(r)}$, in Cross #2 utilized in a cross with an En tester is also crossed to an a_2 tester to verify the presence of the a_2^m allele.) The selected and separated phenotypes resulting from Cross #2 are tested by an a_2 tester (Cross #3).

Cross #3: mutable types resulting from Cross #2 x a_2 bt

non-mutable types resulting from Cross #2 x a_2 bt

Progeny Cross #2 Separated x a_1 into

	mutable		non-mutable	
	I	II	III	IV
Progeny of Cross #3	mutable	non-mutable	mutable	non-mutable
5 1039	11	3	0	7
5 1041	19	2	0	3
5 1044	19	0	0	7
5 1047	11	2	0	8
5 1050	12	0	1	8

Although not all selected a_1-mutable types (and therefore known to carry En) were mutable for a_2 (see column II), only 1 of the non-mutable was found to be mutable for a_2 (column III). This is presently being tested since a low frequency mutable type would escape detection in the a_1 test.

The non-mutable types in Column II are probably $\underline{a}_2^{m(nr)}$ types and crosses with an $\underline{a}_2^{m(r)}$ (a colorless $\underline{a}_2$ that will respond to $\underline{En}$) will test this.

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1. Genetic studies involving homozygous T6-9e: The location of $\underline{Y}_1$ with respect to the break point in chromosome 6 and a reduction in crossing over observed in chromosome 9.

Patterson (1958, Maize Genet. Coop. News Letter 32:54-66) reported on linkage relations of T6-9e (6L.18, 9L.24) in which he indicated that the break point in 6 was probably proximal to $\underline{Y}_1$. This break point position has been confirmed by testcrossing plants homozygous for the translocation and heterozygous at the $\underline{Y}_1$ and wx loci. If the break point on 6 is proximal to $\underline{Y}_1$, wx and $\underline{Y}_1$ should be linked in the homozygous translocation plants. If the break point is distal to $\underline{Y}_1$, independent assortment should be observed. Table 1 gives the results of the testcross.

Table 1
Testcross data of plants homozygous for T6-9e and heterozygous at the $\underline{Y}_1$ and wx loci ($\frac{\underline{Y}_1\ \underline{T}\ \underline{wx}}{\underline{Y}_1\ \underline{T}\ \underline{Wx}}$).

Direction of cross	Phenotypes				% C.O.
	White waxy	Yellow starchy	White starchy	Yellow waxy	
F_1 as males	913	948	34	23	
F_1 as females	919	857	22	24	
	1832	1805	56	47	2.8%

The data indicate that the break point in chromosome 6 is proximal to $\underline{Y}_1$ and that about 3% crossing over takes place between wx and $\underline{Y}_1$. Since the cytological distance between waxy and the break point in chromosome 9

is much greater than that between $\underline{Y}_1$ and the break point in chromosome 6, it is reasonable to assume that most of the observed crossing over takes place in the chromosome 9 segment and that $\underline{Y}_1$ is located cytologically very close to the break point in 6 (L .18).

These data establish that the centromere on 6 is definitely to the left of the $\underline{Y}_1$ locus.

The attachment of a segment of chromosome 6 to chromosome 9 has resulted in a marked decrease in crossing over in the wx-break point region. Linkage data reported at the annual Maize Genetics Conference indicate that the distance between wx and $\underline{gl}_{15}$ (located cytologically at L .1) is 15 units. Since the T6-9e break point (L .24) is distal to $\underline{gl}_{15}$, a minimum of 15% crossing over would be expected between wx and $\underline{Y}_1$ in a homozygous translocation. The observed value of 2.8% represents a considerable reduction in crossing over.

Rhoades (1960, Maize Genet. Coop. News Letter 34:67 and 1966, Maize Genet. Coop. News Letter 40:60-62) reported that the insertion of a duplication for chromosome 3 between bz and wx reduced crossing over between these two loci rather than increasing it as apriori considerations might suggest, since the length of chromosomal material was being increased. However, he did find increased crossing over in the $\underline{C}$-$\underline{sh}_1$ and $\underline{yg}_2$-$\underline{C}$ regions. This latter effect would rule out the possibility that the presence of the homozygous break points somehow acts to reduce crossing over or that the presence of homozygous foreign chromatin of necessity reduces crossing over in adjacent regions.

Contrary to Rhoades' observations, the attachment of a segment of chromosome 6 to the long arm of chromosome 9 creates a marked reduction in crossing over in the region adjacent to the break point in the homozygous translocation. It would be of interest to determine how the presence of this homozygous segment of chromosome 6 affects crossing over between other genes located in the wx-break point region and if this effect extends beyond the wx locus, and also, if a similar reduction in crossing over is observed in chromosome 6. Anderson, Kramer, and Longley (1955, Genetics 40:531-538) found that heterozygous translocation involving the long arm of chromosome 6 often exhibited a marked suppression of recombination in the region between Y and Pl. The work reported here suggests that this suppression may be due to more than just the poor pairing expected in a heterozygous translocation but might involve an effect due to transferring the long arm of 6 and to a foreign environment.

Donald S. Robertson

2. A new opaque gene located on chromosome 7.

This mutant was given me by Dr. Brawn of MacDonald College of McGill University. In his stocks the seeds had pale yellow endosperm which produced yellow-green seedlings when germinated.

The apparent pale color is evidently not the result of less pigment but rather is due to a difference in endosperm texture that, in a flint or dent background, produced an opaque phenotype. The appearance of this

mutant's endosperm is variable. The most common phenotype is that of a typical opaque; but frequently the seeds will be decidedly shrunken, approaching *sh*$_1$ in phenotype. In most stocks (dent or flint) the seed classification has been satisfactory.

The seeds, when planted at about seventy degrees Fahrenheit, produce yellow-green seedlings which are easily distinguishable from normal siblings. Seedlings grown at higher temperatures (e.g. about ninety-five degrees Fahrenheit) will approach normal pigmentation. Under field conditions these seedlings are viable, and stands comparable to normal are common. The mature plants are a foot or two shorter than their normal siblings and mature about a week later. The symbol *o*$_5$ has been given this mutant.

Testcrosses heterozygous for *o*$_5$ and a series of chromosome 9 translocations gave indications of linkage only with T7-9a (7L.63, 9S.07) (Table 1). Although the data are meager, they are sufficient to indicate that the gene is located on chromosome 7. No indication of linkage was found with waxy.

Table 1
Testcross progeny of *wx T7-9a* + / + + *o*$_5$ x ++ ++ *o*$_5$*o*$_5$

T +	+ *o*$_5$	T *o*$_5$	+ +	Total	% recombination
29	32	3	6	70	12.86

Allele tests with *o*$_2$, which is also on chromosome 7, were negative. This is an excellent new marker that can be used either as an endosperm or seedling trait.

Donald S. Robertson

3. Electron microscopy study of plastid development in dim light grown seedlings of *w*$_3$, *pas*$_{8686}$, *lw*$_1$, and *cl*$_1$.

As part of a larger project involving genetic, biochemical, and cytological studies of mutant seedlings of maize, we have been using electron microscopy to study plastid structural differentiation. The white-albino, *w*$_3$; its pastel allele, *pas*$_{8686}$; and their heterozygote (*pas*$_{8686}$/*w*$_3$) were grown, along with a normal control, in the dark for eleven to fourteen days at 26.6 degrees C. The seedlings were illuminated with 2,000 foot candles of light and samples taken in the dark and at intervals up to twenty-four hours after illumination. (Results of these experiments were discussed in last year's News Letter and presently a more detailed report is in preparation for publication.) Tissue was fixed with 3% glutaraldelhyde, post-fixed with 1% osmium tetroxide in phosphate buffer, dehydrated in an alcohol series, embedded in Epon 812 and sectioned on a LKB microtome with a diamond knife. Sections were

stained with uranyl acetate in methanol and examined under an R.C.A. EMU-3F electron microscope.

The dark grown normal plastid, under these conditions, contains one or more large ordered prolamellar bodies, radiating stroma lamellae, and dense osmiophilic globules. With illumination, there is a disorganization and disappearance of the prolamellar bodies, an increase in the number of lamellae and the formation of grana by twenty-four hours within the plastids of normal seedlings. Plastids of the albino, $\underline{w_3}$, after the disappearance of the prolamellar bodies and formation of some lamellae, break down structurally within one to four hours, and after twenty-four hours contain only scattered lamellae, vesicles, and starch grains. Both the pastel and heterozygote proceed initially along similar developmental pathways, but each eventually shows abnormal differentiation. The pastel plastid by twenty-four hours of light contains large grana aggregates and the heterozygote contains large loosely arranged prolamellae bodies.

Both the mutants and normal seedlings studied are capable of producing protochlorophyllide when grown in the dark and show equal ability to convert this to chlorophyllide and chlorophyll after a one-minute exposure to light. Carotinoid contents of these seedlings, however, are not equal. The albino, $\underline{w_3}$, contains no colored carotinoids but does accumulate carotinoid precursors; the heterozygote and the homozygous pastel both contain colored carotinoids in amounts much lower than normal.

Since these mutants are able to produce chlorophyll, they were next grown under a light intensity of approximately one foot candle. This was low enough for the plastids to accumulate chlorophyll, even with carotinoid levels which are insufficient to protect chlorophyll from photo-destruction at higher light intensities. Under these conditions, $\underline{w_3}$ will produce about one hundred times as much chlorophyll as dark-grown seedlings.

Seedlings of $\underline{w_3}$, $\underline{pas_{8686}}$, the heterozygote, and normal, as well as two white-albinos, $\underline{cl_1}$ and $\underline{lw_1}$, which do not accumulate carotinoid precursors, were grown in dim light for fourteen days at 26.6 degrees C. Fixation and embedding procedures were the same as those for the dark-grown seedlings.

The $\underline{w_3}$ plastid contained numerous long lamellae, similar to the large grana aggregate found in $\underline{pastel_{8686}}$ at twenty-four hours, but the discs are separate from one another. Small grana consisting of two discs are also present. The pastel and heterozygote plastids also contain small grana, but these are more numerous. These plastids also contained areas of discontinuous lamellae concentrated in parallel rows. The normal plastid contains numerous lamellae and small grana but no groups of parallel lamellae.

Plastids grown in dim light are much more lens shaped (as are light-grown normal plastids) than those in any of the dark-grown seedlings. Lamellae are long and continuous, not distinctly different from the normal control. Thus, although structural differences were observed in these plastids, they all produced long continuous lamellae with small

grana and attained a more normal shape than dark-grown plastids.

The two mutants which do not accumulate carotinoid precursors are able to produce chlorophyll, but when grown under dim light conditions only retain one-third to one-half as much chlorophyll as $\underline{w_3}$. Plastids of these albinos contain prolamellar bodies, few lamellae and almost no osmiophilic globules. They definitely are less structured than $\underline{w_3}$ under the same conditions. The absence of globules in these non-accumulating albinos suggests that the precursors and/or colored carotinoids when accumulated, as in $\underline{w_3}$, are stored in such globules. The presence of fewer lamellae in $\underline{cl_1}$ and $\underline{lw_1}$ and their inability to form grana probably are related to the lower levels of chlorophyll and possibly relate indirectly to the absence of precursors. Perhaps these precursors accumulated in $\underline{w_3}$ play some role in protecting chlorophyll from photodestruction when plants are grown in weak light.

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1. Reversion frequency of alleles of the $\underline{su_1}$ locus and of some of their compounds.

Seven alleles of the $\underline{su_1}$ locus ($\underline{su_1}$ a-b-c-d-e-f-g) have been obtained by EMS-treatment. The reversion frequency of these mutants is reported together with the rate for a standard allele of presumed natural origin ($\underline{su_1}^{st}$, which is used as a common pollen source) in comparison with the reversion rates of some of their compounds (among which are included also compounds of three mutants with the $\underline{su}$ WMT allele present in the multiple tester of P.C. Mangelsdorf). The data suggest the occurrence of intragenic recombination and a possibility of ordering linearly some of the sites studied.

Both the homoallelic and the compound plants were detasselled and pollinated by a common recessive stock bearing the $\underline{su_1}^{st}$ allele and $\underline{gl_4}$. The data collected from the homoallelic types are presented in the following table:

Table 1

Allele	No. of seeds scored	No. of gametes involved	No. of Su kernels	
su_1^{st}	9,219	18,438	3	Gametic frequency of Su (Backmutation or contamination) = 1.39×10^{-4} (with fiducial limits for P = 0.05 of 0.81×10^{-4} -- 2.22×10^{-4})
su_1^{a}	268	536	0	
su_1^{b}	8,107	16,214	3	
su_1^{c}	10,502	21,004	3	
su_1^{d}	14,918	29,836	6	
su_1^{e}	1,640	3,280	0	
su_1^{f}	6,071	12,142	1	
su_1^{g}	10,440	20,880	1	
Total	61,165	122,330	17	

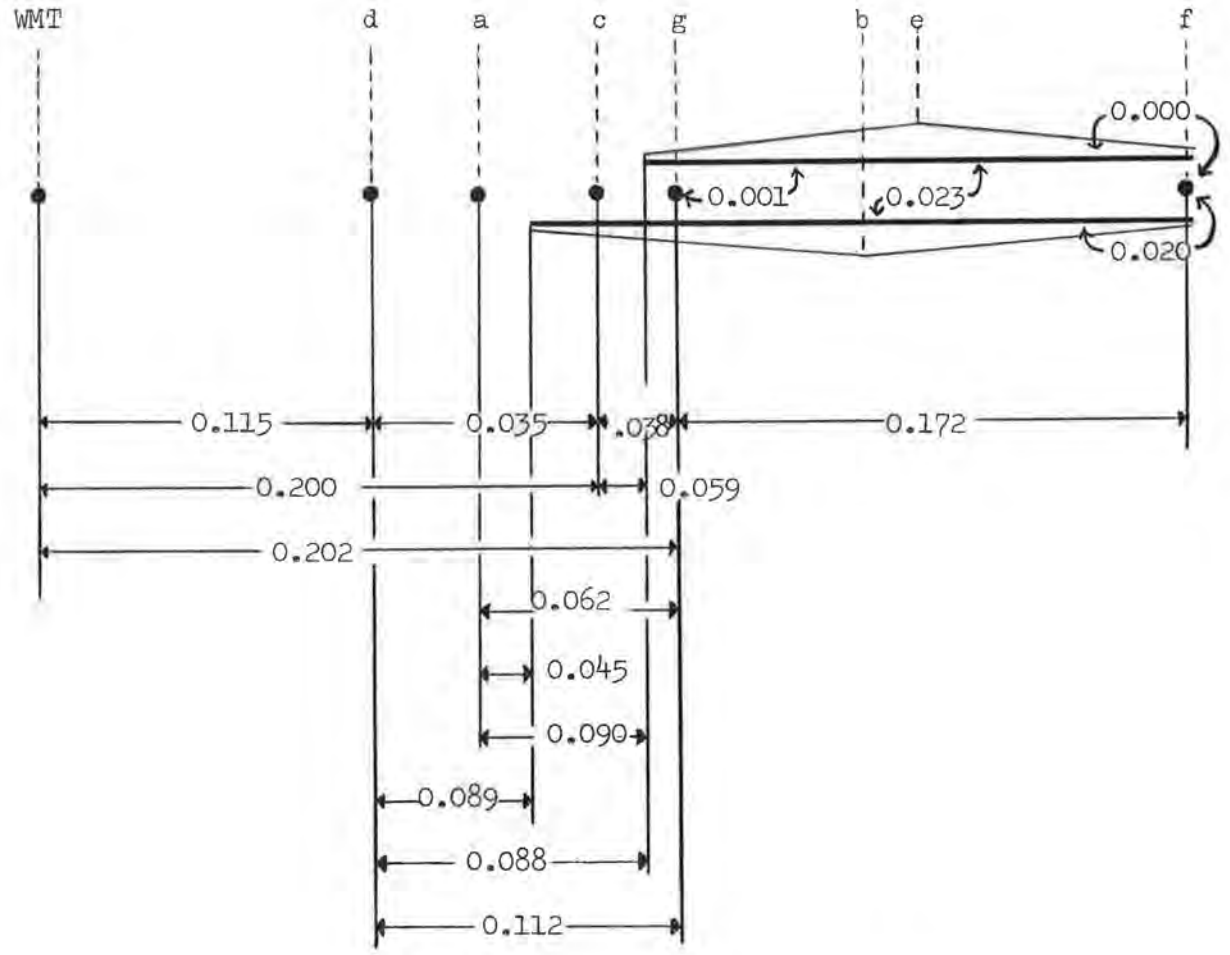
The compound types yielded the following data:

Table 2

Genotype	No. of seeds scored = no. of ♀ gametes involved	No. of Su kernels	No. of reversions arising by backmutation in the pollen or eggs (1)	No. of reversions arising by ♀ recombination	Rate of recombination (1×10^{-4})	Fiducial limits of recombination rate for P=0.05 (1×10^{-4})	Map units
axb	24,681	9	3.43	5.57	2.25	0.89 - 5.29	0.045
axe	17,044	10	2.37	7.63	4.48	2.02 - 9.25	0.090
axg	13,404	6	1.86	4.14	3.09	0.81 - 7.64	0.062
bxe	15,654	4	2.17	1.83	1.17	0.15 - 3.56	0.023
cxd	26,754	10	3.72	6.28	2.35	0.82 - 4.88	0.047
cxe	18,323	8	2.55	5.45	2.97	0.88 - 6.37	0.059
cxg	21,160	7	2.94	4.06	1.92	0.51 - 4.84	0.038
dxb	32,430	19	4.51	14.49	4.47	2.36 - 7.24	0.089
dxc	11,937	3	1.66	1.34	1.12	0.21 - 4.67	0.022
dxe	19,025	11	2.64	8.36	4.39	1.81 - 5.38	0.088
dxg	21,444	15	2.98	12.02	5.61	2.89 - 9.77	0.112
exg	14,041	2	1.95	0.05	0.03	0.00 - 2.62	0.001
fxb	37,826	9	5.26	3.74	0.99	0.29 - 2.71	0.020
fxe	9,060	1	1.26	0.00	0.00	0.00 - 4.07	0.000
fxg	14,006	14	1.95	12.05	8.60	4.43 - 14.96	0.172
WMTxc	16,817	19	2.31	16.69	9.98	5.79 - 16.06	0.200
WMTxd	16,833	12	2.34	9.66	5.74	2.85 - 10.92	0.115
WMTxg	18,230	21	2.53	18.47	10.08	6.05 - 15.94	0.202
Total	348,669	180	48.46	131.54	3.77	3.15 - 4.47	--

1. These values are obtained by multiplying the figures in column 2 by 1.39×10^{-4} reported in the previous table.

The linear order of the alleles studied may be as follows (the b and e mutants are possibly deletions or intragenic inversions):



F. Salamini

2. An unstable locus affecting aleurone and anther color.

In a progeny of a plant yg_2 C bz wx (chromosome 9 tester, originally provided by Dr. B. McClintock) fertilized by X-rayed pollen of the genotype Yg_2 I Sh Bz Wx, an ear was obtained showing a peculiar spotting pattern in the aleurone layer of many kernels. From the sowing of these kernels were obtained two plants which produced one ear each: one with pale colored seeds, and the second segregating for the following seed types which, in turn, give the results described below:

Phenotype of parental seed / Traits in progeny	Self-colored	With large spots	With fine spots	Pale colored (no spots)
Anthers	Self-colored	Speckled red on white background; some are self-colored.	Finely speckled: some are self-colored.	No progeny
Ears	Monohybrid segregation (or no segregation) for: -spotted seeds - pale seeds	With spotted seeds: -large spotting -fine spotting -With some seeds self-colored -Segregating (or not) for pale colored seeds*	With finely spotted seeds Segregating (or not) for pale colored seeds.	

*Ears segregating for wx and pale colored seeds suggest linkage of the latter phenotype with wx (20-25 c.o.).

F. Salamini

3. Weight of opaque (o_2) and normal kernels on the same ear.

The factor o_2 (opaque-2 on chromosome 7) is being used in maize breeding for the improvement of the protein quality. However, this gene affects negatively the weight of the kernel. An attempt is being carried out to find modifying complexes which minimize the difference between the normal and the opaque phenotype. An inbred line homozygous for o_2, derived from an Italian variety, has been crossed with 74 lines (S_2), homozygous for the normal dominant allele obtained from the variety Lierna. Self-pollination of such hybrids has yielded ears segregating opaque kernels. The weight of the two classes of kernels has been measured; the results are as follows:

1. The opaque weight is about 14.5% less than the normal.
2. However, statistically significant differences are detectable among the decreases of different ears. Some of them show a decrease of only 6.9%, whereas others reach the value of 26.3%.
3. The opaque kernels in selfed ears of the opaque Italian line crossed with the inbreds A 158 and WF9 weighed about 10% less than the normal ones.

These results suggest the possibility of selecting modifiers which reduce the gap between the mutant and the normal phenotype.

F. Salamini
T. Ekpenyong

4. Knobs in inbred lines from Italian varieties.

Cytological examination is being made of the pachytene chromosomes in the pollen mother cells of inbred lines derived from 14 Italian open pollinated varieties.

The results of this analysis are presented in Table 1.

Table 1
Knob constitution of inbred lines from Italian maize varieties

Line No.	Variety Source	Chromosome spreading*	Knob position** 1S	3L	4L	5L	6L+	7L	8L	9S	Type of nucleolar organizer#
1	Macario	3				K		2C		C	1
2	"	3				K		(2C)		K	1
3	"	3		(K)	K	(C)		K		K	1
4	"	3				K		2C		K	1
5	"	1				K		(2C)		K	1
6	"	0				not determined					
7	"	0				not determined					
8	"	1				K		2C		K	1
9	"	3				K	Cd	(2C)		K	1
10	"	2				K	Cd	(2C)		K	1
11	"	0				K	Cp?	2C?		K	1
12	"	1				K		2C		K	1
13	"	1				K	Cd	2C		K	1
14	Medoro	2		K		K		(2C)		K	2
15	"	2		K		K		(2C)		K	2
16	"	2		K		K		(2C)		K	2
17	"	2		K		K		(2C)		K	2
48	Nano Succi	4	C				Cd	K			1
49	" "	4					Cd	K			1
50	" "	4						C			1
51	" "	4					Cd	2C			1
52	" "	3					Cd	2C			1
53	" "	0				not determined					
56	Sacra Famiglia	2	C		K			C		C	2
59	Marano	2			K			(2C)	K		2
101	Davini	2				K		2C			2
104	Teso	2				K		2C		C	1
111	Trentinella PE	2				C		2C			1
112	Giallo precoce V.	0				not determined					
113	Giallo precoce V.	2						2C			1
118	Macario x Nodak	2				K	2C	2C		K	1
133	Nano 16	2				C		2C		C	1
134	Barbino di Tort. 14	3		K		K	Cd	2C		K	1
140	Quarantino giallo	2				K	2C	K			1
144	Barbino di Tort. 8	0				not determined					
145	Barbino di Tort. 8	0				not determined					
146	Barbino di Tort. 8	2				K		2C		C	2

*The spreading quality of the chromosomes is indicated by the indexes 0-4; 0 stands for very poorly spread chromosomes; 4 for the best spreading.

**K stands for reasonably large knob; C indicates a consistently prominent chromomere. When they are heterozygous, K and C are accompanied by parentheses.

+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.

#The nucleolar organizer type 1 has its main activity at the distal portion of the body (toward the satellite region); type 2 is chiefly active near the middle of the body.

From the data so far obtained it appears that the lines of different varietal origin are generally characterized by specific knob formulas, whereas the contrary is generally true for lines derived from a given variety.

M. Vetturini

5. Relationships between gametophyte factors and markers of chromosome 9.

Self-pollination of plants derived from normal seeds of Ga Wx/ga wx selfed ears has given, over a period of ten years, 54 ears with about 25% of wx kernels plus 600 ears showing no wx kernels or a severe deficiency of them (about 4% of wx). These figures permit calculation of a crossover rate of about 8.7% for the distance between Wx and the Ga factor detected by the senior Author.

A similar procedure for the repulsion phase Ga wx / ga Wx (independently found by Schwartz and Salamini) leads to an estimate of 13% as a c.o. distance between Wx and ga (566 ears with a large excess of wx, 161 with 25% wx kernels, 105 ears with no wx, and 5 ears with a great deficiency of wx). As reported in the 1966 MNL, this Ga factor has been located between Wx and Bz, at about 2/3 of the Wx-Bz distance from Wx.

Selfed ears of plants Ga sh C/ga Sh c exhibit an excess of sh (about 37.2%), and a deficiency of c (15.6%). If ga gametes are assumed not to function at all (as indicated by other results), these data confirm the median position of ga, at a distance of about 25 c.o. units from sh, and 31 from c. A distance of the same order of magnitude from sh is indicated for ears of selfed plants of the genotype Ga Sh/ga sh that show 12% of sh kernels.

An additional chromosome 9 marker, exhibiting close linkage with ga, is an albino seedling factor (w). Selfed ears of plants Ga Wx/ga wx gave the following results (only 1/3 of the Wx kernels were planted):

Wx kernels		wx kernels	
Normal seedling	Albino seedling	Normal seedling	Albino seedlings
889	1	28	47

The data indicate that the albino factor is closer to Ga than wx; they also permit an estimate of the c.o. value between wx and w (about 2 ± 0.3%).

If ga gametes do not function, the percentage of the w seedlings (about 2%) doubled (4%) provides an estimate of the c.o. value between w and ga.

The distance wx - w is also obtainable from the following data derived from an F_2 in which the Ga - ga pair is absent:

Linkage phase	Wx W	Wx w	wx W	wx w	c.o. ± st. error
R	2021	977	887	4	7 ± 1
C	311	3	0	110	less than 1

Since the wx - w distance varies approximately between 0 and 7 c.o. units, the linkage map of the genes in the short arm of chromosome 9, on the basis of the available data, is tentatively as follows:

C 3 Sh 2 Bz (?) Ga_8 (Schwartz and Salamini) 0 (?) Ga (Bianchi) (=Ga_8?)

4 (?) W 0-7 Wx.

A. Bianchi
R. Parlavecchio

6. Linkage relationships for endosperms and seedling traits.

In the 1966 MNL, data were reported on a shrunken mutant showing a cross-over per cent of 32.5 with gl_3 and 18.7 with gl_4. The data suggested a close linkage between this shrunken type and su_1. This has been confirmed by the scoring of ears obtained from the self-pollination of plants derived from su and sh kernels on selfed ears of plants of the constitution Su sh/su Sh: only 2 ears out of 92 proved to be su su Sh sh or sh sh Su su, with the recovery of the double recessive.

Another case of close linkage is offered by the following data (F_2, repulsion phase):

Gl_1 Ij	gl_1 Ij	Gl_1 ij	gl_1 ij
6042	569	536	0

A second ij-type and a virescent mutant show linkage with chromosome 4 markers:

	X Y	X y	x Y	x y	c.o. ± st. err.
ij-gl$_3$	3367	1471	1369	10	9.2 ± 0.8
ij-su$_1$	4704	1384	1543	125	32.4 ± 0.7
v-gl$_3$	1501	603	598	11	14.8 ± 1.2
v-su$_1$	2213	774	649	111	40.0 ± 0.8

In the 1965 MNL a case was described in which self-fertilization of plants y$_1$ Ij/Y$_1$ij did not yield any double recessives. Further data are reported from selfed progenies studied recently, in comparison with 1965 data:

	Y Ij	Y ij	y Ij	y ij
1965	11365	5802	5735	0
1966	17550	8732	8645	8
Total	28915	14534	14380	8

(c.o. 2.35% ± 0.28 st. err.)

C. Lorenzoni
M. Pozzi

7. Ethyl-methane-sulphonate (EMS) treatment on maize seeds.

Seeds of the single cross A 157 T x W 153 R have been treated with EMS for 12 hours, at 20°C., at a volume concentration of 1.5%. From the analysis of M$_2$ segregations the efficiency of the mutagenic activity of the chemical has been confirmed, as shown in the following table:

Segregations	Semisterile M$_2$ ears		Normal M$_2$ ears		Control ears	
	No. progenies	No. ears	No. progenies	No. ears	No. progenies	No. ears
Endosperm mutants	35	50	77	112	6	7
Chlorophyll mutants	10	16	32	60	4	8
Other seedling traits	15	23	31	42	0	0
Cases examined	30	140	74	282	74	322

Among the induced endosperm mutants more than half were defectives, several were gm, and a few were sh, su, and wx types. The seedling mutants were mainly abnormal growth and chlorophyll types. From the table reported it also appears that the progenies possessing obvious chromosome aberrations (as indicated by the semisterility of the ears) did not contain more "point-mutations" than normally filled ears.

C. Lorenzoni
M. Pozzi

8. Genetical and cytological data on Morocco maize.

Twenty-six samples of Morocco varieties have been studied genetically and cytologically. About six self-pollinations have been made for each sample. In the following table are listed the mutants found, together with the number of the independently occurring cases (when a phenotypically identical mutant was segregating in a given sample, the mutant was listed once):

Mutants			
Endosperm traits		Seedling characters	
Type	No. of cases	Type	No. of cases
Colored	1	Abnormal growth	5
Defective endosperm	8	Adherent	2
Etched	1	Allium type	1
Germless	1	Dwarf	4
Opaque endosperm	1	Fine stripe	1
Vivipary	1	Glossy	5
White endosperm	5	Green mottled	1
		Iojap	1
		Luteus	1
		Narrow leaf	1
		Pale green	5
		Pale yellow	3
		Rootless	1
		Virescent	4
		Yellow green	1
		White	4

For eleven of the samples, cytological study was also carried out, as reported in the following table (when knob shape, size and condition are not specified, the knob is round, medium sized and in homozygous condition):

Variety	Plant examined	Types of Knobs and Remarks
Azzama-Tahala	1	K1L het.; K4L long shaped; K5L small; 2K7L small; K8L + het. dist. chrom; K9L het.; 4 B chromosomes.
Hamra-Skhirat	1	K2L large, het.; K5L het.; K8L + large d.C.; K9S small.
Sefra-Rich	1	K4L het.; K7L het.; K8L; K9S small; 2 B chromosomes.
	2	K5L large; 2K6L small, het.; K7L small; K8L.
Septia-Tahala	1	K1S large; K5L; 3K6L small; K7L small, long shaped; K8L small + small prox. chrom.; K9L large het.; one more knob unidentified.
Beida-Sous	1	K1S small, het.; K4L small, long sh., het.; K5L small; K7L large; K9S large, long shaped.
Hamra-Beuslimane	1	K4L; K6L distal, small, het.; K7L; one more K unidentified.
Sefra-Beni Ahmed	1	2K6L small; K7L; K9S large.
Azougagh-Biougra	1	K1S; K4L het.; K5L; K7L; K9S small.
	2	K1S; K4L het.; K5L; K9S; one more K unidentified.
Sefra-Chichaoua	1	K6L distal, het.; K7L small; one more K unidentified.
Hamra-Dar bouazza	1	K2L long shaped; K6L distal small; K7L; K8L + 2 d.C. (1 het.)
Hamra-Essaouira	1	2K6L, het.; K7L small, het. long shaped; K8L large + d.C. het.; K9L large.
	2	K4L large het.
	3	K4L het. large; K5L small, het.; K7L small het.; K8L small, het.

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JOINT FAO/IAEA
Vienna, Austria
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1. Towards international standardization in crop research data recording.

Studies are now well advanced for tests of a system for computer storage, processing and retrieval of plant germ plasm records. The aim of these studies is to develop a model system for international, national and station records and for studies toward a central international record of world cultivars and useful breeding and genetic stocks. Such a system could serve a central function in an internationally coordinated programme for plant germ plasm exploration, conservation, evaluation and use.

The format for the records is divided into four sections to facilitate such use. This format is based on the use of punched cards, but should be adaptable to other equipment.

The first section of the record is designed for use as a master for other applications. The master record will identify accessions of each crop, by accession number, and by name. A uniform method of numbering accessions is recommended. This method uses letter codes to designate a hierarchal system of international, national and station accession series, as well as numbers to identify the specific accession. In its basic features the method is similar in many respects to methods already in practice.

The second section of the format is for recording information on the station which maintains stocks of the accession and details of its origin, all known synonyms, as well as the complete pedigree of the accession, presented according to a standard method similar to that described by Wiebe (1960 Barley Newsletter).

The third section is for recording information describing the various attributes of the accession. As presently devised, the third section will accommodate sufficient information to describe the more important features of an accession and the purpose for which it is being maintained. This section is to be developed further for recording more complete descriptions of the more useful cultivars, induced mutants, breeding and genetic stocks.

A fourth section will be added later to record in greater detail the performance of individual accessions as demonstrated in different environments by agronomic trials, quality evaluations, and tests of their response to diseases and pests, etc.

As a first step, a form for the records and instructions for entering the input information on each accession has been drafted. These will be used in making test runs early in 1967 using computer programmes designed for the SELECT and ISR systems for storage and retrieval of the information. For these tests, priority has been given to studies

using wheat data. These studies will be followed by tests on barley, oats, and rice. The results will be considered by the FAO-IAEA Working Group which, in December 1965, set up the project under the auspices of the Joint FAO/IAEA Division in cooperation with the FAO Division of Plant Production and Protection. Tests on the use of the master record in field experiment applications have also been initiated. Based on computer programmes and procedures now being applied in the States of Washington and Montana, U.S.A., field record books for recording data have been prepared for use by cooperators in several international field experiments. These trials include FAO/IAEA Coordinated Experiments on Rice Nutrition being conducted in 12 countries of Southeast Asia, FAO/IAEA/IRRI Cooperative Rice Mutant Yield Trials conducted in 8 countries, as well as in the FAO/IAEA Uniform Durum Wheat Mutant Trials conducted in 12 countries, under the FAO Near East Wheat and Barley Improvement and Production Project.

Acceptance of this standardized system by field workers, and the uniformity of results obtained from the studies conducted to date, have been encouraging. The second series of trials is now in progress.

Further information on the progress of these activities and sample copies of the test forms may be obtained by writing to the authors.

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2. International programmes on the use of radiation and isotopes in plant breeding and genetics research.

In the fall of 1964 the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency joined forces for promoting international cooperation to foster the use of nuclear techniques in food and agriculture, by establishing a Joint FAO/IAEA Division located in Vienna.

Engaged in every field of food and agricultural sciences, the Division has a Section dealing with Plant Breeding and Genetics. This Section has three primary field s of interest: (1) to promote and coordinate research leading to the development of more effective methods of inducing and utilizing mutations, (2) to foster cooperation between and

render assistance to mutation workers engaged in breeding some of the world's major food crops, (3) to arrange for systematic international testing of induced mutants in some major food crops, and to standardize and mechanize methods of recording and analyzing data in international trials and mutant collections.

In addition, the Section has technical responsibility for various projects of Technical Assistance to developing countries and technically supervises scientific meetings, training courses and publications in this field. In its work the Section cooperates with other related sections of the FAO as well as with such organizations as EUCARPIA and EURATOM.

A Laboratory Section has also been established at Seibersdorf near Vienna. This Section is primarily engaged in servicing the various international programmes by treating seeds with mutagens, doing basic research for development of new programmes and training fellows in mutation methods.

During the first two years of its operation, the Section, in accordance with its three primary interests, has developed the following programmes:

1. A Coordinated Programme of Research on the Production and Use of Induced Mutations in Plant Breeding. This programme has 17 main participants working under agreement or contract with the IAEA and several associates in countries in Asia, Europe, and North and South America. Coordination is maintained at periodic meetings, the first having been held in Vienna in January 1966, and the second scheduled to be held also in Vienna, in September 1967. The proceedings and recommendations for coordination made at these meetings are published. This group, which is mainly engaged in cereal work (rice, wheat, barley, oats, maize), also serves as a body of advisors to the plant breeding and genetics programmes of the Joint Division and is in addition preparing a Manual on Mutation Breeding which is primarily intended for use in training courses and by plant breeders in developing countries.

A Neutron Seed Irradiation Programme is in the process of getting under way. Its primary objective is standardization of methods of exposing seeds to neutrons in reactors and of measuring and reporting dose. Under contract with the IAEA and in collaboration with FAO/IAEA staff, the Austrian Atomic Energy Research Organization has developed a seed irradiation facility (lead and boron pot with a revolving specimen capsule) for use in pool-type reactors. Recommendations for standardized methods of measuring and reporting of dose have been developed by groups of biology, chemistry and physics experts. Contracts have been concluded with several countries to install the irradiation facility and to carry our coordinated studies. The IAEA laboratory at Seibersdorf is perfecting a technique of using barley seedling growth as an indicator of biological response for comparing different reactors. The first coordinating meeting was held in Vienna in July 1966; a working group meeting was held in December 1966, and the second coordinating meeting is planned for October 1967. Other studies within this programme will include radiosensitivity to neutrons of crop species and use of neutrons for induction of useful mutations.

2. A Coordinated Programme on the Use of Induced Mutations in Rice Breeding. There are nine participants in this programme, who hold research contracts with the IAEA. They are mostly in Southeast Asian countries; one in Latin America. This group cooperates closely with the International Rice Commission in Bangkok and the International Rice Research Institute in the Phillippines. The first coordinating meeting was held in Bangkok in 1965 and the second in Manila in 1966. The proceedings and recommendations are reported to the IRC and published in the IRC Newsletter. The third meeting is scheduled to be held in Taipei in June 1967. Through the work of one of the participants a new mutant rice variety named "Rei Mei" has been released in Japan, excelling mainly in stem strength. Another line produced by him reaches maturity 50 days earlier than the mother variety. Several other promising mutant lines of rice have been produced by the other participants.

In cooperation with the Seibersdorf laboratory, research is being carried out in Africa and Latin America on induction of disease resistance in wheat with emphasis on Septoria.

Plans are being made to develop coordinated research programmes dealing with the use of induced mutation for improvement of protein-rich crops and to improve protein quality and quantity of grain crops.

3. Under the framework of the FAO Near East Wheat and Barley Improvement and Production Project and in cooperation with the Italian Nuclear Research Center at Casaccia, Uniform Regional Trials of durum wheat mutants and controls are conducted in a number of countries of Southern Europe, North Africa and the Near and Middle East. The mutants, developed by Dr. G. T. Scarascia, have shown excellent performance in all these trials, outyielding local and common controls.

Under the framework of the Coordinated Rice Mutation Breeding Programme and in cooperation with the International Rice Research Institute, Uniform Regional Trials of indica rice and observation plot tests of japonica rice have recently been conducted in a number of Southeast Asian countries. The results are not known but the trials will be continued.

In cooperation with the Plant Production and Protection Division of FAO and the International Biological Programme, work is being developed towards standardization of crop research records and mechanization of processing. Several study groups, led by C. F. Konzak, have met to discuss the development of standard record formats and procedures. The Joint Division's Uniform Regional Trials already make use of computer-printed field books under this system. Formats are being developed for recording mutant and other genetic stock collections for computer handling. Adaptation studies, led by K. W. Finlay, are being standardized under the IBP and FAO and the FAO hopes eventually to establish world-wide germ plasm collection records.

Other activities by the Joint Division and its predecessors in this field have included a Technical Meeting on the Use of Induced Mutations in Plant Breeding, held in cooperation with EUCARPIA in Rome 1964, a Symposium on the Use of Isotopes in Plant Nutrition and Physiology held

in 1966, also in cooperation with EUCARPIA, and together with the organizers of the XIth Pacific Science Congress a Symposium on the Use of Isotopes and Radiation in Agriculture. During the first two years of this joint venture of FAO and IAEA, a number of international programmes has been established, which have fostered cooperation among scientists the world over. The resulting coordination in some of the fields dealt with has already contributed to more rapid progress in the use of nuclear methods in agricultural research and has helped to place this technique in its proper perspective as an important and unique additional tool to further research towards more and better food.

Björn Sigurbjörnsson
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1. Spm regulation of Diffuse and mosaic pericarp.

Preliminary evidence presented in the M.G.C. News Letter last year suggested that mosaic pericarp and Diffuse may be regulated by an Spm-like element. Further studies make this suggestion unlikely. Neither P^{mo} nor Idf are consistently associated with regulation of the gene action of a^{m-1}, a gene known to be regulated by Spm. The 1966 test ears were again confusing. Spm-like elements are present in both the stock carrying P^{mo} and the stock carrying Idf, but there does not seem to be a one to one relationship. That is, ears with the Diffuse phenotype do not always regulate the action of a^{m-1} as though they carried Spm, and Spm is not always absent in non-Diffuse ears. The frequent presence of strong Spm-like regulators in these stocks remains unexplained.

R. I. Brawn

2. Pericarp phenotype of a^{m-1}.

The mutable allele a^{m-1} produces a pale aleurone color in the absence of Spm (with A_2 C_1 C_2 R) and colorless aleurone with deep spots when Spm is present. In combination with the pericarp allele P^{rr}, this allele acts as a full recessive to give strong brown pericarp color both with and without Spm and not an intermediate red-brown as its aleurone color interaction would suggest. In the presence of Spm, red stripes are present. One ear of the genotype a^{m-1} P^{rr} Idf Spm has been observed. It has strong brown pericarp with frequent colorless sectors typical of the Diffuse phenotype and frequent red stripes due to the response of a^{m-1} to Spm.

It appears as though the phenotype of $\underline{a}^{m-1}$ $\underline{P}^{mo}$, both with and without Spm, is also brown but the low color level mosaic allele used makes color identification difficult.

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1. On the origin of abnormal 10.

In spite of the considerable interest in abnormal 10 (K10) that has been generated by the studies of Rhoades, Dempsey, and others, the origin of this chromosome has not been established. Ting (Chromosoma 9:286, 1958) postulated that the extra segment of K10 arose by simple translocation between normal 10 and a B-chromosome. This hypothesis was tested by comparing meiosis in haploids with either K10 or normal 10 (k10) and carrying a single B-chromosome. The desired haploids were obtained from diploids of the constitution $\underline{gl_6}/\underline{gl_6}$ K10/k10. The glossy seedlings were selected as putative maternal haploids among the progeny of the glossy female parents crossed with normal males. The male parent used was "stock 6", a high haploid-inducer line discovered and supplied by Dr. E. H. Coe. Sixty-four glossy seedlings were found among a total of 7,100 progeny obtained from this cross. Of these glossy exceptions, 58 were verified as haploids by chromosome counts in root tip squashes prepared by the Feulgen procedure. This is a haploid frequency of 0.82% which is considerably higher than the normal frequency of 0.1%. The chromosome 10 constitution was also determined in each haploid during the examination of dividing root tip cells where K10 can be recognized at metaphase by the acrocentric position of its centromere. Microsporocytes at various stages of division were obtained in the greenhouse from two plants of each chromosome 10 constitution. First division cells were examined for the occurrence of bivalent configurations, that is, associations of two chromosomes joined by a chiasma. Since no metaphase plate is formed during first division in haploid pollen mother cells, it is difficult to distinguish anaphase I from metaphase I. Only those cells in which several univalents were seen passing to the poles were scored. At this stage the two chromosomes of a bivalent can be seen disjoining but connected by a bridge resembling a delayed chiasma. Normally, maize haploids possess ten chromosomes. However, all of the plants used in this study had eleven chromosomes including the normal complement of ten plus one B-chromosome also contributed by the maternal parent.

The frequency of bivalent configurations at metaphase I-anaphase I in k10 and K10 haploids was determined and the data are presented in Table 1. One bivalent occurred in approximately 14% of the microsporocytes from both k10 and K10 plants. Two cells from each type of haploid were found to have two bivalents while one cell from a k10 haploid had three bivalents. A total of 51 bivalents, or an average of 0.15 per cell, was observed among the cells from K10-carrying haploids. This is not

Table 1
The frequency of bivalent configurations at anaphase I in microsporocytes from k10 and K10 maize haploids containing one B-chromosome

Chromosome 10	Plant no.	No. cells with 11 uni.	9 uni. plus 1 biv.	7 uni. plus 2 biv.	5 uni. plus 3 biv.	Total
k10	167-3	120	18	1	1	140
	168-7	169	31	1	0	201
	Total	289	49	2	1	341
	%	84.75±1.88	14.37±1.90	0.59±0.41	0.29±0.29	
K10	167-4	100	20	0	0	120
	168-4	195	27	2	0	224
	Total	295	47	2	0	344
	%	85.76±1.81	13.66±1.86	0.58±0.41	0	

significantly different from the controls with a normal chromosome 10 where an average of 0.16 bivalents per cell was found.

Bivalent associations in haploids have usually been interpreted as resulting from crossing over between duplicate segments present in different chromosomes. If the B and K10 chromosomes had homologous regions that would pair with subsequent chiasma formation, bivalents would be expected to occur more frequently in haploids with K10 than in those carrying the normal chromosome 10. However, the similarity in bivalent frequencies in the two types of haploids fails to lend support to the hypothesis that the extra chromatin of the K10 chromosome came from a B type. Prophase associations have been observed to occur between the two chromosomes at meiosis in diploids (Ting MNL 33:37; Rhoades and Dempsey MNL 33:58). However, these adhesions may represent non-specific attraction of the heterochromatin present in both chromosomes.

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1. Transfer of ae wx to sweet corn by the translocation method.[1]

Dr. R. G. Creech has found that the amylose extender gene (ae), in addition to changing amylose content of the endosperm, causes a substantial increase in sugars and reduction in starch. He found also that ae combined with wx (waxy gene) and du (dull gene) wx, causes a very high increase in sugars and reduction in starch. Preliminary post harvest studies by Dr. E. V. Wann indicate that starch accumulation in the mutant gene types is much lower than that in normal su_1 corn. These findings were of sufficient promise to encourage the transfer of ae and wx to standard su_1 inbred lines.

The transfer of ae and wx requires that after the first backcross to the recurrent su_1 parent, each succeeding backcross must be selfed in order to isolate the Ae ae Wx wx genotype for further backcrossing. At the 5% probability level, at least 10 BC plants must be selfed to be certain of detecting the double heterozygote. In order to save time, paired selfs and backcrosses can be made simultaneously. The efficiency of this system based on the number of ears saved from the numbers of ears needed is 5%.

With the thought of increasing the efficiency of conversion, an ae wx homozygous translocation line was developed at the University of Maryland from an Ae wx translocation obtained from the Maize Genetics Coop. Linkage data show that ae is separated from wx by 11.5 $\pm$ 0.5 units.

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[1]Scientific Article No. A1343, Contribution No. 3903 of the Maryland Agricultural Experiment Station.

When using this translocation for the ae wx transfer, semi-sterility of pollen and scattered kernel set on the ear will identify the Ae ae Wx wx genotype for subsequent backcrossing. Since each backcross progeny segregates 1 Fertile : 1 Semi-Sterile, only 4 plants are needed to be certain of obtaining a semi-sterile plant. The probability of not selecting a semi-sterile plant in which crossing over had occurred between Ae-ae and/or Wx-wx is approximately 9:1. The probability of selecting 2 semi-sterile plants, both having crossovers would be quite remote (1:81). Therefore 2 paired selfs and backcrosses are all that are required to obtain each succeeding backcross. Observation of the selfed ear serves as a check on semi-sterility and whether or not a crossover between the dominant and recessive alleles had occurred. If either ae or wx does not segregate, a crossover occurred and the paired backcross ear would be discarded. Based on the number of ears saved from the number of ears needed, this method is 5 times more efficient than the previous method.

The number of plants necessary to obtain each backcross generation in the first method of transfer would be 20 (10 backcross plants and 10 recurrent parent plants). The number of plants needed in the second method would be 10(8 backcross plants and 2 recurrent parent plants). The translocation method is twice as efficient in regard to the number of plants needed for obtaining backcross populations.

When the desirable number of backcrosses have been made by using either conversion method, the ae wx segregates are selected from the last selfed progeny. However, in the translocation method, the ae wx segregates will also be homozygous for the translocation. The significance of this is that normal inbreds developed by the first method cannot be utilized as parents with inbreds developed by the translocation method. Such hybrids would be translocation heterozygotes and would have semi-sterile pollen and scattered kernel set.

In one way development of homozygous ae wx and ae wx su$_1$ translocation inbreds would be advantageous to a seedsman. It would guarantee that his inbreds could not be used indiscriminately by other seedsmen and would prevent the introduction of large numbers of hybrids that for all practical purposes are similar.

If the breeder wishes to obtain the ae wx combination on normal chromosomes, this can be accomplished by further selection for the crossover type in the following manner. The probability would be very low for selecting a double crossover ae wx type in the first self population after backcrossing is complete. Frequency of this genotype would be .000025 or 1 in 40,000 kernels. The most promising method would be to select wx kernels in this population. Approximately 1 in 225 wx kernels will be of the $\frac{++wx}{ae+wx}$ genotype. In order to obtain this number of wx kernels, 23 self pollinated ears are required; however, at the 5% probability level, it would require 70 selfed ears to provide enough wx kernels (670) to insure obtaining the $\frac{++wx}{ae+wx}$ genotype.

The following year self all 670 wx plants. At seed harvest, throw out all ears not segregating ae wx and also those that are semi-sterile (this will eliminate 50% of the ears).

The following year cross 300 ae wx plants to a normal chromosome line and also self these 300 ae wx plants.

In the following year allow the 300 crosses to open pollinate. Save the seeds of the selfs whose test cross did not have scattered seed set. These plants should have the ae wx combination on normal chromosomes.

The advantages and disadvantages of using the ae wx translocation method have been presented. It can be concluded that if one is satisfied with obtaining the ae wx homozygous translocation, this method is a great deal more efficient in transferring ae and wx to normal su$_1$ lines. If however, one feels it necessary to select further for the ae wx combination on normal chromosomes, the translocation method is inferior and probably should not be used.

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1. A comparison of corn and Tripsacum in genetic composition and chromosome structure.

In connection with our hypothesis that Tripsacum is an amphidiploid hybrid of Manisuris and wild maize, we presented data suggesting that only the chromosomes of the 'maizoid' genome carried alleles to the seven recessive genes in white multiple tester, "WMT", while the other or 'manisuroid' genome did not have such alleles (Galinat, et al, 1963). We now find evidence that the alleles of the recessive loci of corn are distributed on most of the chromosomes of Tripsacum rather than confined to a genome of nine or ten chromosomes. Some chromosomes or chromosome arms still have many loci in common, but differ in their alleles. Perhaps these may be considered homologous. Others have differentiated to various degrees and are no more than homoeologous.

The structure of the Tripsacum chromosome whose long arm bears loci corresponding to the short arm of corn chromosome 2, as reported by Maguire (1957), fits the data for Tripsacum chromosome 5 in the idiogram for T. floridanum diagrammed by Chaganti (1965). A different and very short Tripsacum chromosome has the V_4 locus and probably other linked loci representing at least part of the long arm of corn chromosome 2. If the progenitor of Tripsacum chromosome 5 was originally similar to the present corn chromosome 2, then the transfer of the part of its long arm marked by "V_4" left its centromere in a subterminal relationship instead of its original submedian one. Similar translocations may account for some of the other subterminal positions of centromeres in Tripsacum.

With respect to corn chromosome 4, we have found an even higher degree of differentiation. The Tripsacum chromosome marked by Su_1 on the short arm of corn chromosome 4 does not have the La locus which is on the distal end of that arm in corn nor Gl_3 located on the long arm of the same corn chromosome. Conversely, the Tripsacum chromosome which bears the Gl_3 locus of corn does not have Su_1. Thus, at least some of the longer chromosomes of corn such as 2 and 4 have their loci distributed among several shorter chromosomes in Tripsacum.

Not only are the shorter chromosomes of corn (nos. 7 to 10) similar in length and arm ratios to some of the longer chromosomes of Tripsacum (nos. 1 to 4) (Longley, 1941), but at least two of the shorter chromosomes (7 and 9) have homoeologs in Tripsacum with similar genetic composition. A single Tripsacum chromosome carries at least four loci

(V_5, Ra_1, Gl_1, Ij) in common with corn chromosome 7 and another Tripsacum chromosome has a series of at least 5 loci (Yg_2, C, Sh_1, Bz, Wx) found on corn chromosome 9. The data for constructing idiograms for these two isolated Tripsacum chromosomes have not yet been obtained.

W. C. Galinat
P. C. Mangelsdorf

2. Selection for increased transmission of a Tripsacum chromosome and its resulting homozygosity.

The male and female transmission rates for the Su^d chromosome from T. dactyloides 4n of Florida on a background of a su gl_3 tester stock of corn were originally about 10% for either sex alone instead of 50% and self-pollinated ears had about 19% starchy kernels instead of 75%. By selecting for increased Su^d transmission among hundreds of ears over several generations, the transmission rate for either sex alone was raised to over 40%. Self-pollinated ears from these higher transmission lines yielded some ears (about 25% of the total) which showed 80 to 95% starchy kernels. At least some of these ears with around 90% starchy kernels are assumed to be addition disomics (20+2) with the extra Su^d chromosome from Tripsacum homozygous. The failure to obtain 100% starchy kernels on such ears would result from an occasional loss of both members of a pair of Su^d chromosomes which may not be coordinated in their meiotic behavior with the maize chromosomes. The cytological analysis of this material has not been completed.

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3. Homozygosity of a possible interchange chromosome from Tripsacum.

We did obtain one ear which had 100% Su^d Su^d kernels, but this plant has 20 rather than 22 chromosomes. This may have resulted from a homozygous substitution of a corn-Tripsacum interchange chromosome. Since the Su^d chromosome is known to lack some of the loci of corn chromosome 4 (La and Gl_3), it presumably would not provide a functional substitution in itself. This particular plant was partially male sterile and the ear had 26 per cent defective kernels (out of 133). Here again, the cytological analysis is incomplete.

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4. Additional evidence of somatic mosaicism in corn grass.

In last year's MNL, we reported that certain differences in morphology between two ears borne at different nodes on a corn grass plant were inherited rather than mere physiological variations. Since the action of the corn grass locus seems to involve the phase change process, it appeared that here was evidence for the involvement of a mutational (or paramutational) mechanism, as suggested by Brink on other grounds. However, Brink raised the question that my results could have come from a physiological difference carried through the cytoplasm of the egg.

We have tested this possibility by using pollen from two different forms of inflorescences borne on the same plant. The planting was widely spaced in the field (six sq. ft./plant) and extra fertilizer and water were applied. The plant chosen had a grade two lateral inflorescence that terminated a branch which originated above ground level and could not have been derived from a different embryo than that which produced the main stalk. The terminal inflorescence to the main stalk of this plant was normal (grade 5). The data were gathered from our winter crop planting in Goulds, Florida.

The results (Table 1) of a heritability test of variation through the male side confirm the previous one made on the female side. The hybrid progeny produced from crossing A158 with pollen from a 'vegetative' type tassel (grade 2) borne on a lateral branch was significantly more vegetative than that produced with pollen from the normal type tassel (grade 5) terminating the main stalk ($P = < .01$).

Table 1
Frequency distributions for hybrid progeny from A158 crossed by two grades of corn grass tassels borne on a single plant

	Progeny Tassel Grades				
Parental Tassel	2	3	4	5	Totals
Grade 2 (veg.)	9	18	35	16	78
Grade 5 (normal)	1	9	13	54	77

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1. Genetic instability of R^{st} and derivatives in somatic and germinal tissues.

In a previous report we showed that after introduction of Mp in a homozygous R^{st} R^{st} stock, some kernels are produced exhibiting a new spotting pattern among the standard stippled kernels. These stippled derivatives, that breed true in successive generations, are here symbolized as R^{sk}, $R^{l.sk.}$, $R^{l.st.}$, and $R^{n.c.}$ to indicate respectively smoky, light smoky, light stippled and nearly colorless. Like R^{st} these new forms do not synthesize anthocyanin in the sporophytic tissues and they are strong inducers of paramutation. On the other hand, they differ from stippled in the number and size of dots that they form in the aleurone.

The mosaic phenotype of the aleurone is presumably caused by the frequent somatic reversion of R from an inactive to an active form. Each of the spots therefore registers one such event which occurred sometime

during endosperm development. Accordingly, the various stippled derivatives differ in the time and frequency at which the somatic reversion of R occurs. When the reversion involves germinal tissues it gives rise to self-colored revertants, designated R^{sc}, that are germinally transmissible.

In the present report we analyze the capacity of the various stippled derivatives to undergo reversion toward R^{sc} in the germ cells and in the somatic tissues of the aleurone.

A. Reversion rate toward R^{sc} in the germ cells.

In order to measure the reversion rate in the germ line, plants carrying the stippled allele under test (heterozygous for r and outside markers) were pollinated with a stock homozygous for r and outside markers. The genetic markers here used are the gene golden (g, 14 units proximal) and M^{st} (six units distal to R). The matings made and the non-parental phenotypes selected were as follows:

Cross	Non-parental phenotypes selected
$+\ R^{st}\ +/g\ r^{g}\ m^{st}$	1. colored endosperm and scutellum
$g\ r^{g}\ m^{st}/g\ r^{g}\ m^{st}$	2. colored endosperm only
	3. colored scutellum only

The same scheme has been adopted for the stippled derivatives. Class 1 kernels represent instances in which the megaspore is revertant. If reversion of stippled to self-colored occurs during development of the megagametophyte, class 2 and 3 kernels are the reciprocal types expected. Kernels carrying the non-parental phenotype have been progeny-tested by selfing them. The reversion rate has then been estimated by subtracting the contaminants and germinally non-transmissible revertants from the total number of presumed revertants initially isolated. The figures to subtract were determined by extrapolating from those computed in the tested sample (since not all the revertants isolated germinated). Class 3 revertants have not yet been progeny-tested. The reversion rates of the three classes of revertants are reported in Table 1.

Table 1
Reversion rate of $\underline{R}^{st}$ and its derivatives to $\underline{R}^{sc}$ in the germ cells. (Class 1 = colored endosperm and scutellum; Class 2 = colored endosperm; Class 3 = colored scutellum)

Class 1 and 2 Revertants

Allele tested	# gametes tested	Class 1 revertants	Reversion rate x 10^{-4}	Class 2 revertants	Reversion rate x 10^{-4}
Nearly colorless	4536	21.5	47.4	0.0	0.0
Stippled	3030	9.4	31.1	8.2	27.0
Smoky	4080	3.4	8.4	19.2	46.5
Light stippled	3131	0.9	3.0	6.0	19.1
Light smoky	6790	0.0	0.0	0.0	0.0

Class 3 Revertants

Allele tested	#gametes tested	Class 3 revertants	Reversion rate x 10^{-4}
Nearly colorless	4370	0.0	0.0
Stippled	4888	2.0	4.1
Smoky	6211	3.0	4.8
Light stippled	5403	5.0	9.2
Light smoky	6442	0.0	0.0

Although the population of gametes tested for each stippled derivative is not very large, the results seem to suggest that:

1. Within each of the three classes of revertants, some of the stippled derivatives exhibit striking differences in their capacity to revert toward $\underline{R}^{sc}$.

2. A particular allele can exhibit differential capacity to revert to $\underline{R}^{sc}$ at various times (see the reversion rate of $\underline{R}^{n.c.}$ as measured in Class 1 and 3).

B. Reversion rate toward $\underline{R}^{sc}$ in somatic cells.

In order to compare the reversion rate of a given allele in its somatic and germinal cells, we measured the frequency of somatic reversion as the rate of reversion per cell per division. This is accomplished by scoring the stippled kernels for half seed and quarter seed sectors. These sectors represent reversions occurring when the endosperm was at a two and four cell stage respectively. The estimate of somatic reversion rate has been limited to stippled, smoky and light smoky kernels. In fact, their somatic sectors are frequent enough and their identification easier than

is true for seeds carrying other stippled derivatives. The results are reported in the following table.

Table 2
Reversion rate of different stippled derivatives in germinal and somatic tissues

Allele tested	#seeds scored	Germinal revertants	x 10^{-3}	Half-seed sectors	x 10^{-3}	Quarter-seed sectors	x10^{-3}
Stippled	1397	6.6	4.7	11	3.9	26	4.6
Smoky	3672	3.1	0.8	45	6.1	72	4.9
Light smoky	4080	0.0	0.0	0	0.0	4	0.3

These data suggest that when the frequency of reversion is expressed as reversion rate per cell per generation, it is possible to group the alleles into 3 categories:

1. The stippled allele exhibiting the same chance of reversion for all the cells and at different times of the development.

2. The smoky allele exhibiting an increase of approximately 5 times in its somatic reversion rate when compared to that in the germ cells.

3. The light smoky allele with high stability both in the germ cells and at the first cell divisions during endosperm development.

Giuseppe Gavazzi

2. Paramutagenic action of some stippled derivatives.

The various stippled derivatives can be distinguished by means of different criteria such as their capacity to induce paramutation and their reversion rate toward $\underline{R}^{sc}$ in somatic and germ cells (see previous note). The first criterion is particularly important in relation to the problem of the genetic basis of paramutation. In fact, the mosaic phenotype of the aleurone, conditioned by $\underline{R}^{st}$, could be controlled by a genetic component associated with the $\underline{R}$ region that is also responsible for inducing paramutation. Accordingly, one should find that any genetic alteration leading to a change in the stippling pattern is coupled with an alteration in its paramutagenic potentialities. This possibility has been tested by comparing the paramutagenic capacity of $\underline{R}^{st}$ and $\underline{R}^{n.c.}$ that differ markedly in their phenotypic expression. This test has been accomplished by crossing standard stippled and nearly colorless sib plants with a homozygous $\underline{R}^{r}/\underline{R}^{r}$ stock and then testcrossing them on W23 $\underline{r}^{g}/\underline{r}^{g}$. Individual testcross kernels were scored for pigmentation according to the standard matching technique (see Brink et al., 1960).

From each testcross ear 42 kernels of the appropriate genotype were scored. Since in testcross ears obtained from the cross W23 $\underline{r}^g\underline{r}^g$ x W22 $\underline{R}^{n.c.}$ $\underline{R}^r$, kernels of the two genotypic classes overlap in phenotype, the two classes were separated retrospectively by germinating the seeds after scoring. The results of such a comparison are reported in Table 1.

Table 1

Means of aleurone color scores for $\underline{R}^{rl}\underline{r}^g$ $\underline{r}^g$ kernels from W23 $\underline{r}^g$ $\underline{r}^g$ x W22 $\underline{R}^r$ $\underline{R}^{st}$ ♂ matings in comparison with those of $\underline{R}^{rl}\underline{r}^g$ $\underline{r}^g$ kernels from W23 $\underline{r}^g$ $\underline{r}^g$ ♀ x W22 $\underline{R}^r$ $\underline{R}^{n.c.}$ ♂ matings

Allele tested	# ears scored	Mean aleurone color score	Standard error	Estimated t value (1)
Stippled	15	2.37	0.124	$0.533^{n.s.}$
Nearly colorless	15	2.45	0.087	

(1) n.s. = non significant

No significant difference in level of paramutagenic action between the two classes is detected by the test. These data suggest that there is no straight relationship between the phenotype of the two stippled alleles tested and their paramutagenic potentialities. As previously mentioned, the various stippled derivatives were first isolated following introduction of Mp into the genome of a homozygous $\underline{R}^{st}/\underline{R}^{st}$ stock. Furthermore, it has been noticed that the addition of increasing doses of Mp in a homozygous $\underline{R}^{st}/\underline{R}^{st}$ stock determines an approximately linear increase in the $\underline{R}^{st} \longrightarrow \underline{R}^{sc}$ reversion rate (Gavazzi, 1967). Because of this interaction of Mp with the stippled expression, it seemed appropriate to establish whether the association of a given stippled allele with the Modulator can also affect its paramutagenic capacity. This has been accomplished by determining the paramutagenic action of $\underline{R}^{n.c.}$ and $\underline{R}^{sc}$ individuals isolated from an ear that was segregating for Mp. Each $\underline{R}^{sc}$ and $\underline{R}^{n.c.}$ sib plant has been crossed with a homozygous $\underline{R}^r$ $\underline{R}^r$ stock and then testcrossed on W22 $\underline{r}^g/\underline{r}^g$. The resulting testcross kernels have been scored with the same procedure adopted in the previous test. At the same time, pollen from each plant under test has been put on a $\underline{C}^I$-Ds/$\underline{C}^I$-Ds $\underline{R}^g/\underline{R}^g$ tester stock in order to check its Mp constitution. For each allele thus tested two sublines were derived differing in their Mp constitution. The results of this paramutagenicity test are here reported.

Table 2
Means of aleurone color scores for $\underline{R}^{rl}\ \underline{r}^{g}\ \underline{r}^{g}$ kernels from $\underline{r}^{g}\underline{r}^{g}$ x $\underline{R}^{r}\ \underline{R}^{n.c.}$ (with or without Mp) matings in comparison with those of $\underline{R}^{rl}\ \underline{r}^{g}\ \underline{r}^{g}$ kernels from $\underline{r}^{g}\underline{r}^{g}$ x $\underline{R}^{r}\ \underline{R}^{sc}$ (with or without Mp) matings

Allele tested	Mp constitution	# ears scored	Mean aleurone color score	Standard error
Nearly colorless	1 Mp	9	3.73	0.204
Nearly colorless	0 Mp	10	3.48	0.203
Self-colored	0 Mp	19	2.50	0.097
Self-colored	1 Mp	19	2.32	0.103

In the following table the results of an analysis of variance from the paramutation scores presented in Table 2 are reported.

Table 3
Analysis of variance of the paramutagenic action of $\underline{R}^{n.c}$, and $\underline{R}^{sc}$ with and without Mp in their genome (A = $\underline{R}^{n.c.}$ with Mp; B = $\underline{R}^{n.c.}$ without Mp; C = $\underline{R}^{sc}$ without Mp; D = $\underline{R}^{sc}$ with Mp)

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value (1)
Between genotypes	3	18.489	6.163	24.01**
A vs. B	1	0.300	0.300	$1.17^{n.s.}$
C vs. D	1	0.315	0.315	$1.23^{n.s.}$
AB vs. CD	1	17.873	17.873	69.63**
Within genotypes	53	13.604	0.256	
Total	56	32.938	0.573	

(1) n.s. = non significant ($P > 0.05$)
** = highly significant ($P < 0.01$)

This analysis shows that the $\underline{R}^{sc}$ and $\underline{R}^{nc}$ alleles differ significantly in their capacity to induce paramutation. On the other hand, the introduction of Mp into their genomes does not alter their paramutagenic potentialities. The data here presented indicate that:

1. The paramutagenic action of $\underline{R}^{sc}$ is significantly higher than that of $\underline{R}^{n.c.}$

2. The introduction of Mp in the $\underline{R}^{st}$ genome, while exhibiting an effect upon the stippled phenotype, does not seem to be associated with a change in its paramutagenicity.

The data so far obtained suggest that two functions exhibited by the unstable R alleles, i.e. their capacity to induce paramutation and their production of a variegated phenotype in the aleurone, do not have a common genetic basis. On the contrary the data point to the existence of two independent components associated with the R locus governing these different functions. The paramutagenic capacity of other stippled derivatives and the relationship between paramutagenic potential of $\underline{R}^{st}$ and crossing over in its adjacent regions are now under investigation. The accomplishment of these tests will allow a more general formulation of the conclusions here presented.

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3. Phenotypic stability in maize.

The phenotypic expression of the genotype may vary with environmental conditions and the kind and the amount of that variation cannot be the same for all genotypes. Many efforts have been made to study the genetic control of variability of the phenotypic stability. The object of this study was to examine the phenotypic stability of different genotypes in relation to the effect of plant spacing. This effect is of great interest in plant breeding and in research concerned with the nature of gene action involved in determining quantitative traits.

The aims of this work are twofold: (1) to obtain information about the possibility of selecting strains to be used with high plant density and (2) to get additional information on the genetic control of variation of phenotypic stability.

Sixty-four genotypes from a complete set of diallel crosses between eight inbred lines formed the experimental material. They have been planted at three different levels of plant density, namely 5, 7 and 9 plants per m^2. The experimental design was the following: two blocks were divided into three plots, one for each level of plant density. For each plot, five plants of each family were used. In order to distribute equally the competition effect between genotypes, a single plant randomization was used. The measurements taken in the field were the following: flowering time (tassel), plant height, leaf width and length. Parental and F_1 means of all characters considered for each level of plant density are presented in Table 1. The variance between densities provides an inverse measure of the stability over the range of environmental variation considered in this experiment (Griffing and Langridge, 1963). This parameter was estimated for each family in both blocks. The logarithms of the estimated variances (Sheffe, 1949) have been used for diallel analysis of variance (Table 2) according to the model of Hayman (1954).

The main results of this experiment can be summarized as follows:
Flowering time. The increase of plant density delayed the flowering of both inbred lines and F_1's. However, the experiment did not exhibit any kind of genetic control of the variability of this effect. The parental lines and the F_1's did not show any significant difference in behavior.
Plant height. The biometrical analysis showed that the variability of phenotypic stability between genotypes is genetically controlled. The genetic variance turned out to be of the additive type. On the other hand, the variation in mean values for plant height is due to an increase of plant height in the F_1's and a decrease in the inbreds.
Leaf width. The increase of plant density reduced the leaf width. The mean values presented in Table 1 do not show any consistent difference between inbreds and F_1's in this effect. However, the analysis of variance indicates that the variation of phenotypic stability has a genetic basis of the non-additive type. A significant maternal effect was also noticed.
Leaf length. The data here analyzed show that this character is quite stable within the range of variation considered in this experiment.

Table 1

		I_1	I_2	I_3
1) Flowering time	$\bar{F}_1$	28.31	28.46	30.85
	$\bar{P}$	33.26	33.31	35.50
2) Plant height	$\bar{F}_1$	199.80	207.78	207.76
	$\bar{P}$	145.74	153.76	144.33
3) Leaf width	$\bar{F}_1$	9.04	8.45	8.33
	$\bar{P}$	6.53	6.06	5.47
4) Leaf length	$\bar{F}_1$	76.41	76.10	76.34
	$\bar{P}$	61.41	61.66	61.66

1) Days from the 1st of July
2), 3) and 4) expressed in cm.

Table 2

Items	D.F.	Variances: Flowering time	Plant height	Leaf width	Leaf length
a	7	1.2239$^{n.s.}$	6.1603**	1.5498$^{n.s.}$	2.7192$^{n.s.}$
b	28	0.5884$^{n.s.}$	1.0686$^{n.s.}$	2.8156*	1.9041$^{n.s.}$
b_1	1	0.4243$^{n.s.}$	0.2893$^{n.s.}$	2.0015$^{n.s.}$	0.5303$^{n.s.}$
b_2	7	1.1204$^{n.s.}$	0.7141$^{n.s.}$	4.0483**	3.2292$^{n.s.}$
b_3	20	0.4104$^{n.s.}$	1.2316$^{n.s.}$	2.4250*	1.5089$^{n.s.}$
c	7	1.6970$^{n.s.}$	2.0857$^{n.s.}$	2.7827*	2.3927$^{n.s.}$
d	21	2.2624$^{n.s.}$	2.0390$^{n.s.}$	2.3734	2.4011$^{n.s.}$
Total	63				

n.s. = not significant; * = significant ($P < 0.05$);** = highly significant ($P<0.01$); $\underline{a}$ = additive variance; $\underline{b}$ = unfixable variance; $\underline{b}_1$ = mean dominance; $\underline{b}_2$ = dominance variation between parents; $\underline{b}_3$ = dominance not ascribable to $\underline{b}_1$ or $\underline{b}_2$; $\underline{c}$ and $\underline{d}$ = variances due to differences between reciprocal crosses.

E. Ottaviano

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1. Establishment of knob stocks.

Relatively few maize stocks are available with known knob constitutions. The purpose of this study was to establish stocks with many different combinations of distinctive knobs. Knobs at fifteen positions located on chromosomes 1 through 9 were available from the following Mexican races of maize, listed with their source identifications: Zapalote Grande (Chis. 236) and Harinoso de Ocho (Sin. 7) from Dr. E. C. Johnson; Zapalote Chico (Zapl x^3-1-1-1-1) and Wilbur's Flint from Dr. W. L. Brown. Abnormal chromosome 10 was found to be segregating in Zapalote Grande but was not transmitted to the F_1's used in this study.

The Mexican races were crossed with Wilbur's Flint (knobless) and the F_1's were backcrossed to the knobless stock. Sporocytes were taken from

Table 1
Knob constitutions of the backcross progeny

Source of Knobs and Plant Numbers	Knob Positions+														
	1S	2S	2L	3L	4T	4L	5S	5L	6p	6d	7T	7L	8p	8d	9T
Zapalote Grande															
R-1034-1	x	-	-	-	x	-	-	-	x	*	-	-	x	*	x
R-1034-3	-	-	x	-	x	x	x	x	x	*	-	-	-	-	-
R-1034-6	x	-	x	-	-	x	-	-	x	*	-	x	x	*	x
R-1034-7	-	-	-	-	-	x	x	x	x	*	-	-	-	-	x
R-1034-12	-	-	x	-	x	-	-	x	x	*	-	-	-	-	-
R-1034-14	x	-	-	-	x	x	-	x	x	*	-	x	x	*	x
R-1034-15	x	-	-	-	-	x	-	x	-	-	-	x	-	-	x
R-1034-21	-	-	x	-	x	-	-	-	x	*	-	-	x	*	x
R-1034-24	x	-	x	-	x	-	-	x	-	-	-	x	-	-	x
R-1034-26	-	-	-	-	x	-	x	x	x	*	-	-	x	*	-
R-1034-29	x	-	-	-	x	x	x	x	x	*	-	x	-	-	-
R-1034-33	-	-	-	-	x	-	x	-	x	*	-	x	x	*	x
R-1034-36	-	-	x	-	-	x	x	-	x	*	-	-	-	-	-
R-1034-37	x	-	-	-	-	x	x	-	x	*	-	x	-	-	-
R-1034-40	x	-	x	-	-	x	-	x	-	-	-	-	-	-	x
R-1034-44	-	-	-	-	x	x	-	x	-	-	-	x	x	*	x
R-1037-2	-	-	-	-	x	-	x	x	-	-	-	x	-	-	-
R-1037-3	-	-	x	-	x	x	x	-	-	-	-	-	-	-	-
R-1037-14	x	-	x	-	x	x	-	x	-	-	-	-	-	-	x
R-1037-20	-	x	-	-	-	-	-	x	-	-	-	x	x	*	-
R-1037-23	-	x	x	-	-	-	-	-	-	-	-	x	x	*	-
R-1037-24	-	-	-	-	x	x	x	x	-	-	-	-	-	-	x
R-1037-26	-	x	-	-	x	-	-	-	x	x	-	-	-	-	x
R-1037-31	x	-	x	-	-	x	-	x	x	x	-	-	x	*	-
R-1037-32	-	x	x	-	-	-	x	-	-	-	-	x	-	-	x
R-1037-37	-	x	-	-	-	-	-	x	x	x	-	x	x	*	-

+ S, L, T, p, and d denote the short arm, long arm, terminal end of the short arm, proximal, and distal, respectively.

x, -, and * denote the presence or absence of a knob or the presence of a prominent chromomere, respectively.

(continued)

Table 1 (continued)

Source of Knobs and Plant Numbers	Knob Positions														
	1S	2S	2L	3L	4T	4L	5S**	5L	6p	6d	7T	7L	8p	8d	9T
R-1037-42	x	-	-	-	-	-	x	x	x	x	-	-	x	*	-
R-1037-47	-	x	-	-	-	-	-	x	-	-	-	x	-	-	-
R-1037-49	-	-	x	-	x	-	-	-	-	-	-	x	-	-	x
R-1040-1	x	x	-	-	x	x	x	x	x	x	-	-	-	-	x
Zapalote Chico															
R-1043-31	-	-	x	x	x	-	-	-	x	x	-	-	*	*	-
R-1044-8	-	-	-	-	-	-	-	-	-	-	x	-	*	*	x
R-1044-13	-	-	x	x	x	-	x	-	-	-	-	-	*	*	-
R-1044-15	-	-	x	-	x	-	-	-	-	-	-	-	-	-	-
R-1044-27	x	x	x	-	-	-	x	-	-	-	x	-	*	*	-
R-1044-32	x	-	x	x	x	x	-	x	-	-	x	x	-	-	-
R-1044-33	x	-	x	-	-	x	-	x	-	-	-	-	-	-	-
R-1046-20	-	-	x	-	x	-	-	x	-	-	-	-	-	-	x
R-1047-4	-	-	x	-	x	-	x	x	-	-	-	-	*	*	x
Harinoso de Ocho															
R-1049-5	x	-	x	x	-	x	-	-	*	x	-	-	x	*	-
R-1049-6	-	x	-	-	x	-	-	-	*	x	-	-	x	*	-
R-1049-9	-	-	x	-	-	x	-	-	*	x	-	-	x	*	x
R-1049-10	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
R-1049-11	-	-	-	x	-	-	-	-	-	-	-	-	-	-	x
R-1049-16	-	-	x	-	-	x	-	-	*	-	-	-	x	*	x
R-1049-22	x	-	-	-	-	-	-	x	x	*	-	x	x	*	-
R-1049-25	-	-	x	-	-	-	-	x	-	-	-	-	x	*	-
R-1049-27	x	-	x	-	-	-	-	x	*	x	-	-	x	*	x
R-1049-28	x	-	x	-	-	x	-	-	-	-	-	-	x	*	-

tillers of the backcross progeny while the main stalk was selfed and backcrossed to the knobless stock again. Cytological determination of the knob constitution of 49 plants was made with aceto- or propiono-carmine smears. Table 1 lists which knobs were present in each of these plants. When only one knob was present on chromosome 5 in plants from crosses involving Zapalote Grande or Zapalote Chico, the placement of the knob in 5S or 5L may not be correct. When both knobs are present, it is apparent that the knob in 5S is not as close to the end of the chromosome as is the one in 5L.

Seed from self-pollinations and backcrosses to the knobless stock is available from the Department of Agronomy and Plant Genetics, University of Minnesota.

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2. Progress on big rings in corn.

Two rings of 10 were observed in F_1 plants from crosses between stocks homozygous for interchanges involving 3-2-4-9-10 and 1-5-6-7-8. No pollen was shed, but open pollinated ears set 0 to 6 seeds (ears with about 600 ovules). Backcrosses were made to both parents as the first step in establishing a line homozygous for both groups of interchanges.

Lines homozygous for 3-2-4-8-6 were established also and crossed with a 5-7-1-9-10 stock.

C. R. Burnham
R. L. Phillips
J. Stout

3. Chromosome 3 linkage test.

Tests between the W7748 albino and ba$_1$ failed to give any indication of linkage.

C. R. Burnham

The following reports are based on studies supported by N.S.F. Grant GB 1586 and GB 5543, Renewal of GB 1586. Those assisting in the work were Dr. Ronald L. Phillips, Dr. Gary R. Stringam, Joseph N. Neubauer, John T. Stout, and during the summer, Alan Novak.

4. Notes on the 2-6 interchanges.

We now have all but two of the 24 stocks listed plus two additional ones not listed. The following stocks listed as 2-6 interchanges in the 1961 Crops Research ARS 34-16 list of interchange break points are shown by linkage tests with lg gl B V_4 not to involve chromosome 2:4394, 6671, and 5648. The break points for three which do not have the breaks in 6L as listed are: 2-6 (027-4): 2L.1-6 org.; 2-6e:2S.18-6S.20; and 2-6 (5648): (not 2)-6S.19 .

C. R. Burnham
J. Stout
R. L. Phillips

5. Notes on the functioning of Dp-Df classes from interchange heterozygotes involving chromosome 6.

The following interchanges when heterozygous give a ratio of about 1 partially sterile: 2 fertile through the ♀ , probably a result of the functioning of one Dp-Df class:

Interchange	Probable Df-Dp
listed as 2-6 (4394) but does not involve 2	2S-6L
2-6 (001-15)	2S-6 sat.

Of the three 2-6 interchanges with the break in the nucleolus organizer, two have been tested. Both give normal 1:1 ratios, indicating that the deficiency which includes at least part of the organizer of chromosome 6 does not function.

C. R. Burnham
R. L. Phillips

6. Notes on the 1-5 interchanges.

We now have all but 7 of the 40 stocks listed in the 1961 ARS 34-16 publication by Longley. Seven of the stocks have not been checked in intercrosses or linkage tests. Multiple-point linkage tests that include bm$_1$ as one of the markers served as a test to determine genetically whether the break was in the short or the long arm of chromosome 5. The genetic data and the results of intercrosses agree on the following changes in placement of the breaks in chromosome 5. Cytological observations alone are the basis for the changes in positions made for chromosome 1. Those with breaks found to be in a different arm from that listed:

6899	S.40 - L.10	not S-S
6197	S-S	not S-L
e	S.01-S.12	not L-L
7219	S.20-L.42	not L-S
a	L.64-L.49	not L-S
8041	L-S	not L-L

The information is not complete for 1-5 (6401).

J. Stout
C. R. Burnham

7. Chromosome pairing in intercrosses between stocks of interchange that involve the same two chromosomes.

Type 2a, interchange points in opposite arms in both chromosomes. In the intercrosses involving T1-5 interchanges, the frequencies of "pairs" at diakinesis ranged from 5 to 100%. When the interchange points in both chromosomes in both interchanges were at .4 or closer to the centromeres, the diakinesis configurations were all or mostly 10II. When one or more of the interchange points was at .5 or farther away from the centromeres, fewer of the configurations were pairs and more were chains, rings, or other types of associations of the 4 chromosomes. Complex configurations of 4 were observed which are probably the result of crossovers in both differential segments. Often these can be described only as a clump. Similar configurations in Pisum have been pictured by Lamm and Miravalle (1959, Hereditas). The frequencies

of pairs became progressively lower for intercrosses with breaks farther out on one or more of the arms.

At pachytene in intercrosses showing 15-30% of "pairs" at diakinesis, most of the cells had an association of 4 chromosomes with 2 +-shaped pairing configurations, one in each arm of the two chromosomes. When the breaks were at .4 or closer to the centromeres (100% "pairs" at diakinesis), "pairs" were also frequent at pachytene. In an occasional figure an association between the two in regions near the centromeres could be recognized. When certain of the interchange points were close to the ends, only one "cross" was observed in many of the figures. Although no intercross combination has been studied in which the 4 breaks were close to the ends, one can predict that in the resulting "pairs," homologous ends would not be paired.

The evidence from the intercrosses in which the interstitial segments are relatively short, with breaks at .4 or less, indicates that pairing is not initiated at the centromeres. If it were, the "pairs" observed in these intercrosses would then be associated homologously in the middles. This is not the case. However, when the interchanged segments are short, pairing may be initiated on either side of the break points. There may be several sites at which pairing may be initiated. In the type 1a intercrosses, the centromere is not one of these sites.

Hence, in maize the "intercross method" of locating break points proposed for barley by Kasha and Burnham, 1965 (Canad. J. Genetics and Cytol.) fails for the Type 1a intercrosses when one or more of the interchange points is at .5 or greater. Interchanges with breaks known to be at distal positions in the chromosomes are needed in barley to test the method.

J. Stout
C. R. Burnham
R. L. Phillips
J. Neubauer

8. Crossing over in intercrosses involving T5-6c.

The T5-6c (5L 0.89-6S 0.0) stock homozygous for $\underline{bm_1}$ $\underline{pr}$, $\underline{ys}$, $\underline{v_2}$, $\underline{y}$ was crossed with five T5-6 stocks with breaks in 5S and 6L, i.e. the opposite arms, or Type 1a intercross. The F_1's were backcrossed to $\underline{bm}$ $\underline{pr}$ $\underline{ys}$ $\underline{y}$, in some cases $\underline{v_2}$, in others $\underline{v_2}\,\underline{v_2}$. Crossovers in both of the between-breaks segments in chromosome 5 and in 6 will result in two kinds of combinations, one with normal chromosomes 5 and 6 and one in which chromosomes 5 and 6 carry both interchanges. These will appear among the backcross progeny as fertile and semisterile plants, respectively. The latter is the type desired for the new marker method described below, but cannot be distinguished from the non-crossovers which are also semisterile. The frequencies of the fertiles from 5-6c crossed with five 5-6 (S-L) interchanges are shown in the following tabulation:

	5S-6L parent	Break position in chromosome 5	Break position in chromosome 6	Total plants	% fertile**	Recombination values bm-pr	Recombination values pr-ys	Recombination values total plants
1.	5-6 (5622)	S.87*	L.47*	376	0.8	24.1	9.6	270
2.	5-6 (6522)	S.87	L.7	400	19.0	36.2	7.5	213
3.	5-6 (4933)	S.23	L.89	420	26.3	16.3	11.6	190
4.	5-6 (5765)	S.19	L.32	381	0.0	5.4	3.1	353
5.	5-6 (5906)	S.28*	L.28*	408	3.6	36.4	4.1	406
Checks 5-6c/bm pr ys						24.8	13.3	1030
Checks N/bm pr ys						17.5	11.2	268

*Phillips (1966).
**The other plants were all semisterile.

The two intercrosses that had 19 and 26% of fertile plants are the only crosses in which a long differential segment (in this case, also interstitial) in 6L was available for the crossing over needed for the recovery of the crossovers in the differential segment in 5. In the other three intercrosses, the interstitial segment in 6L is a region in which crossing over frequency is low.

Recombination values in the pr-ys region are all lower than for the 5-6c check, some considerably lower. In the bm-pr region, two are high, two low and one about the same as the T5-6c check. Since single crossovers within only one of the differential segments are not recovered, one might expect reduced recombination within those segments, and this in turn to be related to the recovery of fertile progeny. No explanation of the data can be offered at present.

C. R. Burnham
J. Stout

9. Crossing over in intercrosses involving T1-5 interchanges.

Intercrosses in which the parents had genetic markers for chromosome 5, mostly pr ys_1 yg_1, were backcrossed to the multiple recessive. The frequencies of fertile progeny for three of the crosses are as follows.

	total plants	% fertile
1-5 (4597) x 1-5 (5525)	446	18.6
1-5 (5045) x 1-5 (4597)	151	0.7
1-5 (4597) x 1-5b	542	0.0

Since many fertile plants were observed in one of the crosses, one would expect to recover the complementary semisterile class which carries both interchanges on the same two chromosomes. The recombination values were similar to those in the check, but the data are limited, since only part of the crosses segregated for the 3 markers.

C. R. Burnham
J. Stout

10. A new method of using interchanges as chromosomal markers.

The single interchange stocks that have been used in the past for genetic analysis of complex characters require a large number of stocks for an adequate coverage of the chromosomes. In the test with each interchange only two of the four arms of the chromosomes involved in the interchange are marked with breakpoints. If the two chromosomes had a second interchange with breaks in the other two arms (breakpoints in each of the four arms of the two chromosomes), one should be able to detect the presence of any gene located in those two chromosomes. A method of developing such stocks is as follows: Cross two interchange stocks that involve different arms of the same two chromosomes and then cross the F_1 with a standard normal stock. Progeny that come from crossovers in the two differential segments, and which bring the two interchanges together in the same two chromosomes, would be semisterile. They could be distinguished from the semisterile noncrossover sibs by testcrosses with the parental interchange stocks, and established as a double interchange stock that is homozygous. These could be used in linkage tests in a manner similar to that used for the single interchange stocks. In backcross linkage tests, the parental classes would be semisterile or fertile. Single crossovers in either of the differential segments would not be recovered. Also even when single crossovers occur in both differential segments, both complementary crossover classes are semisterile, and hence only one is recognizable as a recombinant. This should furnish a more efficient test for linkage.

A set of five such double interchange stocks, e.g. 1-2, 3-4, 5-6, 7-8 and 9-10 should completely test the ten chromosomes and narrow the location of a major gene to two chromosomes. A set of nine such double interchange stocks in which one of the two chromosomes interchanged was the same, e. g. 1-9, 2-9, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9 and 9-10, should locate a major gene to an individual chromosome. If on 9, it should show linkage with all or most of the stocks in the series.

An extension of the method to 3-chromosome interchange stocks is possible also. As an illustration, if there are three interchange stocks T1-2 (1S-2L), T1-7 (1L-7L), and T2-7 (2S-7S), the cross of T1-2 x T1-7 will have a ⊙ 6 at meiosis. Crossing over in the differential segment of chromosome 1 can give rise to a combination of chromosomes (T1-2 + T1-7) that includes both interchanges. This stock marks arms 1S, 2L and 7L, but does not mark arms 2S and 7S. If T2-7 (S-S) could be added, all arms of the three chromosomes would be marked. This can be accomplished by producing a second 2-interchange stock, e. g. 1-7 + 2-7. If

T1-2 + 1-7 is crossed with T1-7 + T2-7, crossing over in the differential segment in chromosome 2 will produce a new chromosome which in one combination of chromosomes will include the three chromosomes with the six arms marked by interchanges.

C. R. Burnham

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1. On the status of the Stadler-Uber "r-x_2" deficiency.

The "r-x_2" deficiency is known to be transmitted only through the egg and includes the r locus. What has not been established is whether it is terminal or intercalary, and what other loci are situated in the segment concerned. Individuals having the constitution G R Sr_2/ ? r-x_2 were employed as female parents in a cross involving g r sr_2 males. None of the plants obtained from the colorless aleurone kernels was striate or golden. Thus the genetic data indicate that this deficiency is intercalary and does not include the g and the sr_2 loci.

Gary Y. Kikudome

2. Aleurone color intensity.

During the course of an experiment to synthesize altered abnormal chromosomes 10 through the action of maleic hydrazide, the following observations were made: When R Sr_2 K10/ r Sr_2 k10 females were pollinated by yg c sh wx/ yg c sh wx; R/R and wd C Sh wx/wd C Sh wx; R/R pollen, both deeply colored and pale colored aleurones were obtained. The frequency with which the pales were obtained was in agreement with the expected value for r transmission through the female. In fact all of the pales appear to exhibit a mottling phenotype. The pales will be tested for verification. When the R K10 chromosome was involved in the production of r/r/R aleurone, the color produced was deep.

One possible reason for the appearance of the pale colored aleurone is the existence of several R (S component) alleles, each having a different degree of efficiency in color production or expression. Not to be ignored is the possibility of induction of factor(s) by maleic hydrazide which influences color expression. Tests are being constructed to determine the cause of paleness.

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1. Two new B-type translocations.

A B-type translocation with breakpoint proximal to $\underline{d}_1$ on the short arm of chromosome 3 has been isolated. Cytological observations confirm the presence of the translocation, but further observations are required to determine the exact position. One known hyperploid ($3\ 3^B\ B^3\ B^3$) and three probable hyperploids gave the following progenies when crossed as males onto $+/\underline{d}_1$:

Male	Seedlings D	Seedlings d	Seedlings Total	Per cent hypoploids
1060-3	85	16	101	31.7
1060-6	88	15	103	29.1
1060-23	81	14	95	29.5
1060-63	89	14	103	27.2

Because the female tester was heterozygous for $\underline{d}_1$, the frequency of hypoploids was obtained by doubling the frequency of dwarf plants.

The long arm of chromosome 5 appears to be involved in another B-type translocation. The translocation appears to be proximal to pr, but genetic and cytological confirmation is still required.

J. B. Beckett

2. Pollen selection experiments.

The pollination media reported last year (News Letter 40:108) permit tests of selection techniques parallel to the enrichment procedures that are used in microorganisms. An extensive series of trials was made this year; the most interesting of the results are presented below.

Selection for "resistance" to the media was tested on a pilot scale. Following self-pollination with a given medium, seed sets on controls (no previous selection) were compared with seed sets on plants derived from pollinations made with the medium in the previous generation. Out of 12 pairs of comparisons (differing in medium or dilution or exposure

time), 4 showed slightly higher average seed set in the once-selected group, 5 equal (no seeds in either), and 3 lower set in the selected than in the control group. However, of 11 ears with high set (over 15 kernels), 7 were in the once-selected class. Among aqueous-medium trials, 5 ears with high set were found out of 49 tests in the once-selected group, as compared with 0 out of 50 in the controls. Indications are strong that resistance to exposure to the aqueous medium and to dilution (as high as 100 ml aqueous medium to 1 ml pollen) can be selected.

Tests for selection involving mutants with known biochemical effects ($\underline{o}_2$, $\underline{fl}_2$, $\underline{sh}_2$, $\underline{sh}_1$, $\underline{su}$, $\underline{wx}$) were carried out with a series of agents chosen for their relations to the biochemical effects. An emulsion medium (aqueous medium: Tween: paraffin oil, 30 : 0.1 : 30) was used to dissolve the agents; 6 ml of the solution was mixed with 1 ml of pollen and used to pollinate 3 or more ears. In retrospect, the emulsion was a poor choice, since the pollen grains would be exposed variably, rather than uniformly, to the dissolved agents. For $\underline{fl}_2$, pollen from $\underline{+/fl}_2$ was suspended and used to pollinate $\underline{+/+}$, as suggested by O. E. Nelson, because the dominant floury expression of $\underline{fl}_2$ is classified more clearly in a single dose. The other 5 mutants were testcrossed onto recessive females. The results were highly variable, but they do suggest that the method may be successful with solutions in uniform media (i. e., oil or aqueous). The most promising indications (though requiring confirmation) for each mutant were as follows (numbers following agents are ppm):

Mutant	Favors wild type	Favors mutant
$\underline{o}_2$	DL methionine 1000 anthranilic acid 100	DL tryptophan 10000 DL 5-fluorotryptophan 1000
$\underline{fl}_2$	DL 5-fluorotrytophan 10	L arginine 10000 DL ethionine 1000
$\underline{sh}_2$	2, 4-dinitrophenol 10	
$\underline{sh}_1$ $\underline{wx}$	5-hydroxy-DL-tryptophan 100	DL 5-fluorotryptophan 1000 oligomycin 10
$\underline{su}$	pyridine-3-sulfonic acid 1000, oligomycin 10	gibberellic acid 5000 DL 5-fluorotryptophan 1000

In a broader series of tests, 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 $\underline{r}$ was suspended and used on testers for each marker. Either the known marker or unknowns (linked, heterozygous) could result in deviant ratios due to selection. The most striking deviations for one or more markers were found with L arginine 10000, acridine orange 100, gibberellic acid 5000, lysine 10000, and quercitrin 5000.

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3. Chemical treatment of pollen in vivo by the plastic bag method.

In the course of recent chemical mutagenic investigations (G. Ficsor, Ph.D. thesis, University of Missouri, 1965) indicator dyes were used to compare various methods of chemical treatment of pollen in vivo. The routine was to treat pollen by the various methods with a 0.1 per cent solution of methylene blue (MB) followed by visual examination of the plant and by microscopic examination of some anthers in quest of colored pollen grains. Five methods of pollen treatment were compared: treatment through leaf uptake, by stem or tassel injection, by the wick method, by the cut tassel method and by the cotton packing injection method. The result was that all methods stained the vascular systems to some degree, but only with the cotton packing injection method were a few colored pollen grains observed. Four of the five methods were also tested genetically, using the mutagen ethyl methanesulfonate (EMS) in place of MB to determine if the method which produced colored pollen grains would also produce increased mutation rates. The result was, that only with the cotton packing injection method were mutation rates increased significantly (Science 139, p. 1296, 1963). Thus one finds a close agreement between the penetration of methylene blue in the preliminary trials and the mutagenic efficiency of EMS with the various methods of treatment. But even with the cotton packing injection method, mutation rates were much lower than obtainable with optimum doses of x-rays in spite of the fact that in seeds EMS was capable of inducing mutation rates several times higher than x-rays (MNL 37, p. 104, 1963). In search for clues to explain the chemical's low mutagenic efficiency in the pollen it was noted that with the cotton packing injection method the tassel received uneven treatment due to the flow of MB or EMS solutions to the bottom of the pack. Moreover, with MB one could detect differences between florets in degree of pigmentation, in that younger florets were penetrated better than older ones which were protected by well sealed waxy glumes. Also, the leaking of the pack made the method risky to use with toxic chemicals.

To overcome these difficulties a new method of treatment was developed using 0.1 per cent MB as indicator with a few drops of Tween 20 as a wetting agent to break down the waxy protection of the glumes. As a first step the tassel to be treated was stripped of leaves exposing the upper part of the last internode. Then a long, narrow, seamless plastic bag was pulled down over the tassel (just like a tassel bag) and its mouth was firmly tied around the stem with a strong string so that all possibility of leakage down the stem was excluded. The dye was then poured in from the top of the bagged tassel through a 1" to 2" cut in the bag. The volume can be adjusted on the tassel by wrapping it with adhesive tape until the desired volume is obtained. In our experience 50-150 ml of solution was needed to submerge the tassel completely. Before pouring in the chemical the tassel should be supported with a stick to prevent it from breaking. Within 3 hours of treatment all the pollen grains examined from any part of the tassel and from any floret were stained, indicating that the difficulties of the cotton packing injection method were overcome.

Mutagenic tests with the plastic bag method have not yet been completed but it is expected that the correspondence found previously between indicator dye tests and actual mutagenic experiments will hold for this method as well.

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1. Classification of maize lines and selection of breeding materials by the application of multivariate statistical analysis.

Classification of local maize lines and selection of breeding materials were successfully carried out by the application of principal component analysis. Biological meanings of the extracted principal components in this study and the classification of the lines on four principal component axes were discussed in relation to plant breeding.

Materials used in this study were the data reported on the characteristics of 57 local Caribbean flint lines collected from Shikoku, Japan (Suto et al, unpublished). A part of the data were preliminarily reported in M.G.C.N.L. 33:84-88 . Out of 65 botanical and agronomical characters observed, we selected ten characters which were of significance in plant breeding, and among which correlation coefficients were not so high. They were silking date, stalk length, leaf length, number of leaves, tassel length, ear length, ear diameter, ear weight, number of husks and 100 grains weight.

The correlation matrix of these ten characters was calculated, following principal component analysis. Twenty-eight, twenty-two, nineteen and thirteen per cent of the total variation of ten characters were accounted for by the first four principal components respectively, and hence more than 80 per cent could be explained in total (Table 1).

Table 1
Eigen values (λ_j) and associated eigen vectors (l_{jk}) obtained from principal component analysis

Principal component	1	2	3	4	5	6	7	8	9	10
Eigen value (λj)	2.825	2.139	1.819	1.248	0.634	0.530	0.340	0.224	0.179	0.062
$\Sigma \lambda j$	2.825	4.964	6.783	8.031	8.665	9.195	9.535	9.759	9.938	10.000
Eigen vector	l_{1k}	l_{2k}	l_{3k}	l_{4k}	l_{5k}	l_{6k}	l_{7k}	l_{8k}	l_{9k}	l_{10k}
Silking date	.457	-.286	-.034	.199	.026	-.178	.558	.198	.525	.101
Stalk length	.497	-.083	-.102	.189	.273	.302	-.453	-.384	.349	-.244
Leaf length	.460	.155	.253	-.206	-.096	-.151	-.475	.613	-.155	.020
Number of leaves	.412	-.253	-.334	.162	.254	.030	.202	-.031	-.698	.187
Tassel length	.241	.046	.447	-.530	-.212	-.046	.087	-.591	-.235	.014
Ear length	.213	.269	.374	.462	-.261	.408	.400	.128	-.098	-.329
Ear diameter	.134	.423	-.478	-.084	-.142	-.449	.099	-.090	-.056	-.570
Ear weight	.193	.580	-.171	.215	-.185	-.043	-.005	-.175	.155	.679
Number of husks	-.037	.234	-.361	-.551	.027	.685	.093	.176	-.005	-.008
100 grains weight	-.055	.422	.291	-.068	.827	-.113	.177	.030	-.002	-.023

Table 2

Average squared distance between varieties and within a variety classified by using principal component analysis and squared distance between lines[1] in the four-dimensional space

Variety	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
Number of lines	4	1	5	6	15	3	3	3	1	5	5	3	2	1
V1	3.5[2]	9.8	12.5	16.8	14.6	35.8	30.8	33.5	51.6	33.2	28.8	38.4	44.0	26.8
V2		0	22.0	16.2	15.3	29.5	25.9	41.5	46.6	35.0	45.6	54.1	46.2	50.6
V3			4.2	12.7	8.9	21.5	23.5	23.0	27.3	12.2	11.2	13.2	16.9	34.1
V4				4.6	9.3	11.7	19.0	18.2	14.9	20.8	20.3	21.0	20.6	32.2
V5					4.3	10.3	8.6	13.4	22.0	10.1	14.0	22.5	24.6	32.2
V6						4.0	8.1	11.3	8.2	12.5	19.3	24.4	22.8	45.9
V7							4.0	10.1	25.3	13.4	21.3	35.8	40.1	40.1
V8								2.3	22.4	18.1	11.1	24.2	39.1	19.4
V9									0	19.8	24.1	17.2	11.9	61.5
V10										4.2	11.5	16.9	17.4	52.2
V11											4.2	8.8	22.6	26.5
V12												3.9	10.0	43.0
V13													4.7	71.9
V14														0

1) Squared distance between lines was calculated from the scores of lines for the first four principal components

2) Figures in the diagonal indicate average squared distances within a variety

So as to classify the lines into line groups or varieties having similar characteristics, squared distances between lines in the four-dimensional space were calculated from scores of lines for the first four principal components. The smaller the squared distance between lines was, the more similar the characteristics of lines were expected to be. So the lines among which squared distances were very small were grouped as a variety. The criterion of grouping lines was that the average squared distances within a variety were always smaller than ones between varieties. In consequence, 57 lines were classified into 14 varieties (Table 2).

The classification based on the principal component analysis and distance method agreed generally with the previous one based on the conventional method. Furthermore, these varieties were classified into four major varietal groups by using the same procedure mentioned above (Table 3).

Table 3
Average squared distance between varietal groups and within one

Varietal group	A	B	C	D
A	6.0	22.2	37.4	31.5
B		11.4	18.9	38.1
C			10.6	40.5
D				0

Note: Varieties belonging to the respective varietal groups are as follows: A (V1-V2), B(V3-V10), C(V11-V13), D(V14)

These calculations were made on electronic computer OKITAC 5090C.

For the purpose of understanding the relation between characters and principal components, the characters were assorted into three classes, plus, minus and zero (in Table 4, class zero was omitted).

Table 4
Identification of characters by the degree of contribution to the first four principal components

Principal component	Class[1)]	Corresponding character
Y1	+	Stalk length, leaf length, silking date, number of leaves
	-	None
Y2	+	Ear weight, ear diameter, 100 grains weight, ear length
	-	Silking date, number of leaves
Y3	+	Tassel length, ear length, 100 grains weight, leaf length
	-	Ear diameter, number of husks, number of leaves
Y4	+	Ear length
	-	Number of husks, tassel length

Note: 1) l_{jk} in Table 3. + ; $l_{jk} \geq 0.250$, - ; $l_{jk} \leq -0.250$

This assortment was based on the value of eigen vectors corresponding to the respective characters in Table 1. In the case of the first principal component, characters concerned with the size of vegetative parts of a plant and earliness, such as stalk length, leaf length, number of leaves and silking date, contributed greatly to the principal component. So the biological meaning of the first principal component appeared to correspond to the general size of vegetative characters of a plant in relation to the duration of the growing period. In the case of the second principal component, the characters concerned with grain yield and earliness, such as ear weight, ear diameter, ear length, 100 grains weight, silking date and number of leaves, contributed greatly to the principal component. So the biological meaning of the second principal component appeared to correspond to the yielding ability, especially to the efficiencies of photosynthesis and translocation in the plant. By a similar consideration of biological meaning, the third principal component appeared to correspond to the degree of differentiation in organs.

Corresponding to the respective principal components, several plant types, i.e. forms of variation represented by the compound characters, were made clear. The first principal component corresponded to an early and small plant type vs. a late and large, the second an early and high yielding plant vs. a late and low yielding one, and the third a plant with short conical ear and short tassel vs. one with long cylindrical ear and long tassel.

Varieties or lines which seemed to be most suitable for breeding materials could be selected by choosing the principal components which were important in connection with the breeding objectives. In this study the first and the second principal components were most important in connection with the objectives of breeding early and high yielding hybrids.

Thus, classification of maize lines and selection of breeding materials were achieved by the application of principal component analysis and distance method.

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1. X-ray induced mutations during pollen tube growth in maize.

X-ray induced mutations in maize pollen were first discovered by Stadler (1928). Recently, the 24-28 hours old developing embryos in maize have proved to be a suitable system for a study of radiation- and chemical-induced mutagenesis (Singleton, 1961; Verma et. al., 1962; Caspar, 1965; Chatterjee et. al., 1965). However, very little is known about the frequency of X-ray induced mutation during pollen tube growth or early stages of fertilization. In order to obtain some information in this respect, ears having the recessive gene (su) were pollinated with Su pollen and were irradiated by X-rays at different times after pollination ranging from 0 to 30 hours. Total dose was 1210r $\pm$ 110r, with a dose rate of 96.1 r per min. (173 kVp, 25mA, 0.5^{mm}Cu + 0.5^{mm}Al filter, 45-50cm distance). Whole and chimeral endosperm mutations at the Su locus were scored in the kernels resulting from these pollinations and data thus obtained are shown in Figure 1.

The frequency of whole mutations increased rapidly (from 2.91% to 5.81%) when ears were irradiated 0 to 12 hours after pollination; the frequency decreased slightly (from 5.81% to 5.60%) when X-irradiation was given 12 to 18 hours after pollination; the frequency declined rapidly (from 5.60% to 3.41%) from 18 to 30 hours after pollination. On the other hand, the frequency of chimeral mutations increased gradually (from 0.83% to 1.39%) when treatment was in the interval from 0 to 12 hours after pollination; the frequency increased rapidly (from 1.39% to 3.94%) in the interval from 12 to 30 hours after pollination. The data for the per cent of chimeral mutations are summarized in the following table:

	Hours after pollination					
	0	6	12	18	24	30
% chimeral endosperm mutations	21.6	18.3	19.5	28.1	41.0	50.4

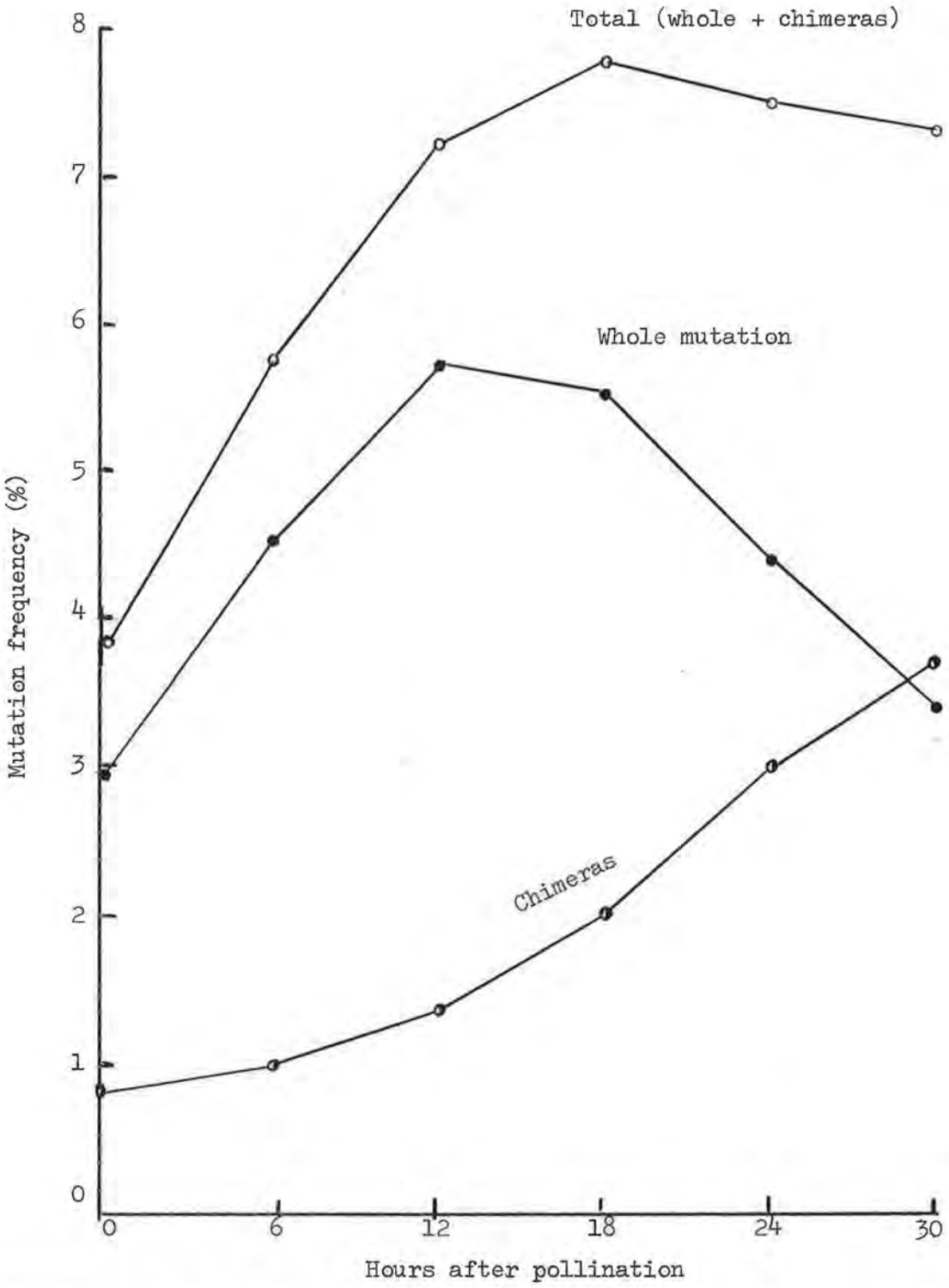

Figure 1. Mutation frequency resulting from X-irradiations during pollen tube growth in maize.

It is significant to note that total mutation frequency when ears were irradiated 12 hours after pollination increased two-fold over the frequency found with ears irradiated immediately after pollination.

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1. The genetics and environmental interaction of a new pale midrib mutant.

A new chlorophyll mutant, tentatively designated pale midrib-2, was found in 1965 at The Pennsylvania State University in a line of "supersweet" corn. The leaves of the affected plants are green at the margins, with the midrib and adjacent tissue chlorotic from the base of the leaf to the tip. In the region near the margin the chlorosis is in the form of a fine stripe so that there is a gradation of yellowing from the completely green tissue to the completely yellow chlorotic tissue. In the field-grown plants the trait did not appear until about a month after planting. However, plants germinated in a growth chamber segregated for the chlorosis at the time of germination. The expression of the trait by the plants grown under artificial light closely resembled that of field-grown plants.

The two original mutant plants were selfed and outcrossed to inbred W153R. The following summer seeds from these pollinations were planted and both were found to segregate for the pale midrib character. The selfs resulted in 11 green and 24 pale midrib plants while the outcrosses resulted in 46 green and 31 pale midrib plants. Furthermore, among the chlorotic plants in the population resulting from selfing, six plants appeared to be more severely affected than the others. These data suggest that the expression of this character is controlled by a single incompletely dominant gene and that the severely affected plants were perhaps homozygous for the pale midrib gene. Because of the inbred background of the shrunken-2 line from which this mutant was obtained, the progeny from the selfing lacked vigor. The chlorotic plants were even less vigorous and the severely affected plants were extremely stunted and did not set seed. However, the mutant plants which resulted from the outcrosses were quite vigorous, with excellent seed set.

Seeds from the above selfs and outcrosses were grown in a growth chamber on a 16-hour photoperiod and on several temperature regimes. When the night temperature was 15°C and the day temperature was maintained either at 21°C or 26°C, 1:3 and 1:1 segregations, similar to those observed in the field, were obtained. It was also possible under these conditions to distinguish mildly and severely affected plants. However, when the day and night temperatures were held at 29°C, the progeny of the self

pollinations segregated in a ratio of three green to one pale midrib and the seedlings from the outcrosses were all completely green. These data are summarized in the table below.

	Temperature	Expression: Green	Expression: Pale midrib	Ratio	p
Selfed	Field	11	24	1:3	0.40
	21°-26°C day 15°C night	12	21	1:3	0.10
	29°C day and night	25	7	3:1	0.70
W153R Outcross	Field	46	31	1:1	0.08
	21°-26°C day 15°C night	19	14	1:1	0.35
	29°C day and night	48	0	---	----

It appears that at higher temperatures such as those encountered in the field, the pale midrib-2 gene acts either as a dominant or an incomplete dominant, while at lower temperatures it acts as a recessive. This difference may be controlled by the night temperature alone or by a combination of day and night temperatures. The expression of this mutant was compared to that of pale midrib-1 and was found to be considerably different. The expression of the recessive pm_1 is characterized by white streaks in the vicinity of the midrib. Allele tests and chromosome location studies are presently being conducted.

David K. Shortess

2. Inheritance, environmental and preliminary linkage studies of the lutescent maize mutant.

It had previously been reported (MNL 39:146) that the expression of the lutescent character in maize resulted from a single recessive gene, probably located on chromosome 5. The location of a lutescent gene locus on the fifth chromosome has been confirmed. However, the expression of the character appears to depend on two recessive genes rather than on one. Furthermore, the expression of this trait was found to be temperature dependent.

The original lutescent material was outcrossed to two inbred lines, Oh51A and Pa423, and to one single cross, Pa32 x CMD5. F_2 and testcross populations were grown in Pennsylvania in the field and in the greenhouse in January and April, and in a growth chamber on a 16-hour photoperiod. The

temperatures maintained in the growth chamber were (a) 21°C day and 15°C night, (b) 26°C day and 15°C night and (c) 29°C day and night. Linkage studies were initiated using chromosome 5 markers $\underline{a}_2$, $\underline{bt}_1$ and $\underline{pr}$.

F_2 and testcross populations resulting from the several outcrosses did not provide consistent ratios of segregation. These data are summarized in the table below.

Outcross	Population	Phenotype		Ratio	p
		Green	Lutescent		
Oh51A	F_2 (all)	136	9	15:1	0.90
	Testcross (all)	372	138	3:1	0.30
Pa32 x CMD5	F_2 (all)	39	4	15:1	0.50
	Testcross (all)	110	42	3:1	0.40
Pa423	F_2 (field)	63	7	15:1	0.40
	F_2 (greenhouse or growth chamber)	94	31	3:1	0.95
	Testcross (greenhouse or growth chamber)	50	41	1:1	0.35
Waxy-marked translocations	F_2 (field)	259	78	3:1	0.45
Chromosome 5 markers	F_2 (greenhouse)	369	111	3:1	0.50

The segregations resulting from the Oh51A and the Pa32 x CMD5 outcrosses indicate that the lutescent expression depends on two recessive genes. These data include field, greenhouse and growth chamber populations. On the other hand, the F_2 populations resulting from the crosses with the waxy-marked translocation series, reported previously, produced a 3:1 segregation, suggesting a single gene. These were field-grown. The segregations of the progeny from the Pa423 outcross appeared to depend on the environment. When grown in the field, a 15:1 digenic F_2 segregation resulted, while in the greenhouse or growth chamber monogenic 3:1 F_2 and 1:1 testcross segregations were observed. The crosses between the chromosome 5 markers and the lutescent mutant resulted in a 3:1 segregation of normal to lutescent when grown in the greenhouse. However, when this material was grown in the field, a segregation of 2031 green to 298 lutescent plants were observed. This approximate 7:1 ratio suggests a combination of mono- and bigenic inheritance.

It is concluded from these data that two recessive genes are necessary for the expression of the lutescent character. They have been designated $\underline{lu}_1$ and $\underline{lu}_2$. One of these appears to be homozygous in some maize lines such as the waxy-marked translocation series. Furthermore, in certain genetic backgrounds, one of these genes (not necessarily the same one) appears to be affected by those growing conditions which differ between the field and the greenhouse or growth chamber.

Environmental studies carried out in a growth chamber indicate that temperature is a factor in the overall expression of the lutescent mutant. F_2 and testcross populations grown at 21°C day and 15°C night temperatures could not be scored visually. All plants appeared green. At 26°C day and 15°C night some plants in these populations were slightly paler than others, although it was not possible to determine clear-cut patterns of segregation. At 29°C, the lutescent expression was unmistakable and the populations could be scored with relative ease. These observations paralleled those obtained in the greenhouse. During the winter months when the temperature seldom rose above 21°C, F_2 and testcross populations could not be scored, while those populations planted in the greenhouse in the spring, when increased sunlight brought higher temperatures, could be scored. The expression of the lutescent trait under these conditions was not as intense as that observed in the field, however. To date all field work on this mutant has been carried out in Pennsylvania. It will be interesting to observe the effect of the New Mexico summer on the expression of this trait.

The small F_2 greenhouse population from the cross of lutescent with a stock carrying several chromosome 5 markers gave a definite indication of linkage between $\underline{lu}_1$ and $\underline{a}_2$, $\underline{bt}_1$ and $\underline{pr}$. Because the population was small, no accurate map distances could be calculated. However, based on the tables of Immer, the closest linkage appeared to be with $\underline{a}_2$.

David K. Shortess

3. A low temperature-chlorosis effect in Oh51A.

When seeds of inbred line Oh51A were germinated at low temperature, the leaves formed during the germination were completely devoid of chlorophyll. Seeds were planted in trays of moist vermiculite and kept in a cold box in the dark at 11±1°C for 21 days. By this time the coleoptiles were from 1 to 3 cm long and the trays were transferred to an illuminated growth chamber at about 29°C. The initial leaves which subsequently developed were completely chlorotic, while the second leaves were chlorotic at the tips. The chlorotic regions did not become green but remained very pale yellow and eventually died. Those leaves which formed later at the higher temperature were all green. Oh51A plants germinated in an illuminated growth chamber maintained at 10°C displayed the same pattern of chlorosis, so that temperature rather than light appeared to be the determining factor.

A number of other lines listed below, including both eastern and Texas inbreds, were tested for this low temperature effect.

A509	Pa11	Tx61M	Tx303	Tx601
CMD5	Pa32	Tx102A	Tx305	Tx602
Mich1334	Pa36	Tx127C	Tx403	W37A
NY16	Pa54	Tx203	Tx585	W153R
NYD410				W182B

In no case did a chlorotic condition result from the germination at the low temperature. All seedlings appeared to be normally green. Both F_1 and F_2 populations of crosses in which Oh51A had been a parent were available. The other parent was a U.S.D.A. plant introduction which did not result in a chlorosis from the low temperature treatment. When 13 F_1 and 44 F_2 seeds were germinated at low temperature, all resulting seedlings were green. Apparently there is not a simple genetic explanation for this phenomenon.

The Oh51A seeds used in this study were obtained from the Pennsylvania Agricultural Experiment Station. I am in the process of obtaining seeds of this inbred line from a variety of sources in order to determine whether or not this is a universal trait of this line.

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1. Surface waxes of maize.

Preliminary investigations have begun on the surface waxes of maize. Surface waxes are extracted from the leaves by immersion in chloroform for 15 seconds. Several other lipid solvents have proven equally effective but a personal preference exists for chloroform. Immersion time can be varied; however, if too long, internal lipid extraction will also occur. For this same reason, broken or cut ends of leaves are avoided. Inbred lines, CI 21, Kys and T 204 were used as the normal or wild type for maize while 13 stocks carrying different glossy genes represent the mutant types. The 13 different glossies were $\underline{gl}_1$, $\underline{gl}_2$, $\underline{gl}_3$, $\underline{gl}_4$, $\underline{gl}_5$, $\underline{gl}_6$, $\underline{gl}_7$, $\underline{gl}_8$, $\underline{gl}_9$, $\underline{gl}_{11}$, $\underline{gl}_{14}$, $\underline{gl}_{15}$ and $\underline{gl}_{16}$.

Initial analysis of the surface lipid constituents has been by thin layer chromatography. The extract, after reducing by evaporation, has been spotted on silica gel G plates and developed in benzene for a distance of 10 cms. Spots were located by spraying with 50% sulfuric acid and heating on a hot plate for about ten minutes. Four spots are located by this technique when the sample extract was taken from a seedling in the two or three leaf stage. Two of the spots have been identified as fatty acids

and paraffins by co-chromatography of knowns. Positive identification has not been made of the remaining two spots, but they are tentatively identified as primary alcohols and either ketones or esters. These four spots have been found in extracts from all the normal and glossy seedlings. However, the glossies in general produce much lighter spots than the normal inbred lines. Thus, a quantitative difference was found between the normals and glossies, but no qualitative differences were detected by this method. Based upon these results, the visual difference between normals and glossies lies in the relatively greater amount of surface waxes on the leaves of normal plants than on glossy.

Several other observations are worth noting. First, although the same four spots were developed from the 13 different glossies, a wide range of quantitative differences was noted. It seems apparent, therefore, that the various glossy genes are acting at different sites in the synthesis of surface waxes. Secondly, glossies exhibit their mutant phenotype while in the seedling stage and ultimately develop a normal wax covering. A difference was evident in the rate of developing the normal wax complement among glossies. Lastly, leaves taken from plants at the time of anthesis developed six spots by the previously described chromatographic technique. Four of these were identical to those described for the seedling extracts, but the other two were new. Therefore, the surface waxes of mature plants are more complex than those of seedlings.

C. S. Levings, III

2. Application of plant hormones to cytoplasmic male steriles.

To determine the effect of hormones on cytoplasmic male sterile tassels, GA_3, IAA and kinetin were applied individually to male fertile and sterile plants of the inbred line T 204. Sterility was due to the Texas type male sterile cytoplasm. Treatment was begun at the onset of tassel differentiation (42 days after planting) and continued until tassel emergence. The late treatment start was chosen deliberately to coincide with the beginning of tassel development. Ten milligrams of hormone were pipetted into the plant whorl every 3 days. A season total of 60 milligrams of hormone was applied to each treated plant. Alterations of sterility or fertility were not detected on the treated plants when compared with appropriate checks. No differences were noted in plant height or shape between treated and untreated plants. Since similar quantities of GA_3 have been reported to cause misshapen plants, taller plants, tassel silks and pollen sterility when the treatment was initiated at the time of plant emergence, it is believed that the treatment was begun too late. Therefore, although the late treatment was ineffective, it is doubtful that an adequate test of hormone effects on pollen fertility and sterility has been performed.

C. S. Levings III

3. Random self-fertilization in a finite population of autotetraploids.

Wright (1938)* has derived the formula for determining F, the coefficient of inbreeding, for a finite population of monoecious autopolyploid individuals with random self-fertilization. In working with autotetraploid maize, the need arose to extend his formula to take into consideration alpha, the coefficient of double reduction. Double reduction does occur in autotetraploid maize, and therefore would contribute to inbreeding. The inclusion of alpha, double reduction, leads to the formula,

$$P_n = \frac{1}{6N} \left\{ (8N - 3 - 2N\alpha) P_{n-1} - (1 - \alpha) (2N - 2) P_{n-2} \right\}$$

where P is the panmictic index and is equal to 1 - F, N is the number of monoecious autotetraploid individuals, and n is the generation. When alpha equals zero, the above expression reduces to Wright's formula,

$$P_n = \frac{1}{6N} \left\{ (8N - 3) P_{n-1} - (2N - 2) P_{n-2} \right\}$$

If N equals one, complete self fertilization occurs.

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*Wright, S. The Distribution of Gene Frequencies in Populations of Polyploids, P.N.A.S. 24:372-377. 1938.

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Proposal: It is proposed that a collection of digital computer programs of value to practicing maize geneticists be started and maintained. Such a collection might encompass programs suitable for:

1. field notebook production, and plot arrangement and layout
2. statistical reduction of plot data
3. useful data manipulative procedures for geneticists (both common and somewhat uncommon).

The Computer Center, Ohio University volunteers to be the repository and distributive center for this, if desired. It should be noted, however, that a strong research effort in maize genetics is not extant here.

It is suggested that programs submitted to such a repository should be freely available for distribution and that the repository agency cannot be responsible for accuracy or correctness of the programs or algorithms. It is further suggested that only 'higher level' languages such as FORTRAN, ALGOL, or PL/1 be accepted.

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1. Analysis of factors controlling chiasmata in maize.

Evidence regarding the genotypic control of chiasmata in maize is provided by (1) differences between inbred lines and (2) the greater genetic rather than the environmental (error) component of variance (Rao, 1966: M.S. Thesis, Orissa University of Agriculture & Technology, Bhubaneswar, India). The genotypic control appears to be exerted by a polygenic system besides the few known major genes. Environment is divisible into at least two major components: (1) general or external environment comprising factors like temperature, nutrition, etc. and (2) special or internal environment to which meiocytes are directly exposed inside the plant body. While appreciable information is available on the action of the first component, much less is known about the contribution, if any, of the second component. One approach to this problem of the internal environment appeared to be offered by the observed asynchrony of PMC division in many lines of maize (Rao, 1966). For example, within individual anthers one could find (1) about half of the PMC's in pachynema-diplonema or earlier stages and half at diakinesis, (2) cells mostly in diakinesis, (3) half in diakinesis and the rest in later stages. In case the groups of anthers provided different extra-cellular environment for the PMC's at diakinesis, any difference in chiasma frequency should lend information about the role of the internal component, i.e. inter-cellular influences. The study undertaken with this objective appeared to reveal the operation of at least two further components of the 'internal environment': one temporal and related to the onset or progress of the stage of division, the other spatial due partly to interaction with the neighboring cells.

Eight inbred lines and three single crosses were taken for the present study. The total number of anthers examined from each line is indicated in Table 1. Chiasma frequency of PMC's at diakinesis was noted in aceto-carmine squash preparations. Anthers were grouped into three classes according to the frequency of division stages: (1) about 1:1, diakinesis: early stages, (2) mostly diakinesis, and (3) about 1:1, diakinesis: late stages. For convenience these will be referred to as Groups 1, 2 and 3, respectively.

From the data presented in Table 1, a regular trend may be noted in all inbreds except one (Ext 127). In these there is a decreasing order of

chiasma frequency: Group 1 > Group 2 > Group 3. Contrasted with this trend is the reverse picture in the two hybrids, Ext. 355 X Ext. 357 and Ext. 357 X Ext. 139, in which Group 3 > Group 2 > Group 1. But the trend in the remaining cross (Ext. 139 X Ext. 127) is comparable to that in the inbreds. Further comparison between the hybrids and their inbred parents in case of the two crosses showing the different trend reveals the following: (1) In Group 1 anthers chiasma frequency of the PMC's is higher in the inbreds than the corresponding hybrid, and (2) in Group 3 anthers the chiasma frequency is higher in the hybrid than the parents.

The three groups of anthers represent different stages of development of the anther, Group 1 being slightly early and Group 3 being relatively more advanced in development. Diakinesis in these groups would be correspondingly in the early, middle and late stages. One would then expect to find the changes associated with the progress of the division stage. One important change is terminalization. The observed decrease in chiasma frequency in inbreds may be partly due to terminalization as diakinesis advances. Besides this temporal factor, there must be some other factor(s) affecting observed chiasma frequency. For in the two hybrids, instead of a decrease as expected due to terminalization, there is a consistent increase. Even assuming that there is no terminalization (although some must have occurred as a normal event) in these two hybrids, the excess will still have to be accounted for. Whatever be the cause of this increase, it is apparent that two opposing factors control diakinesis chiasmata--one reducing the number through terminalization and the other tending to counteract it. Further, the relative effects of the two factors are different in inbreds and hybrids. While the first appears to predominate in most inbreds, it is obscured in some hybrids, in which the second appears to prevail. The rate and/or the degree of terminalization appears to be different in inbreds and hybrids in general.

The exact nature of the second factor can at best be speculated at this stage. The condition in which high frequency of chiasmata is observed would suggest the possibility that the neighboring cells in an advanced stage of division may be exerting an enhancing effect. It is perhaps simpler to view the role of the neighboring cells as negative rather than positive. In case there is any competition between meiocytes for any material limited in quantity but essential for chiasma formation, then PMC's entering into the appropriate division stage later than others may have more chiasmata due to lack of competition. The possible role of such a spatial factor along with the temporal one in controlling chiasma number needs further investigation. Since Group 3 anthers are likely to produce pollen earlier than Group 1 anthers, it may be expected that in many hybrids earlier formed pollen will yield recombinants in a greater frequency than later formed pollen. Whether this would apply to the eggs is doubtful, but perhaps worth exploring.

Table 1
Variation in chiasma frequency at diakinesis in anthers of Groups 1-3 (No. of anthers studied is given inside parentheses)

Line (#1 - #8 are inbred lines)	Anther group based on the frequency of division stages		
	Group 1 (diak. & early stages)	Group 2 (mostly diak.)	Group 3 (diak. & late stages)
#1 Nayagarh	18.73 (4)	18.38 (4)	18.09 (7)
#2 Ext 357	18.40 (2)	17.35 (4)	17.14 (5)
#3 Kenduguda	---	18.75 (4)	17.90 (4)
#4 Ext 139	---	17.38 (4)	17.34 (3)
#5 Jhadgan	18.47 (3)	18.20 (4)	17.50 (1)
#6 Jeypore	18.30 (1)	18.30 (4)	17.80 (3)
#7 Ext 355	19.00 (1)	18.63 (3)	--
#8 Ext 127	18.30 (3)	17.83 (4)	17.97 (3)
#9 Ext 355 X Ext 357	17.80 (1)	19.15 (4)	19.18 (4)
#10 Ext 357 X Ext 139	17.50 (2)	17.58 (4)	17.97 (3)
#11 Ext 139 X Ext 127	19.20 (1)	18.05 (4)	--
All inbred lines (Pooled)	18.52	18.10	17.72
All hybrids (Pooled)	18.00	18.26	18.66

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2. Dissociation of the nucleolus from the organizer site in a few lines of maize.

In the course of a study of the comparative cytology of inbred and hybrid maize particularly in relation to developmental homeostasis, the nucleolus was found to be dissociated from the nucleolus organizer in a few PMC's at diakinesis. The condition was not due to any mechanical damage during squashing, nor could it be considered as a normal situation associated with the late stage of diakinesis. In the PMC's exhibiting this abnormality, the nucleolus was found to be appreciably larger than those in the normal cells in the comparable stage of division. A survey of several local inbred lines and a few hybrids appeared to reveal this abnormality more frequently in the vigorous hybrids rather than inbreds. The frequency of appearance of this condition in different lines has been indicated in Table 1.

Table 1
Frequency of nucleoli dissociated from the organizer site at diakinesis in PMC's

	No. of spikelets studied	No. of PMC's examined	No. of PMC's showing dissociation		
			Wide apart	Slightly apart	Total
Inbred lines:					
Jhadgan	1	600	1	0	1
Gandasahi	8	4,790	0	0	0
Mahabirapur	10	8,956	0	0	0
Guali	6	2,240	0	0	0
Chheliguda	2	754	0	0	0
Hybrids:					
Ganga 101 (double-cross)	6	2,920	33	16	49
(105 X 101) X (115 X 111)	5	590	11	0	11
Kenduguda X Jhadgan	12	720	1	0	1

There are reasons to believe that the size of the nucleolus is directly proportional to its synthetic activity, particularly RNA and protein synthesis. Since the nucleoli dissociated from the organizer sites are often large, it is necessary to examine whether these cells and their nucleoli are more active in RNA/ protein synthesis, and whether this activity has any relationship to vigor. We are particularly interested to ascertain if synthetic activity can be used as a measure of combining ability of inbred lines. In case some kind of easy-to-detect morphological cellular manifestation (e.g. the nucleolar condition reported here) is related to synthetic activity and vigor, means would be provided for studying combining ability at the cellular level and thus to understand the cellular basis of the phenomenon.

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3. The nature of variation in some quantitative traits in terms of adaptation.

The contention that heterozygosity would lead to developmental stability or homeostasis at least in outbreeding species has been examined by us in respect to several quantitative characters including different aspects of meiotic chromosomal behavior. These studies have revealed that heterozygosity per se may not ensure developmental homeostasis. But the phenomenon is more likely to be encountered in heterozygotes rather than homozygotes. Our interest in the study of phenotypic variation in inbred

and single-cross hybrid maize (MNL 40:119-120) has been partly prompted by the opportunity that this study would provide to understand the biological significance of variation in different characters in terms of adaptation. By comparing the magnitude of variation in a large number of inbreds and hybrids it may be possible to ascertain whether (1) the variation in a particular trait is a reflection of developmental instability arising from 'accidents in development' and without having a role in adaptation, or (2) the variation has an adaptive significance so that it is a measure of 'developmental flexibility' arising in adapted individuals in response to the variable environment. In the present report we will refer to these as Type 1 and Type 2 variation, respectively. In case of characters showing Type 1 variation, the variance in hybrids (average of a number of hybrids) would be expected to be less than that in inbreds; and in case of traits showing Type 2 variation the reverse would hold good.

To begin with, we have focused attention on (1) seedling traits and (2) certain aspects of meiotic chromosomal behavior. We have purposely chosen the former in order that the experiments can be repeated as often as necessary and if required under varying conditions and thus the premise underlying the operational approach to understanding variation can be put to a rigorous test.

Tentative inferences regarding the nature of variation (Type 1 or 2) in several traits are indicated below (Table 1).

Table 1

Comparisons of phenotypic variances in inbreds and hybrids. (The figures represent average values of squared coefficients of variation)

	Inbreds*	Hybrids**	Remarks (Type 1/Type 2 variation)
(a) Seedling traits:			
Radicle length	2508	1114	Type 1
Average length of seminal roots	3924	1441	Type 1
Coleoptile length	519	304	Type 1
Mesocotyl length	1174	1460	Type 2?
Length of the first leaf	897	403	Type 1
Number of seminal roots	2029	956	Type 1
No. of vascular strands in radicle	218	247	??
(b) Chromosomal traits:			
Chiasma frequency per PMC at diak.	0.0012	0.0011	??
Sixth chromosome chiasma frequency (per PMC at diak.)	0.0141	0.0095	Type 1
Univalent frequency	0.3341	0.1715	Type 1

*Average of 6 inbreds in case of seedling traits; average of 4 in case of

chromosomal traits.
**Average of 6 hybrids (including reciprocal crosses) in case of seedling traits; average of four (including reciprocals) in case of chromosomal traits.

It is evident that for most of the traits studied the variation is due more to developmental instability rather than to adaptive changes. For three characters it is difficult even to make tentative inferences. Further work is in progress to detect characters exhibiting adaptive changes.

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4. Evidence for genes controlling pollen grain development in chromosome 9 and an attempt at locating similar genes in other chromosomes.

A study of pollen grains segregating for waxy and starchy phenotypes in plants heterozygous for Inversion 9a has given the following picture regarding variability in shape and size associated with the two phenotypes (Table 1).

Table 1
Comparison of shape and size of grains segregating for Wx and wx in plants heterozygous for Inversion 9a

	% of grains of different shape		Size (in divisions of the ocular micrometer)	
	Spherical	Oval	diameter of spherical grains	length of oval grains
Starchy (Wx)	44	56	37.0	37.6
Waxy (wx)	72	28	33.6	37.0

It appears that a block of genes associated with spherical shape and small size are linked to the wx allele. A part of this block may be located in the inverted segment and another part may be close to the same gene but on the side opposite to the inverted segment so that these genes may be free to enter into recombination with the genes in the homologous segment linked to the Wx allele.

We are further studying the variation associated with the starchy-waxy phenotypes in plants heterozygous for T 6-9b as well as a few other translocations involving chromosome 9 marked by wx. It is hoped that this study, when completed, will reveal the distribution of genes controlling pollen grain development in segments of different chromosomes in the

vicinity of break points. The rationale of this analysis is but an extrapolation of Dobzhansky and Rhoades' approach for locating favorable dominant genes.

We are also planning to extend this analysis in order to locate, if possible, the components of the polygenic system controlling aspects of meiocyte development.

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1. Protein and some amino acid composition of Indian hybrid maize.

The six leading hybrids Ganga-3, Ganga-101, Ganga sufed-2, Ranjit, Deccan hybrid, and high starch as well as the opaque-2 mutant have been analyzed for crude protein and for lysine, leucine, tryptophan, and methionine content. The hydrolysis of the protein was carried out for six hours in an autoclave in 2.5N NaOH in the case of tryptophan and 2.5N HCl in the case of the other amino acids. The amino acids were estimated microbiologically with Leuconostoc mesenteroides P-60 (Steele, B. F. et al, J. Biol. Chem. 177:533, 1949) in collaboration with the microbiology Section of the Nutritional Research Laboratory. The results are presented in Table 1.

Table 1
(grams/16gms.nitrogen)

	Lysine	Leucine	Tryptophan	Methionine	Crude protein (in percentage)	Yield in Kg/Ha
1) Opaque-2	5.92	8.00	0.55	1.74	9.97	
2) Ranjit hybrid	2.95	12.30	0.44	1.91	10.19	4500
3) Hi starch	2.76	12.95	0.39	1.88	10.47	4500
4) Ganga-3	3.50	13.50	0.43	2.24	9.60	4000
5) Deccan hybrid	3.52	13.15	0.53	2.36	7.96	4900
6) Ganga saffeed-2	3.00	13.30	0.41	1.88	9.99	4500
7) Ganga-101	2.60	12.50	0.45	1.65	11.13	4100

There seems to be some relationship between methionine and crude protein content. The studies are in progress with inbred lines which might give some information regarding the protein content and the pattern of these essential amino acids.

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1. Alpha-amylase in developing maize endosperm.

An amylase-like enzyme was detected in developing maize endosperm during investigations of the phytoglycogen-forming branching enzyme. Investigations were then initiated to develop a reliable technique of extraction and fractionation of the enzyme, to determine the activity, nature and classification of the enzyme, and to determine the activity of this enzyme in several genetic endosperm mutants.

Fractionation and partial purification of the enzyme was accomplished through gradient elution with NaCl in Tris-maleate buffer from a DEAE-cellulose column. The products of enzyme action on amylose, glycogen and beta-limit glycogen were examined by thin-layer chromatography. The enzyme reaction was measured by the decrease in iodine-staining ability of an amylose solution that was being degraded by the enzyme. The enzyme activity was calculated by the rate of decrease in color of the iodine-starch complex.

The enzyme was found to degrade amylose, glycogen and beta-limit glycogen. Maltose, maltotriose and maltotetraose were produced in about equal amounts when amylose was used as a substrate. Action on glycogen and beta-limit glycogen gave less maltose than maltotriose and maltotetraose and the rate was slower than on amylose. No isomaltose or glucose were ever detected, even after extended periods of incubation, on linear or branched substrates.

Copper, iron, lead, mercury and p-chloromercuribenzoate inhibit enzyme activity. The enzyme was active after extended periods of dialysis against EDTA; therefore, a requirement for calcium was not demonstrated. The enzyme was very stable in water at 10°C for several weeks.

The enzyme was found to have a pH optimum near 6.8 in Tris-maleate buffer, which is higher than most amylases of plants. The pH of alpha-amylase in germinating corn kernels has been reported to be about 4.6-5.4; therefore, the amylase in developing maize endosperm was much higher. The Michaelis-Menten constant was calculated to be 0.08 per cent amylose.

Enzyme was detected in normal dent and the mutants ae, bt_1, bt_2, du, fl_1, o_2, sh_1, sh_2, su_1, su_2 and wx. Results of analyses of kernels at 16, 20, 24 and 28 days after pollination and at maturity (air dry) indicated that activity increased from 16 to 24 days and then began to drop. Very little activity was detected in dry kernels.

The enzyme may play an important role in starch synthesis even though it is a degradative enzyme. Possibly, the enzyme degrades the long molecules formed by ADPG- and/or UDPG-transferase to form more acceptors for glucose transfer, thereby increasing the efficiency of the transferases. The enzyme was not demonstrated to synthesize higher polymers. It was concluded that the enzyme is an alpha-amylase because it cleaved the glucosidic linkages of amylose and cleaved beyond the branch points of beta-limit glycogen to produce small molecular weight oligosaccharides. The other characteristics of the enzyme are similar to alpha-amylases. This is the first time that alpha-amylase has been characterized in developing maize endosperm.

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1. Modification of Anderson's method for transferring genes via translocations.

Anderson's method (see Brookhaven Symposia in Biology No. 9 pp. 30, 31) involves three main steps as follows: Finding a translocation with a breakpoint close to the locus of the gene to be transferred, backcrossing the translocation into the inbred to be improved, and backcrossing the desirable gene into the inbred that temporarily carries the translocation. Two complete backcrossing programs are necessary to obtain the recovered inbred that contains the desired trait, a normal chromosome complement, and a minimum amount of foreign chromosome.

With the advent of an increasing proportion of single cross commercial hybrids, it might be advantageous to permanently place the translocation into the converted inbred along with the desired trait. This would reduce the number of backcross programs from two to one per line.

The main points of the modified scheme might be summarized as follows: Cross the suitable translocation (one with a breakpoint near the desired gene) to the stock carrying the desirable gene; then make the appropriate backcross in order that both the translocation and the desirable gene are segregating; identify and maintain crossover plants that link the desirable gene to the breakpoints of the translocation. Once the desirable gene translocation stock has been made, it may be used as a source for backcross programs to inbred lines; selection may be for the desirable gene and/or semi-sterility due to the translocation.

The modified scheme differs from Anderson's method and standard (without translocations) backcross methods in that converted inbreds must be used with other converted inbreds carrying the same translocation to avoid semisterility in the farmer's field. Consequently the modification's use is less cumbersome for single cross hybrids than where more inbreds are used per hybrid.

The modified translocation scheme differs from backcross programs without translocations in other ways: As in Anderson's method the suitable translocation stock must be found that has its breakpoints in close proximity to the location of the desired gene and in addition crossover plants that link the trait to the breakpoints must be produced. Semisterility may be used for selection in lieu of the trait in unfavorable environments (disease resistance in absence of disease for example), and fewer testcrosses (to insure the gene's presence) are necessary when a recessive trait is involved. Extra generations of backcrossing will be necessary (to obtain a minimum amount of foreign chromosome) in order to compensate for the reduction of crossing over near the desired gene due to the presence of the translocation. One less generation will be required at the end of the backcrossing program for a dominant trait because plants homozygous for the trait can be discerned from those heterozygous since the former will have normal and the latter semisterile pollen and ovules.

The following Mankato 1964 backcross data illustrate the method of obtaining the desired stocks that link breakpoints of translocations with Ht (a dominant gene that reduces sporulation of Helminthosporium turcicum leaf blight in corn):

Trans.	breakpoint	ht S	ht N	Ht S*	Ht N	% C.O.
2-6d	2L.41	146	37	30	155	18.2
2-4L	2L.59	184	10	21	153	8.4
2-4b	2L.81	144	21	36	179	15.0

*Crossover class linking Ht and the translocation.

These data indicate that Ht is located cytologically near the middle of the long arm of chromosome two. More translocation stocks were obtained with breakpoints in this general area. The 1966 Mankato backcross data follow:

Trans.	breakpoint	Ht S*	Ht N	% C.O.
2-10 (8219)	2L.50	3	133	2.2
1-2d	2L.56	9	154	5.5
2-6 (9002)	2L.57	36	108	25.0
2-8 (051-15)	2L.62	21	105	16.7
2-3d	2L.67	19	97	16.4

*Crossover class linking Ht and the translocation.

The Ht S class for 2-10 (8219) or the progeny from it that also link Ht and the breakpoints would be a desirable source to initiate a

backcrossing program for Ht.

The data for 2-6 (9002) appear to be somewhat out of line with the other stocks; this may be due to sampling error or faulty cytological determination of breakpoints.

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2. A second modification of Anderson's method.

A similar, but somewhat different modification of Anderson's method has been used to move $\underline{Rf}_1$ into genotypes of $\underline{rf}_1$ constitution. Selection of a close linkage between a translocation and the desired gene is made, as in the above scheme, but the intention is to break the linkage after sufficient backcrossing has been done. This will require selfing and testcrossing large numbers of individuals at the end of the backcrossing series, in order to pick up the infrequent recombinations of normal chromosomes with desired gene. In this respect the scheme is more cumbersome than the first one, but it does give one a recovered line with normal chromosomes, should one desire this.

Translocation 3-9c has about 3% recombinations with $\underline{Rf}_1$. The recombination of T3-9c and $\underline{Rf}_1$ was identified and has been placed in three inbred lines, by continual backcrossing (in normal cytoplasm) with selection (by examination of pollen and ears) for semi-sterility. Two plants per inbred were selected in each generation. At BC^4, testcrosses revealed that all plants tested still had the desired linkage of T3-9c and $\underline{Rf}_1$. At BC^7 an attempt was made to identify and self (with testcrossing) large numbers of normal-chromosome backcross plants in one of the lines ($WF9^7$), hoping that some 3% of the normal-chromosome plants would have $\underline{Rf}_1$. Due to hot weather at pollinating time nearly all BC^7 plants were partially sterile and it was not possible to classify their tassels for presence vs. absence of semi-sterility (presence of 3-9c in heterozygous condition). A few random selfs and testcrosses were made successfully, however, all going back to two semi-sterile BC^6 plants. One of the two BC^6 plants whose BC^7 progeny was tested proved still to have $\underline{Rf}_1$ linked with T3-9c (genotype $\frac{T\ \ \underline{Rf}_1}{N\ \ \underline{rf}_1}$). The other plant appeared to have an undesired recombinant, having $\underline{rf}_1$ linked to T3-9c (genotype $\frac{T\ \ \underline{rf}_1}{N\ \ \underline{rf}_1}$). Further attempts will be made to identify desired recombinants.

This scheme also is being used to "cure" inbreds of partial restoration. T3-9c with $\underline{rf}_1$ is being transferred to plants of partial restorer genotype ($\underline{Rf}_1^P$) with the intention of selecting recombinants with normal chromosomes and $\underline{rf}_1$ genotype at the end of the backcrossing period. This method obviates the need for testcrossing backcross plants, to distinguish those of $\underline{Rf}_1^P\ \underline{rf}_1$ genotype from those of $\underline{Rf}_1^P\ \underline{Rf}_1^P$ genotype.

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1. Base ratios of maize DNA's.

It was reported in last year's News Letter that highly unusual base ratios had been found in DNA extracted from young endosperm of two lines of Black Mexican sweet corn (one with and one without B-chromosomes). In view of these peculiar results, determinations on these lines and a number of other maize lines were made again this year. In addition to the extracts of young endosperm which give very good yields of DNA, extracts of seedlings and leaves were also prepared from the two Black Mexican lines.

The results are presented in the following table:

Line	% C-G		
	endosperm	seedlings	leaves
Black Mex. with B-chromosomes	70	44	42
Black Mex. without B-chromosomes	55	43	42
White sweet corn	42		
A C R pr	42		
A C R^{st}	41		
A C R^{mb}	41		
Peruvian flint, yellow endosperm	42		
T2, white dent inbred	44		
K64r, white dent inbred	44		
lt. variegated pericarp on T2 background	43		
red pericarp dent	42		

The table shows that the C-G proportions from seedlings and leaves of the two Black Mexican lines give typical values for maize as do the endosperm determinations for all the other lines tested. These values confirm those reported by Rinehart in last year's news letter.

Abnormal values were obtained again for the endosperm of the two Black Mexican lines. The DNA's from these two lines differ in their behavior during extraction both from each other and from all the other maize sampled. It appears that in the young endosperm of the Black Mexican

lines some substance which appears to contain starch and a phenolic compound as well as nucleotides is firmly bound to the nuclear DNA. Modification of the extraction process has on one occasion produced a 42% C-G DNA precipitate after the enzymatic removal of RNA. However, the supernatant was still strongly positive for DNA and presumably contained the contaminating substance which produces the distorted base ratios. These results were obtained only for the line lacking B-chromosomes. Identical treatment had no effect on the B-chromosome line.

It can be seen from the table that neither the B-chromosomes, the aleurone color genes nor the sugary gene alone can be responsible for the unusual behavior of the DNA in the endosperm of the Black Mexican lines. Further studies are in progress.

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1. Cytoplasmic differences in respect of some important plant characters in a maize composite.

Current trends in maize breeding techniques are mainly based on increasing the frequency of superior genes in the base population and selecting for specific interaction of these genes determining better preformance than the parents. This lays all the emphasis on the manipulation of Mendelian inheritance controlled by nuclear genes and the fact that the differences in cytoplasm may alter the expression of the genes to a considerable extent has not received attention. As early as 1933 Rhoades discovered that male sterility in maize was inherited only from the female parent. In recent years the finding of Rf genes (Restorer genes) has clearly shown that sterility or fertility in maize is conditioned by certain genes in the nucleus and the cytoplasm with which they interact.

Bauman in 1950 (quoted from Stringfield 1958) reported that ear length was significantly different in two three-way crosses differing in their source of cytoplasm only. Later Stringfield (1958), Fleming et al. (1960), Brown (1961), Dhawan and Paliwal (1964), Dhawan et al. (1965) and Mukand Singh (1965) reported high significant differences in yield and other plant characteristics in reciprocal crosses of various types.

Recently in India and in Mexico highly diverse germplasm complexes are being used in maize improvement programmes. Observations on one germplasm complex (J_1) having a very wide genetic base indicated that there is a great deal of variability for cytoplasmic effects and selection for superior combinations of genes and cytoplasm may be useful.

Observations on 46 reciprocal biparental comparisons were recorded for per plant yield, silking period, plant height, ear placement, ear length, ear girth, number of rows per ear, number of grains per ear and 100-grain weight. The analysis of variance for all the characters was done as for the randomized plot design and the differences between reciprocal progenies were compared with C.D. values at the 5 per cent and 1 per cent levels of significance.

The analysis of variance showed that the differences for the characters studied were highly significant in many of the reciprocal pairs. Since the female parent contributes almost all the cytoplasm, the observed differences between reciprocal crosses are therefore due to cytoplasmic differences. As the base population of the J_1 composite is constituted of highly diverse sources of maize germplasm from U.S.A., South America, Peru, Columbia, Venezuela, the Caribbean region, Kenya and India, it abounds in high genetic variability and different sources of cytoplasm. The magnitude of the differences for the characters in various reciprocal crosses is, therefore, an indication of the interaction of a particular cytoplasm with a specific genotype. Out of 46 reciprocal pairs, 43 pairs exhibited significant mean differences for one character or more. The details of the cytoplasmic effects on various characters are as follow:

Highly significant differences for per plant yield (range 1.4 to 40.2 grams) were observed in 15 reciprocal cross pairs. This indicates that cytoplasmic effects were quite vital in at least one-third of the population in determining the yield performance, and selection on the basis of superior interacting cytoplasm could certainly improve the yield potential of the complex. Besides yield, differences were significant for plant height in 23 progenies (0.20 to 53 cms.), silking date in 4 progenies (0.20 to 3.0 days), ear placement in 19 progenies (0.20 to 22.5), ear length in 5 progenies (0.1 to 3.6 cms.), ear girth in 2 progenies (0.1 to 2.3 cm.), number of rows per ear in 8 progenies (0.1 to 3.1), number of grains per ear in 11 progenies (1 to 181), and 100-grain weight in 3 progenies (0.1 to 6.4 gms.). The characters, yield per plant, plant height, ear placement and number of grains per ear, seem to be most affected by the cytoplasmic differences, whereas these differences did not affect the individual yield components to the same extent. The cytoplasmic effects between different reciprocal pairs are not consistent for the characters studied, which indicates that genic cytoplasmic interactions are not the same in all the reciprocal pairs and depend on the specificity of the genotype and cytoplasm for which the composite has a great deal of variability. Inconsistencies in cytoplasmic effects for various agronomic characters in different sources of cytoplasm have been reported by Fleming et al. (1960) and Mukand Singh (1965). According to Dhawan and Paliwal (1964) and Mukand Singh (1965), genetic diversity of the parents is important in the expression

and manifestation of quantitative differences for agronomic characters influenced by specific cytoplasmic effects in the crosses. Hence in a composite like J_1 constituted of genetically diverse sources of germplasm from different geographical areas, the cytoplasmic differences offer great scope for selection and putting together cytoplasm and genotype which give the most desirable plant types in the derived population. The approach of selection between reciprocal biparents would combine the ease and rapidity of different mass selection procedures to exploit the additive gene effects in such populations and at the same time would insure selection for superior interacting cytoplasmic effects.

The observations in the present study also point out that in practical breeding programmes where such composites are being used, a larger population than generally used should be involved as female parent to represent the entire range of the variability of cytoplasm in the composite, and it may be desirable to get an idea about the extent of cytoplasmic variability and the combining ability for cytoplasmic effects in such composites, so that one may have a sound basis for determining which composite should be used as the female parent. The present findings also suggest that the maintenance of composites should be based on fairly large plant populations.

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1. Verification of R^{st} reconstitution in $R^{sc}/r^{g}(I)^{3}$ heterozygotes.

A report in the 1966 MNL (pp. 135-137) described the recovery of four R^{st} mutants from heterozygous combinations between two R^{sc} and a near-colorless aleurone allele, $r^{g}(I)^{3}$, originally isolated from an $R^{r}R^{st}$ plant. The male parent in these tests was r^{g} r^{g}, wx wx, and the four isolations of R^{st} segregated wx, as expected. However, there were several plantings in the field of an $R^{st}R^{st}$, wx wx stock culture, and the possibility existed that the R^{st} alleles isolated as mutants could have been pollen contaminants from this source. Conclusive verification required the identification of the r^{g} allele brought in from the male parent. A R^{st}, wx contaminant gamete would produce a $R^{st}/r^{g}(I)^{3}$, Wx wx kernel, whereas a mutation would produce a R^{st} r^{g}, Wx wx kernel. The $r^{g}(I)^{3}$ and r^{g} alleles are identifiable by phenotype and paramutagenic action.

The four stippled kernels selected as possible mutants were self pollinated when grown out. The nonstippled kernels from each ear were planted, and plants were self pollinated and crossed to R^{r} R^{r}. Selfed ears from the four progenies were examined for a near-colorless aleurone phenotype but

all kernels were colorless; kernels homozygous for the $\underline{r}^{g}(I)^{3}$ allele show some pigment on about 75% of the kernels.

Seed from two ears crossed to $\underline{R}^{r}$ $\underline{R}^{r}$ from each progeny was planted and 3-4 plants from each were used as males in testcrosses to $\underline{r}^{g}\underline{r}^{g}$. The testcross ears were examined for evidence of paramutagenic activity and none was found; the $\underline{r}^{g}(I)^{3}$ allele is known to be as paramutagenic as $\underline{R}^{st}$.

The results from the above two tests exclude pollen contamination as a possible source for the four stippled alleles isolated and support a mutation origin.

Some additional data are now available on the characteristics of these four $\underline{R}^{st}$ mutants. One of the four clearly has a phenotype different from that of the stippled allele maintained in our stock cultures; the pattern of spots is finer, frequent but small in size. The three other mutants have a phenotype similiar to that of our stock allele, although one appears to be somewhat lighter (less frequent spots).

The two $\underline{R}^{sc}/\underline{r}^{g}(I)^{3}$ heterozygous combinations from which $\underline{R}^{st}$ mutants were recovered were also heterozygous for $\underline{M}^{st}$, a modifier of the stippled phenotype about six units distal to $\underline{R}$, with $\underline{M}^{st}$ being carried on the $\underline{r}^{g}(I)^{3}$ chromosome. The $\underline{R}^{st}$ mutants were tested for the presence of $\underline{M}^{st}$ by pollinating several $\underline{R}^{r}$ $\underline{R}^{st}$ plants representing each of the four mutants with $\underline{r}$ pollen. The $\underline{R}^{r}$ chromosome is known not to carry $\underline{M}^{st}$, and a lighter stippled phase of each mutant, indicating a loss of $\underline{M}^{st}$, occurred with a frequency of about 6%. These results show that the mutants carried $\underline{M}^{st}$ and are responsive to its modifying effect.

Testcrosses of $\underline{R}^{r}$ $\underline{R}^{st}$ plants representing each mutant to $\underline{r}$ $\underline{r}$ ears have shown all four mutants to be paramutagenic. Detailed scorings have not been completed, and it is not known whether the level of paramutation is comparable to that of $\underline{r}^{g}(I)^{3}$ and our stock $\underline{R}^{st}$ allele.

The $\underline{r}^{g}(I)^{3}$ allele is unstable for plant color and mutates to a form having red plant; these mutations often are somatic and evidenced as tassel sectors in which the anthers are red. About 60 plants homozygous for each of the four $\underline{R}^{st}$ mutants were examined for such tassel sectors but none were found. Evidently, the unstable plant characteristic of $\underline{r}^{g}(I)^{3}$ was not retained by the $\underline{R}^{st}$ mutants even though all carried a distal genetic marker from the $\underline{r}^{g}(I)^{3}$ chromosome.

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1. Genetic analysis of the absence of aleurone color in South American maize.

One method of analyzing the structure of populations of indigenous races of maize consists in the analysis of characters with simple inheritance. This report is concerned with the genetics of absence of anthocyanin in the aleurone in South American Highland and Lowland races of maize, as part of a project testing a large number of indigenous races for the genetic basis of colorless aleurone, started by Brieger several years ago (M.N.L. 28:74, 1954). Plants of indigenous races with strong modifier complexes for colorless aleurone were crossed with plants of Negrito, a race from Northern Colombia, which is completely homozygous for all the genes for aleurone color and which has a strong modifier complex for coloration. By selfing the F_1 plants, segregating F_2 ears were obtained. These ears give normal Mendelian ratios owing to the apparent randomization of the two types of modifier complexes. A statistical analysis was elaborated for the observed segregations in 1,764 F_2 ears from Highland races and 704 F_2 ears from Lowland races. The results are given in Table 1 on the following page.

From the lowland data, we may conclude that the colorless races were homozygous for at least one recessive inhibitor gene; a second recessive inhibitor was present in all races with a frequency of approximately f = 60%, and the presence of the third recessive inhibitor varied from race to race. Future analysis with genetic testers should elucidate more clearly the situation, especially with regard to the exact nature of the loci involved but it is interesting to note that there seems to be a certain stability in the gene frequencies of two of the loci involved.

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1. The Navajo phenotype at the R locus - an example of phase change or a cell differentiation?

In a recent interesting paper P.A. Peterson (Genetics 54:249, 1966) described two unusual phenotypic patterns of mutability, designated "flow" and "crown", at the A_1 locus. These were interpreted as due to phase variation of two different regulatory elements. It was implied also, that the Navajo allele (R^{nj}) at the R locus could be a further example of phase variation in maize.

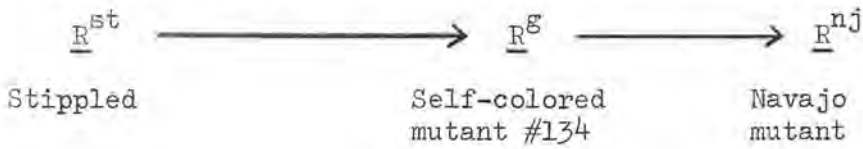
An alternative, though not readily distinguishable, concept of the Navajo phenotype is that it is a manifestation of a cell differentiation

Table 1
Frequency of Mendelian ratio in F_2 ears from the crosses between colorless races x Negrito

	3:1	9:7	27:37	1:3	3:13
HIGHLAND					
CAPIA	13	15	--	--	--
	13	19	15	15	--
	--	9	9	4	16
DENT BRANCO	16	49	26	--	--
	13	57	61	18	--
	--	54	31	--	--
	--	50	25	9	--
	--	23	16	5	6
FLINT BRANCO	11	6	--	--	--
	27	134	53	--	--
	11	89	38	16	--
	--	174	77	--	--
	--	6	15	7	--
FLINT AMARELO	15	108	31	3	--
PISINCHO	12	--	--	--	--
	33	45	2	--	--
	41	111	61	13	5
	--	13	2	2	--
LOWLAND					
CAINCANG	50	71	--	--	--
	3	39	24	--	--
LENHA	--	6	13	10	--
GUARANI YELLOW Soft Corn	30	68	95	--	--
	--	29	49	--	--
	--	3	15	10	--
CALCHAQUI	--	74	58	--	--
	--	10	14	6	--
PIPOCA PAULISTA	--	53	95	--	--
CRISTAL	--	+	+	--	--

phenomenon. That is, the aleurone cells which become pigmented in $\underline{R}^{nj}/-/-$ kernels are differentiated with respect to the non-pigmented cells. This does not necessarily imply that the cell types be morphologically distinguishable. The concept implies that there is a master differentiation process, to which the $\underline{R}^{nj}$ alleles respond, probably under the direction of a specific genetic element (operator like?) located at or near the R locus, by expressing the synthesis of anthocyanin. The phase change concept reaches the same phenotype by implying that the "differentiation" observed results from the specific activation to expression of the R gene in the pigmented cells.

Both concepts, "phase change" and "cell differentiation" imply that R-Navajo alleles be R in basic R locus constitution. Evidence for this has been obtained with one particular $\underline{R}^{nj}$ allele. The $\underline{R}^{nj}$ allele studied is designated $\underline{R}^{nj}$(scl 34)-14, and was obtained by mutation of a self-colored ($\underline{R}^{g}$) allele by the sequence of mutation events (M.G.C.N.L. 35:142, 1961);



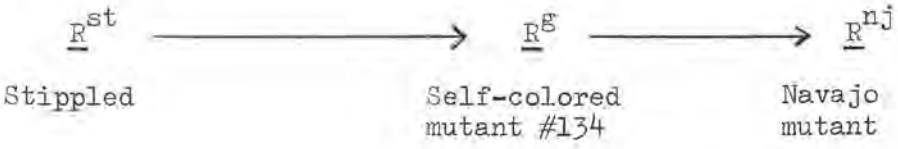
The $\underline{R}^{nj}$ mutant thus isolated has been observed to mutate to $\underline{R}^{g}$ (self-colored). Seven proven $\underline{R}^{g}$ (self-colored) mutants have been obtained from $\underline{R}^{nj}/\underline{r}^{r}$ genotypes (rate ca.30 x 10^{-4}), and one proven $\underline{R}^{g}$ (self-colored) mutant has been obtained from $\underline{R}^{nj}/\underline{R}^{nj}$ genotypes (rate ca. 1 x 10^{-4}). Additional putative mutant kernels are currently being progeny tested, and a suggestive association of mutation of $\underline{R}^{nj}$ to $\underline{R}^{g}$ in $\underline{R}^{nj}/\underline{r}^{r}$ genotypes, with crossing over in the G - R region is being tested further.

The phenotype of the mutant $\underline{R}^{nj}$ allele studied is quite similar to that of some $\underline{R}^{nj}$ alleles of natural occurrence. Its origin by the mutation sequence outlined above may mean, however, that it is constituted differently from other naturally occurring $\underline{R}^{nj}$ alleles. The study of $\underline{R}^{nj}$ alleles, with emphasis on an attempted recombination analysis of the structure of the R region, is being extended to include alleles $\underline{R}^{nj}$ (Illinois), $\underline{R}^{nj}$ (Cudu), $\underline{R}^{nj}$ (Anderson), $\underline{R}^{nj}$ (New Mexico) and R. A. Brink's $\underline{R}^{nj:st}$(stippled-Navajo). I would appreciate receiving seed of any $\underline{R}^{nj}$ allele of different origin to those listed.

K. S. McWhirter

2. A paramutagenic Navajo allele at the R locus.

A mutant allele with the Navajo phenotype was obtained by mutation of a self-colored ($\underline{R}^{g}$) allele, by the sequence of mutations (M.G.C.N.L. 35:142, 1961):



The $\underline{R}^{nj}$ mutant thus obtained was tested for paramutagenicity, in $\underline{R}^{nj}/\underline{R}^{r}$ heterozygotes, and compared with the parent $\underline{R}^{g}$ (self-colored) allele and $\underline{R}^{st}$ (stippled).

Genotype of staminate parent	Mean aleurone color score of $\underline{R}^{r}/\underline{r}^{g}/\underline{r}^{g}$ kernels* from testcrosses on W23 $\underline{r}^{g}\underline{r}^{g}$ $^{oo}_{++}$
$\underline{R}^{nj}$ (mutant) $/\underline{R}^{r}$	3.66
$\underline{R}^{nj}$ (mutant) $/\underline{R}^{r}$	3.56
$\underline{R}^{sc}$ 134$/\underline{R}^{r}$	5.10
$\underline{R}^{sc}$ 134$/\underline{R}^{r}$	5.16
$\underline{R}^{st}/\underline{R}^{r}$	2.99
$\underline{R}^{st}/\underline{R}^{r}$	3.66
$\underline{R}^{r}/\underline{R}^{r}$ (Control)	5.89

*Based on scores of 300 individual kernels (50 kernels from each of 6 testcross ears in each male family).

These data show that the $\underline{R}^{nj}$ mutant is paramutagenic, and is significantly more paramutagenic than the $\underline{R}^{sc}$134 parent allele. There have been few instances in which a mutant allele at the R locus has been more paramutagenic than the parent allele. In the present sequence both the observed mutation events involved coincident alteration of R locus phenotype and level of paramutagenicity. In the Navajo mutant full paramutagenicity is restored, and this observation lends support to the idea that in certain self-colored ($\underline{R}^{g}$) mutants derived from $\underline{R}^{st}$, the unaltered genetic region determining paramutagenic action is suppressed by an extragenic element.

K. S. McWhirter

3. A dominant partial inhibitor of yellow endosperm.

A dominant gene which acts as a partial inhibitor of Y (yellow endosperm) has been isolated from Dr. A. L. Hooker's Source A Helminthosporium turcicum resistance stock. One plant from this stock, when crossed with W22 ($\underline{AC}\underline{r}^{g}/\underline{AC}\underline{r}^{g}$, Y/Y), produced a progeny in which half the plants segregated 3 dark yellow: 1 white endosperm, on selfing, and half the plants segregated dark yellow, pale yellow and white endosperm kernels, on selfing. On the latter ears the kernel types occurred in the ratio of 9 pale yellow: 3 dark yellow: 4 white. The segregation totaled for samples from 10 ears was:

1057 pale yellow: 331 dark yellow: 416 white.
Segregation (X^2 (2d/f) = 4.63, P = 0.20; heterogeneity
X^2 (16 d/f) = 14.1, P = 0.50).

These results can be interpreted as indicating the segregation of a dominant gene (Iy), which causes partial inhibition of yellow endosperm to produce a pale yellow kernel phenotype. On this basis the observed classes of kernels have the phenotypic formula 9 pale yellow (Iy Y): 3 dark yellow (iy Y) : 4 white (Iy y and iy y). There is some variation within the colored classes, presumably due to dosage effects, but the segregation is nevertheless readily recognized.

This interpretation is supported by the results of progeny tests of 115 plants grown from kernels on ears segregating 9 : 3 : 4. These data are summarized in Table 1.

Table 1
Progeny test results obtained by self-pollination of plants grown from kernels on ears segregating 9 pale yellow : 3 dark yellow : 4 white endosperm

Parent kernel type	Description of selfed ears	Number of plants observed*	Number of plants expected
White endosperm	Homozygous white	14	14
Pale yellow	Seg. 3 pale : 1 white	12	15
	Seg. 9 pale : 3 dark : 4 white	32	30
	Seg. 3 pale : 1 dark yellow	14	15
	Homozygous pale yellow	9	7
Dark yellow	Seg. 3 dark yellow : 1 white	21	23
	Homozygous dark yellow	13	11

†

*These results are approximate, counts have yet to be completed.
+Excluding 1 & 4 exceptional ears, respectively, probably due to heterofertilization.

After excluding a few exceptional ears, which were probably due to heterofertilization, these data show a good relation of parent kernel phenotype and the genotypic array expected within each class. Also there appears to be a reasonable correspondence of observed and expected frequencies of the different genotypes.

All four homozygous genotypes, Iy Iy Y Y, Iy Iy y y, iy iy Y Y and iy iy y y, have been established and the inhibitor (Iy) has no obvious pleiotropic effect.

The plant from Source A was of the genotype Iy/iy, y/y and W22 has the genotype iy/iy, Y/Y.

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1. Studies on the effect of temperature treatment on intergenic and intragenic recombination.

Plants heterozygous for T6-9b or for $wx^{90}wx^{c}$ were grown in a growth chamber with controlled temperature, light period and humidity. Temperature was maintained at 70°F (±2°F) at floor level (close to 75°F at sporocyte level); relative humidity varied between 85% (with light on) and 95% (with lights off); diurnal light period was 14 hours with a mixture of cool white fluorescent and incandescent lights. Each plant was grown in fresh "Carl Pool" potting soil; starting at the three-leaf seedling stage, each was fertilized once a week by addition of one teaspoon (in solution) of "Rapid Gro." Reasonably vigorous growth was achieved under these conditions. At sporocyte stage bracketing spikelet samples were removed from some tassel branches and fixed for stage determinations; the remainder of each tassel was carefully returned to the stalk and was either immediately heat treated, as described elsewhere (P.N.A.S. 55:44-50, 1966), or maintained at constant temperature as a control. Tassel branches (both bracket-sampled and intact) were then removed and fixed at intervals following initial sampling by 5, 24, 48, 72, 96 and 120 hours from plants heterozygous for T6-9b. Pollen samples were collected and fixed from treated and control $wx^{c}wx^{90}$ plants and will be examined for effects of treatment on interallelic recombination. Quartet stages from heterozygous T6-9b samples have been scored for frequency of normal nucleolus quartets. This quantity may be related to crossover frequency if it is assumed that the frequency of adjacent II distribution (from the ring of four translocation configuration) is inversely related to crossover frequency in the interstitial segment of the limb carrying the chromosome 6 centromere and if 6^{9} univalents are distributed at random with respect to the normal chromosome 6. Results are shown in Table 1. The results are not suitable for standard statistical analyses because of interplant heterogeneity and because of the number of sampling times from various plants where data are missing (where samples did not contain any quartet stage cells). The greatest and most consistent departures from means of treated plants and from control values were found in the 72 hour samples of treated plants. Cells fixed at quartet stage 72 hours after initial sampling are estimated to have been at premeiotic interphase at the time of treatment although the possibility that some were at early synizesis cannot be excluded. Data so far available from studies of stage duration, based on samples of adjacent spikelets removed at the beginning of the experiment and at the various intervals following, and on relative frequencies of the various stages, are

Table 1
Frequency of Normal Quartets

	Plant No.	pretreatment	5 hours	24 hours	48 hours	72 hours	96 hours	120 hours
		No. %	No. %	No. %	No. %	No. %	No. %	No. %
treated plants	1	1882/2608 = 72.2	1088/1517 = 71.7	949/1339 = 70.9	1194/1615 = 73.9	280/373 = 75.1	----------	----------
	2	1516/2060 = 73.6	----------	----------	----------	1947/2522 = 77.2	2009/2655 = 75.7	1769/2419 = 73.1
	3	1896/2483 = 76.4	741/960 = 77.5	----------	1769/2323 = 76.2	1899/2350 = 80.8	1630/2042 = 79.8	----------
	4	1623/2181 = 74.4	1118/1536 = 72.8	1174/1611 = 72.9	846/1165 = 72.6	1801/2311 = 77.9	1748/2287 = 76.4	1546/2092 = 73.9
	5	1586/2160 = 73.4	1541/2098 = 73.5	1508/2162 = 69.8	1550/2187 = 70.9	1847/2444 = 75.6	1580/2262 = 69.8	----------
	Total	8503/11492 = 74.0	4488/6111 = 73.4	3631/5112 = 71.0	5359/7290 = 73.5	7774/10000 = 77.7	6967/9246 = 75.4	3315/4511 = 73.5
controls	1	1894/2528 = 74.9	1554/2127 = 73.1	1885/2604 = 72.4	1679/2283 = 73.5	3324/4568 = 72.8	1797/2502 = 71.8	520/706 = 73.7
	2	1817/2499 = 72.7	----------	662/924 = 71.6	3151/4296 = 73.3	2599/3621 = 71.8	1507/2042 = 73.8	1545/2116 = 73.0
	3	1495/2124 = 70.4	----------	1262/1832 = 68.9	1396/2005 = 69.6	1575/2260 = 69.7	1401/1989 = 70.4	814/1166 = 69.8
	Total	5206/7151 = 72.8	1554/2127 = 73.1	3809/5360 = 71.1	6226/8584 = 72.5	7498/10449 = 71.8	4705/6533 = 72.0	2879/3988 = 72.2

inadequate but suggest that the total duration of meiosis was of the order of 68 hours (synizesis 50 hours, pachytene 6 hours, diplotene through telophase II 6 hours, and quartet stage 6 hours). All of the figures are rough estimates of means with unestimated variances. (On the average the longer stemmed spikelet of a pair is slightly more advanced than the short stemmed, but is often found at the same or a slightly earlier stage.) The durations of the pachytene (exclusive of synizesis) through quartet stages may be such that some cells scored at quartet stage were treated at least partly at pachytene while others were past pachytene. Some scored at quartet stage in 5 hour samples, are thought to have passed through metaphase during treatment, and these may show a tendency for treatment at this stage to depress the frequency of normal quartets (effect on disjunction); data are too sparse for significance in this respect. Further tests for this effect are planned. Because the quartet stage scoring method has poor resolution and probably inherent sources of error, studies have been initiated to search for effects of temperature treatment on crossing over within and coincidentally within and proximal to heterozygous inversion 5083 where a more direct method of scoring crossover frequency and much better resolution are available. Other inversions may also be used.

M. P. Maguire

2. Incorporation of tritiated thymidine by microsporocytes of maize.

Preliminary experiments on tritiated thymidine (H^3-TdR) uptake in maize microsporocytes have indicated that a major period of incorporation precedes the synizesis stage. Following submergence of freshly cut ends of tassel branches in a culture medium containing H^3-TdR, label was frequently found in nuclei at premeiotic interphase and less frequently at synizesis in sporocytes from the first two or three spikelets above the cut. Nuclear label was found after incubation periods of 5 hours, 22 hours and 72 hours at temperatures of 23°C and 27°C in medium containing tritiated thymidine in concentrations of 6 μc/ml (6.7C/mM), 10 μc/ml (6.7C/mM), 3 μc/ml (1.9C/mM) or 10 μc/ml (1.9C/mM). The culture medium contained 4% sucrose, 1% casein hydrolysate, 2% Vogel's solution (containing trace elements, minerals and buffers) and 1% vitamin mixture (containing thiamin, riboflavin, pyridoxamine, pantothenic acid, PABA, nicotinamide, folic acid and lipoic acid). After the incubation period the tips of branches were washed and then submerged in test tubes of medium supplemented with 20 μg/ml unlabeled thymidine for two hours. The material was then fixed in alcohol:acetic 3:1 mixture; anthers were squashed in aceto-carmine and prepared for autoradiography as described by Schmid (in Human Chromosome Methodology. Academic Press, New York, 1965). Maximum exposure of film was 20 days. The approximate stage of treated microsporocytes at the beginning of the experiment was estimated by observation of microsporocytes from bracketing spikelets, collected at that time and presumed to be nearly the same stage. No significant chromosomal incorporation of tritiated thymidine was found during pachytene or the latter half of synizesis. It is uncertain whether some of the label found in cells at synizesis might have been incorporated during earlier phases of this stage or in the

premeiotic interphase. Attempts to induce incorporation of label into sporocytes of excised whole or chopped up anthers submerged in medium containing H^3-TdR have been unsuccessful.

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3. Further studies on disjunction at anaphase I of the chromosomes of a trivalent configuration.

It was reported in the 1965 M.G.C.N.L. that progeny of 21 chromosome plants carrying reciprocal maize-Tripsacum interchange chromosomes appeared to show a deficiency of 21 chromosome plants from non-disjunctive distribution for the distal region of the maize chromosome 2 short arm. The preliminary results were consistent with the interpretation that a tendency existed for trivalents destined to have non-disjunctive distribution to orient so that only the 2^T chromosome was directed toward the basal position. After addition of data from the 1966 season there is no significant difference in numbers of 20 and 21 chromosome progeny from non-disjunction as compared to disjunction and, therefore, no cause to suspect non-random metaphase I orientation of trivalents:

	disjunction	non-disjunction
20 chrom. progeny -	430	260
21 chrom. progeny -	448	251

(Correction for estimated 8% non-disjunction of maize centromeres is included).

Frequencies of disjunction and non-disjunction for distal chromosome 2S from the trivalent described above have been studied from two lines of descent, one which had been outcrossed to L289 and the other repeatedly backcrossed to a Coop chromosome 2 tester. These have been found to differ significantly in non-disjunctive frequency (19% and 37% respectively) although each was internally homogeneous.

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1. Evidence for the inheritance of acquired characters.

The R gene conditions aleurone pigment in the endosperm of maize. When R is removed from a heterozygote with its allele R^{st} (such R alleles are symbolized R^1, one generation with R^{st}), less pigment is produced. This phenomenon is called paramutation and has been reported on by the Wisconsin Maize Laboratory over the past ten years.

In Vol. 40 (under Defiance College) we reported that when $\underline{RR}^{st}$ heterozygotes were given environmental treatments LL or LD (constant light or 12 hr. light and 12 hr. darkness, respectively) for the first four weeks of seedling development, differences in amount of pigment could be observed in the testcrosses of treated plants. LD environments conditioned more cells to form pigment; LL conditioned fewer pigmented cells. Our evidence now shows that these differences in R expression are found in the generation following the administration of LD and LL treatments--the differences are male transmissible.

Inbred W22 plants, $\underline{RR}^{st}$, were raised under growth chamber conditions, LL and LD, for the first four weeks of plant development (70°F; light supplied by 14, 200W, cool white, fluorescent tubes supplemented by 12, 60W incandescent bulbs; in a 4' x 8' Percival Growth Chamber) in 1965. Plants so treated were removed to the field in June for the completion of their life cycle. Treated plants were sibbed or selfed to produce the seeds to be used in 1966; at the same time the treated plants were testcrossed to colorless inbreds. The testcross-kernels were scored by matching them against a set of standard kernels ranging in classes from 0-22, colorless to completely pigmented, respectively.

Table 1 shows that $\underline{R}^1$ expressions from $\underline{RR}^{st}$ plants which received LD conditions are darker than those which had received LL conditions the previous year. The testcross scores represent $\underline{R}^1$ alleles from W22/W23 hybrid background ($\underline{R^1r}$) produced in 1965; this $\underline{R^1r}$ hybrid was testcrossed onto inbred W23 in 1966. The differences represented in Table 1 required that such differences accompany the $\underline{R}^1$ allele through the pollen in 1965 in the W22/W23 hybrid; another pollen transmission was again required to account for the score differences in Table 1, in 1966. Thus the differences resulting from the LL and LD treatments in 1965 have survived two passages through the pollen.

Differences attributed to the LD and LL conditions are shown within a different subline in Table 2. $\underline{R^2R^2}$ segregates from $\underline{R^2R}^{st}$ plants which had received LD and LL treatments show that darker phenotypes result from the LD conditions the previous year; LL cause $\underline{R}^2$ to produce fewer pigmented cells. This difference can also be seen when the $\underline{R}^2$ (Table 3) allele is transmitted through the female, and thus is present in the endosperm cells in two doses. Where $\underline{R^2R^2}$ segregates are pollinated with $\underline{rr}$ to produce the triploid endosperm $\underline{R^2R^2r}$, fewer kernels are found which show a mosaic phenotype on ears resulting from the plants whose $\underline{R}^2$ allele traces back to the LD treatment the previous year. On the other hand, nearly twice as many kernels show a pigment mosaic in the endosperm when the $\underline{R}^2$ allele which is present traces back to the LL treatment.

Table 4 shows that the effects of LL and LD environments on $\underline{R}^1$ from $\underline{RR}^{st}$ heterozygotes, reported in 1965, are again repeated in a test conducted in 1966. LD conditioned darker $\underline{R}^1$ phenotypes than the LL environment; mixed treatments show that it was the environment which the plants received during the third and fourth weeks of development that determined the phenotype which $\underline{R}^1$ produced in the testcrosses.

It is concluded that the LD and LL environments are capable of conditioning heritable changes in R expressions and that these changes are determined sometime during the third and fourth weeks of seedling development. While it may be objected that the environments above affect only the paramutation process, it must be pointed out that the changes in the endosperm pigment mosaic attributed to the $\underline{RR}^{st}$ combination are to be found when R is made heterozygous with any of its alleles (see below). The change in mosaic pattern, called paramutation, is simply a more extreme mosaic with greater standard deviations among pollen samples from a single plant. Our data in MGCNL 40 showed that R from either the RR or Rr combinations showed tassel mosaicism and thus variation in R expression, depending on the tassel sectors from which the gametes originated. The RR and Rr combinations differ from $\underline{RR}^{st}$ heterozygotes by showing a much narrower range of variation for R mosaicism from a single tassel. To argue therefore, that LD and LL effects are to be found only in the presence of paramutation is not meaningful because now it appears that no conditions exist in which the R expression is free of paramutation (mosaicism) when testcrossed.

This work was made possible by an equipment grant from the Charles F. Kettering Foundation; more extensive evidence will be published elsewhere.

Table 1
Growth Chamber Treatments, Spring 1965

	LD	LL
	17.56	15.44
	15.92	12.62
	15.78	15.80
	16.94	13.62
	19.10	13.94
	18.64	15.28
	17.30	15.50
	17.08	15.44
Pooled $\bar{X}$	17.29	14.71

Testcross scores of $\underline{R}^1$ from hybrid $\underline{R^1r}$ background (Inbred W23, rr, x Inbred W22, $\underline{RR}^{st}$). Plants scored above were raised under greenhouse conditions in 1966. Scores represent ear means based on 50 kernels/ear.

Table 2
Growth Chamber Treatments, 1965

	LD	LL
	5.52	2.62
	8.00	1.88
	11.20	5.28
	5.26	3.10
	5.86	3.08
	6.40	3.58
	5.88	3.34
	7.96	2.84
	8.94	3.10
	9.22	1.40
$\bar{X}$	7.42	3.02

Testcross scores of $\underline{R^2R^2}$ segregates from seeds tracing back to LL and LD treatments of $\underline{R^2R^{st}}$ the previous year. Above scores are ear means based on 50 kernels/ear from testcrosses made in the field in 1966. Endosperm genotypes are $\underline{rrR^2}$.

Table 3
Growth Chamber Treatments, 1965

LD		LL	
Mosaic	Total	Mosaic	Total
3	113	35	252
3	246	69	254
17	347	40	233
15	278	47	382
11	344	32	308
15	212	11	252
7	224	12	189
39	313	13	262
47	350	26	174
38	360	19	259
2	130	41	347
2	294	46	296
2	336	42	337
3	132	28	269
2	253	41	271
3	286		
209	4218	502	4085
5.0%		12.3%	

Numbers of mosaic kernels/ear on $\underline{R^2R^2}$ segregates tracing back to $\underline{R^2R^{st}}$ which received LD and LL treatments and had been self pollinated the previous year.

Table 4
Growth Chamber Treatments, Spring of 1966

	LD	LL	Field	LL-LD	LD-LL	t-Test Comparisons	P
Ear means based on 50-kernel samples/ear. Fall, 1966.	17.14	8.38	13.98	15.36	11.58	LD vs LL	<.001
	18.52	8.34	14.56	17.04	11.70	LL-LD vs LD-LL	<.001
	15.78	9.92	16.38	16.20	13.70	LD vs Field	<.05
	13.60	9.98	13.56	15.92	9.36	LL-LD vs Field	<.05
	17.20	11.78	9.58	15.60	11.22	LD-LL vs Field	<.20
	17.26	11.78	14.52	16.52	13.82	LL vs Field	<.01
Pooled $\bar{X}$	16.58	10.03	13.76	16.11	11.90		

Testcross data for $\underline{R}^1$ expressions from $\underline{RR}^{st}$ heterozygotes given environmental treatments during the first four weeks of seedling development in 1966. A repeat of experiments performed in 1965.

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2. Selection for different states of R^9 when transmitted through the female.

In MGCNL 40 we reported that it was possible to select heritable light and dark phenotypes from among testcross kernels when $\underline{R}^1$ was removed from the $\underline{R^1R}^{st}$ heterozygote. Such differences in phenotype, following paramutation, suggest that the R alleles have different heritable states which are reflected in different degrees of mosaicism in the endosperm of testcross kernels. In advanced stages of paramutation, when R is introduced through the pistillate parent and is represented in endosperm cells by two chromosomes with R, one can observe many near colorless kernels. In Vol. 38 we reported that the lightest and darkest of such phenotypes (when $\underline{R}^6$ was introduced through the female parent) did not respond to selection.

It was noted, in 1965, that upon self-pollination of $\underline{R^9R}^9$ homozygotes (nine generations with $\underline{R}^{st}$) the upper halves of ears produced 20% more kernels with mosaic patterns of pigment in the aleurone than did the lower halves of ears. Kernels from the upper half of the ear were grown out along with kernels from the lower half in an effort to check for the heritability of these mosaic sectors in the female inflorescence. Table 5 shows the percentage of mosaic kernels observed in the upper and lower halves of ears in 1965; 1966 results show nearly the same numbers of mosaic kernels were found in both groups of progeny from each of the

ears sampled. It is concluded that where R is introduced through the female and differences in frequency distribution of light and dark kernels exist over the length of the ear, such ear mosaics will not result in heritable differences if selection is practiced on the light and dark kernels. Thus far, selection for heritable differences in R expression has been possible only when R is introduced to the endosperm by the male gamete.

Table 5

	Ear #1 % Mosaic Kernels		Ear #2 % Mosaic Kernels		Ear #3 % Mosaic Kernels	
	Lower ½	Upper ½	Lower ½	Upper ½	Lower ½	Upper ½
Year, 1965	15.9	35.4	16.6	37.3	20.2	35.2
Progeny from above, 1966	68.0	70.7	62.2	65.8	59.3	59.3
Number of seeds scored in thousands	1.5	2.4	2.0	2.6	2.8	3.1

Heritability of light and dark kernels from the upper and lower halves of R^9R^9 ears, self-pollinated in 1965. Samples of kernels representing upper and lower halves of three ears were grown out in 1966 and mated using W22, rr, pollen. Frequencies of mosaic kernels on offspring derived from the light and dark halves of ears are recorded above.

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3. Progressive paramutation of R-locus expression through ten generations.

In MGCNL 38 we reported that when R was kept heterozygous with R^{st} for eight generations, a progressive reduction in number of cells with pigment could be observed with each generation. Since the amount of pigment which R is capable of producing, when transmitted through the pollen, rapidly reaches the null level by the third or fourth generation, it was necessary to follow changes in level of pigmentation when R is introduced into endosperm through the pistillate parent. When brought into the endosperm through the female, two R genes are contributed to the triploid endosperm cells. With two paramutated R genes present, a considerable increase in pigment is realized and it is possible to continue to follow the effects of R^{st} on R through many more generations. Table 6 below shows that the

R pigment over a ten generation period with R^{st} shows a steady and measurable decrease with each generation--that is, the effects of R^{st} continue to be additive as measured through the phenotype of R pigmentation. It is interesting that the effects of R^{st} on R at this 2-dose level of R has continued to show a linear increase in numbers of kernels which show a mosaic pattern. After 10 generations with R^{st}, one can see in Table 6 that a mosaicism can be detected in over half of the kernels. No other effects of R^{st} have been observed on our inbred lines. It is likely that this reduction in pigmentation can be followed for at least ten more generations since considerable pigment still remains when two paramutated R alleles are present. From the existing data, it appears likely that R can eventually be converted to the completely colorless form and thus it seems possible that a dominant phenotype can be converted to the recessive.

Table 6
Number of generations that R has been heterozygous with R^{st}

	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}
Kernels scored (in thousands)	2.0	6.9	7.8	6.1	6.3	6.1	5.6	4.8	2.6
% mosaic kernels	1.2	10.1	7.7	17.4	23.1	30.1	32.7	42.7	50.4

Progressive conversion of R expression (paramutation by R^{st}) through ten generations. Scores represent the percentage of kernels showing endosperm mosaicism (pigmented and nonpigmented cells) when R is contributed to the endosperm tissue through the pistillate parent. Scored kernels represent the RRr genotype.

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4. High level repression of R expression in the presence of two R^6 alleles.

When R has been kept heterozygous for six generations with R^{st} (by self-pollination), one observes a progressive reduction in the number of aleurone cells with pigment (Vol. 38). When R^6R^6 segregates are self-pollinated so that endosperm cells are $R^6R^6R^6$, one can note some 20-25 kernels whose aleurone pigmentation is considerably reduced--near the colorless level. The presence of such kernels with highly repressed pigment furnishes a striking contrast to the otherwise heavily pigmented kernels on the same ear. If pigment is repressed in certain kernels, as such lightly pigmented kernels suggest, then it may be possible to place into cells of such kernels a "normal" R and detect repression in the resulting endosperm on a standard (unparamutated) R introduced through the pollen. The

genotypic constitution of such cells will be $\underline{R}^{6}\underline{R}^{6}\underline{R}$; with $\underline{R}$ introduced through the pollen, a dark kernel would be expected. At the same time it was also of interest to ask what degree of repression might be observed if the same standard $\underline{R}$ allele were introduced into aleurone cells against the background of highly paramutagenic alleles such as $\underline{R}^{st}$-- endosperm cells with $\underline{R}^{st}\underline{R}^{st}\underline{R}$.

The 25 lightest kernels were removed from each of six ears from the crosses $\underline{R}^{6}\underline{R}^{6}$ x $\underline{RR}$ and $\underline{R}^{st}$ x $\underline{RR}$. These selected kernels were scored by matching them against a set of standard kernels as described above. In Table 7 it can be seen that the weakest $\underline{R}$ pigment expressions for standard $\underline{R}$ are found on $\underline{R}^{6}\underline{R}^{6}$plants and not on the highly paramutagenic $\underline{R}^{st}\underline{R}^{st}$ plants where $\underline{R}$ pigment scores were uniformly dark. Table 7 confirms the results of the Wisconsin Laboratory which reported that no difference in $\underline{R}$ expression was found when standard $\underline{R}$ pollen was placed on plants which carried a highly paramutagenic colorless allele (compared to $\underline{R}$ on nonparamutagenic controls). It is highly interesting, therefore, that such a marked reduction in pigmentation can take place in the presence of two $\underline{R}^{6}$ alleles in the endosperm, especially when the paramutagenic alleles appear to be ineffective in this respect.

It would be premature to call this reduction of $\underline{R}$ pigmentation paramutation, if by paramutation we mean a transmissible change in the mosaic pattern of $\underline{R}$ pigmentation. There is no way of testing for transmissibility of the altered $\underline{R}$ expression in the endosperm. Whether this change represents paramutation or is simply a repression of the normal $\underline{R}$ in the presence of two highly paramutated $\underline{R}$ genes remains to be determined.

Table 7

	Endosperm genotype $\underline{R}^{st}\underline{R}^{st}\underline{R}$	Endosperm genotype $\underline{R}^{6}\underline{R}^{6}\underline{R}^{6}$
	19.96	17.00
	19.92	17.32
	20.16	17.00
	20.04	15.96
	19.52	15.48
	20.12	16.60
		16.88
Pooled $\overline{X}$	19.95	16.61

Comparison of R repression in the endosperm in the presence of two R^{st} and two R^{6} alleles. Each of the above figures represents ear mean scores based on 25-kernel samples. The lightest 25 kernels were selected from each of the ears for the above comparisons.

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Richard Sherman

5. Heritability of light and dark phenotypes from RR and Rr tassel mosaics.

In MGCNL 40 we reported that tassel mosaics could be found from RR and Rr combinations and that such mosaics parallel variations to be found for R from RR^{st} heterozygotes. Such differences in R expression when R is removed from the RR and Rr combinations may involve the same mechanisms responsible for differences in R from RR^{st} combinations. In Vol. 40 we reported that the differences in R expression from RR^{st} tassel sectors were heritable. Table 8 below shows that heritable differences, though very much smaller than from RR^{st}, can be found for R from RR and Rr backgrounds. Dark kernels, resulting from pollen collections made on the first day pollen was shed, were planted for comparison with the lightest kernels resulting from a pollen sample taken six days later from the same tassel. Scores of testcrossed plants from these light and dark seed selections show their means separated by two standard deviation units. Likewise, selected light and dark phenotypes from testcrosses from Rr plants show heritability. Seeds in this last case were provided by pollination from a single pollen sample from an Rr plant in 1965. Plants resulting from selected seeds were tested in 1966; the light and dark selections produced scores whose means were separated by two standard deviation units. In another test, in Table 8, it can be seen that where tassel samples were separated by six days in 1965 and where testcross scores were nearly alike from these pollen samples, again, the scored seeds produced plants whose testcrosses, in 1966, show no significant difference for pigment scores. This variation in R expression from the RR and Rr combinations must be considered to originate in somatic sectors arising during the course of tassel differentiation. These somatic sectors, in turn, result in pollen transmissible levels of mosaic expression (different states of R) visible in the aleurone layer of the endosperm.

Heritability of different states of R (light and dark pigment mosaics) from RR and Rr backgrounds. Seeds were selected from 1965 testcross ears reported in MGCNL 40. Selected Rr seeds were grown and resulting plants were testcrossed in 1966 for the scores given on the next page.

Table 8

Source of R	Ear $\bar{X}$	Stan. Dev.	Ear $\bar{X}$	Stan. Dev.	Pollen Collection Detail 1965
RR	20.73 n=9	.29	20.13 n=9	.35	Pollen from same tassel; collections separated by 6 days; darkest and lightest seeds selected from darkest and lightest ears, respectively.
Rr	21.35 n=6	.23	20.42 n=5	.41	Single pollen sample; darkest and lightest seeds selected from single ear.
Rr	20.82 n=6	.58	20.81 n=7	.37	Pollen samples separated by six days; randomly selected seeds from two testcross ears whose pigment scores were alike.

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6. Ranges of R expression (paramutation) from RR, Rr, RR^{st} and R^1R^1 plants.

Brink and his students have reported that different levels of R expression from RR^{st} heterozygotes can be attributed to the somatic sectors which occur during tassel formation. Our data in MGCNL 39 and 40 confirm the Wisconsin reports for tassel mosaics--our methods of sampling pollen from tassels differed from those used by the Wisconsin group. By sampling RR^{st} tassels daily during the time of pollen shed, usually a period of seven days, we have found that earliest pollen collections produce the lightest R^1 expression; the darkest R^1 expressions come from the last pollen samples from the tassels. It would appear, therefore, that paramutation can be defined as somatic mosaicism which is manifested as heritable changes in R expression. The level of R expression--the state of R--depends, in turn, on the position in the tassel from which the gamete carrying R emerges. It is hypothesized that different ranges of mosaicism result from different allelic combinations; evidence for this hypothesis is presented below.

Samples of pollen from single plants representing the different allelic combinations, RR, Rr, RR^{st} and R^1R^1, were taken over a period of seven days and applied to colorless inbreds. Such testcrosses were scored by the method of matching testcross kernels against a set of standard kernels, detailed in earlier reports above. Each test plant was represented, on the average, by four testcrosses (four pollen samples made on different

days over a seven day period) from which standard deviations for tassel samples were computed for each plant. Standard deviations for each of the four genotypes are averaged for comparison purposes. It can be seen in Table 9 that single plants of the Rr and RR combinations show only a third of the range of variation for R expression when compared to the standard deviations for R^1 from RR^{st} combinations. It is appropriate to suggest, therefore, that the four allelic combinations represent variation from a continuum of possible states of R; any particular state of R is dependent on its previous history of association with its alleles (as well as its position of origin in the somatic mosaic of the tassel, discussed below).

Such differences in R scores reflected in the standard deviations, suggest that somatic sectors from different positions in the tassels determine the particular state of R. The Wisconsin Laboratory has not been able to demonstrate somatic sectors for either the RR or Rr combinations, though they have reported heritable differences in R expression from RR and Rr combinations. Our evidence for tassel mosaicism (Vol. 40) and its heritability (Table 8) together with evidence for a continuum of variation in R expression (Table 9) depending on allelic combinations, requires that we consider RR, Rr, RR^{st} and R^1R^1 as paramutagenic combinations.

Samples of pollen from a single plant, made over a seven day period, show a "polarity" which is reflected in an orderly change in R expression during development. We have reported, for instance, that pollen collections from tillers produce R expressions which score darker than those of the main tassel; this confirms a report of Brink and his students. From the RR^{st} combinations, we find almost invariably, over the past three years, that the earliest collections show R^1 expressions to be lighter from the upper part of the tassel than pollen collections made lower (later) on the tassel. On the other hand, in the case of R^1R^1 segregates, from the RR^{st} sibs, we find the opposite "polarity"; the earliest pollen collections from R^1R^1 plants produce darkest testcrosses. Table 10 shows somatic sectors can result in opposed gradients with respect to the state of R^1--from RR^{st}, the earliest pollen yields the lightest testcrosses, later pollen samples become progressively darker; from R^1R^1 segregates of RR^{st} the darkest testcrosses came from the earliest pollen samples, while the later pollen samples produced progressively lighter kernels. It is quite possible that the environment may make some contribution to the differences in these tassel mosaics. In 1965 we reported for RR and Rr that the earliest pollen collections gave the darkest testcrosses and the latest collections showed the lightest testcrosses. In 1966 testcrosses of RR (Table 10) and Rr, lightest testcrosses are found from among the first pollen samples, the reverse of the data of 1965.

Since pigment mosaics are characteristic of the R gene for all allelic combinations with R, it may be well to give some consideration to terminology to be applied to the conditions affecting mosaics controlled by the R locus. At the present time these phenomena are being described by such terms as: reversion, mosaicism, induction, paramutation, enhancement, conversion, variegation, sectoring, states of R,

mottling and still others. Operationally, the result observed, is either an increase or decrease in the numbers of cells in the aleurone layer with pigment. The simplicity of the observations may be obscured by the multiplicity of terms for the observations. One may also be left with the impression that the paramutation alleles are a class apart from "normal". As the data above suggest, this implication cannot be defended. The increase or decrease in numbers of cells--the state of R--is a function of the allelic combinations and the position of origin in the tassel of the gamete carrying R. There are no allelic combinations (even the hemizygote) where mosaicism is absent in the testcrosses of the R allele, therefore paramutation (mosaicism) is universal for R.

The genetic apparatus reflected by the mosaic expression of the R alleles above, represents a system of biological "memory", a "summing device", whose current phenotypic range of mosaicism represents an expressive summation of its genetic history. The expressivity of this system responds to both allelic and environmental regulation in its memory-storage capabilities. The availability of the many states of R and the ranges of expression which show heritability provide a mechanism of extreme genetic versatility of the individual plant. In so far as a single gene is concerned, the choices of R expression available to the next generation from a single plant or from a few plants would be very great; still further opportunity for increase in the range of expression for a particular R would take place with each generation. Should this system examined for R prove to be at all applicable to other genetic systems, the problem of accounting for sources of variation for evolutionary purposes would cease to exist and the behavior of the R locus will have made possible the opening of a Pandora's box the biological consequences of which stagger the imagination with all its implications.

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Table 9
Standard deviations of testcrosses for pollen samples of single plants

	Rr	RR	R^1R^1	RR^{st}
	.37	.33	1.01	.90
	.23	.39	2.11	1.42
	.35	.50	1.13	1.84
	.33	.24	.67	1.32
	.30	.55	.93	1.61
	.60	.47	1.55	1.37
Pooled $\bar{X}$	.36	.41	1.23	1.41

Comparison of standard deviations for R-expression from four different genotypic backgrounds. Each standard deviation figure above represents, on the average, four or more pollen samples (50 scored kernels/sample) from the same plant taken over a period of from four to seven days.

Table 10

		Plant Number					
	Day	#1	#2	#3	#4	#5	#6
rr x R^1R^1	1	19.00	13.82	18.26	19.94	19.88	20.18
	2						
	3	17.96	13.52	15.40	13.26	17.56	19.78
	4	17.20	11.82	14.78	18.36	18.02	19.26
	5	16.00	11.46	14.74	16.58		17.74
	6					16.82	
	7		11.62	12.38			
rr x RR^{st}	1	11.76	9.92	11.28	13.96	13.82	15.56
	2	12.74		10.10	14.36	17.06	14.22
	3	14.84	6.78			15.42	17.42
	4				13.74		18.14
	5	14.00	11.60	14.22	15.16	14.28	17.18
	6			13.18	17.76	16.60	
	7	15.68	10.88		15.82		
rr x RR	1	20.70	20.74	20.70	20.34	20.44	19.94
	2			21.74	21.44		
	3		21.90	20.86			
	4	20.46	21.00	21.88	20.46	20.06	20.10
	5	20.96	21.00		21.22	20.26	20.64
	6	21.24	21.20			21.06	20.82
	7	21.36				21.10	20.98

Selected examples of tassel gradients for R expression from three different allelic backgrounds, R^1R^1, RR^{st} and RR. Pollen samples were made over a seven day period and put on rr for scoring purposes.

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1. Enzyme treatment of chromosomes.

A preliminary investigation into the effects of trypsin on maize chromosomes is being undertaken as an undergraduate research project. The experiments were suggested by the work of Trosho and Wolff (J. Cell Biol. 26, 1965), who succeeded in observing multistrandedness in metaphase chromosomes of Vicia faba root tips.

Modified Trosho and Wolff techniques were adopted to microsporocytes; the anther smear was made in a trypsin solution (0.1 mg/ml of 0.01 M phosphate buffer, pH 7.2), incubated for one hour at 32°C, air dried and stained in Feulgen. Compared to controls, incubated in the buffer and treated similarly, the enzyme treated chromosomes showed in all stages of division a "fraying" and "blurring", the effect being most noticeable for cells in diplotene. Although multistrandedness may occur, no cells demonstrated an organized arrangement of strands.

Studies are also being conducted on mitotic chromosomes and interphase nuclei from root tip and tapetal cells. A cursory examination has not revealed an effect as found for the sporocytes.

It is hoped that further observations may be performed on sporocytes exhibiting anaphase bridges and large translocation regions. Comparative digestion studies on metaphase chromosomes of mitosis and meiosis I and II are also underway.

Edward J. Ward

2. Refined smear technique for obtaining large numbers of metaphases in corn root tips.

This is an amended recipe of the technique which appeared in the Maize Genetics Cooperation News Letter, 40:146-147, 1966:

Step 1: The seeds can be grown on 2% agar or on vermiculite in a Petri dish. A 10 mm radicle will be produced within 40 hours.

Step 2: Transfer 10 mm radicles to a Petri dish containing a 0.2% aqueous tween 80 colchicine solution and incubate 8 hours.

Step 5: Hydrolyze in 1N HCl at 60°C for 25 minutes.

Step 6: Stain in leuco-basic fuchsin until the root tip is deeply colored.

Step 8: The enzyme is a 5% cellulase - 5% pectinase solution.

Step 11: Add a 22 x 50 mm coverslip.

New Step 12: Heat gently.

Step 13: Seal with gum mastic. The coverslip should be held down by a flat block of marble or other suitable material while sealing.

R. M. Brown

3. Karyotype of Zea mays.

Karyotypes of maize have been composed from several commercial varieties, translocation stocks, a trisomic 6 stock and normal stocks. Metaphase chromosomes from root tip cells were employed.

The relative lengths of the mitotic chromosomes are very similar to the meiotic chromosomes, with chromosome 10 being approximately one half the length of chromosome 1. Chromosome 1 in mitotic metaphase is easily distinguished because of its length. Chromosome 2 is also usually distinguished from all others. Chromosomes 3, 4, and 5 can be identified one from the other in exceptional preparations only. Chromosome 6 does not possess an easily discerned satellite at mitotic metaphase, although it is observed during mitotic prophase. Root tips germinated in .02% 5-bromodeoxyuridine contain cells with satellites at mitotic metaphase. Two chromosomes possess satellites, and the length of the stalk varies. From preliminary studies this appears to be chromosome 6. Chromosomes 6, 7 and 8 cannot always be distinguished. Chromosomes 9 and 10 can usually be identified.

Graphs have been made of 10 cells using the length and arm ratios as axes. The points were grouped, and compared with the karyotypes which had been grouped visually. The measurements for the graphs were taken from projections of the negatives. The groupings were in most cases similar in graphs and the karyotype.

Measurements of the longest two and the shortest two chromosomes in projected cells were taken and used to determine the mean, standard error, and variance among cells. A group of cells was measured independently eleven times to calculate the experimental error. Confidence limits were calculated; analysis of variance, and t tests were completed.

The mean arm ratio for the longest two chromosomes was 1.19. The standard error was .00279.

The mean arm ratio for the shortest two chromosomes was 1.59. The standard error was .0264.

A t test showed that the two samples (long and short chromosomes) were not from the same population.

An F test showed that the experimental error was not significant. A second F test yielded a highly significant difference between the variance of the longest and shortest chromosomes.

A table of the arm ratios of the 10 pairs is below. The measurements were done on enlarged photographs, and the arm ratios given are the averages from fourteen cells.

The arm ratios are not always the same as those observed in meiosis. Chromosomes 1, 2, 3, 4, 5, and 9 have ratios in mitotic metaphase similar to pachytene. No ratio has ever been observed greater than 2.8: 1.0 in any chromosome. The ratio of chromosome 6, 7, 8, and 10 varies from cell to cell. This variation of 6 might be accounted for by the degree of condensation of the satellite region.

Chromosome No.	Arm ratio
1	1.2
2	1.2
3	1.8
4	1.6
5	1.1
6	2.2
7	1.7
8	2.1
9	1.8
10	1.6

Our karyotype is routinely presented as follows:

Group A - chromosome 1
Group B - chromosome 2
Group C - chromosomes 3, 4, 5
Group D - chromosomes 6, 7, 8
Group E - chromosomes 9, 10

R. M. Brown

4. Influence of temperature on pollen germination and tube growth.

The effect of temperature on the germination and growth of "Seneca 60" ($\underline{su}_1/\underline{su}_1$) hybrid corn pollen was investigated. The pollen was germinated in the concavities of well slides on a medium consisting of 12% sucrose, 100 ppm H_3BO_3, 300 ppm $CaCl_2.2H_2O$ and 1% methyl cellulose (MGCNL 39:169 and 40:147). Germination was allowed to proceed for varying times in a water-saturated atmosphere either within covered

glass dishes in incubators or on a Wild temperature controlled microscope stage. Both per cent germination and pollen tube lengths were measured from photomicrographs of random fields.

Optimum temperature for per cent germination was in the range 20-23°C. Germination was low and variable above 35°C and below 15°C. Much bursting occurred at the higher temperatures. No germination took place at 10°C and below; the grains remained intact and a small bubble formed at the germ pore. If the temperature of the stage was then raised by 5C° a tube formed from the bubble.

Temperature influences the average time for germ tubes to appear--the latent period of germination. The latent period is a hyperbolic function of temperature. As the temperature was lowered below 10°C the time for germination became infinite; at an infinitely high temperature the latent period approached zero, but bursting of the grains, of course, actually terminated the curve between 35°C and 40°C.

Rates of growth of several hundred pollen tubes over periods up to 60 minutes were determined at 9 temperatures between 10°C and 45°C. Periods of maximum growth rate varied with temperature and seldom exceeded 35 min. Maximum rates of growth increased linearly between 12°C and 30°C with a Q_{10} of 2.1 and fell off rapidly below 12°C and above 35°C.

The lengths of tubes after a measured period of germination are functions of the latent periods of germination, the growth rates, and the times at which these rates level off. The average tube growing at 30°C was 1.5 X as long when it began its plateau phase of growth as the average tube length at 38°C. No significant difference was observed between those at 20°C and 30°C.

F. S. Cook
D. B. Walden

5. Tests for chemotropism in pollen tubes.

Two methods for testing the chemotropic response of "Seneca 60" ($\underline{su}_1/\underline{su}_1$) hybrid corn pollen were tried. The first used the method of Mascarenhas and Machlis (Nature, 196:292, 1962) in which pollen and test materials were placed in depressions in a 1% Noble agar medium containing 100 ppm boric acid and 12% sucrose but no added calcium. Directions of pollen tubes were observed under a microscope.

The second method consisted of soaking Whatman 3MM filter paper discs in test material and placing one disc in the centre of a slide well containing the methyl cellulose supplemented medium lacking calcium (MGCNL 39:169 and 40:147). Pollen was shaken onto the surface after a suitable diffusion period. The pollen grains and tubes were photographed and the distance of each grain from the edge of the disc and the direction of growth of the tube relative to the disc were measured. The quantitative data from the second experiment were subjected to regression analyses.

No germination was observed in the germination depressions in agar when solutions of various calcium salts, filter paper soaked in calcium chloride solutions, blocks of agar containing calcium, pieces of style or pieces of endosperm were added to test depressions. Some germination occurred when crystals of $CaCl_2 \cdot 2H_2O$ were added to the test depression and allowed to diffuse for 17 hours, but the direction of growth of the tubes appeared to be random.

Germination was obtained around the filter paper discs soaked in calcium chloride or mashed styles and endosperm. The best results were obtained after a 40 min. diffusion period from discs soaked in 1500 ppm $CaCl_2 \cdot 2H_2O$. The analysis of the numerical data, however, indicated a random orientation of the tubes. Further details, photographs and illustrations may be found in the Canadian Journal of Botany, 45: (in press).

F. S. Cook
D. B. Walden

6. A plant with opposite leaves.

A single plant, hemizygous for the short arm of chromosome 9 distal to but not including bz$_1$ was found in 1965 from a culture of 12 siblings to possess opposite leaves at every node and in addition twin ear shoots at each of three nodes. Prior to extensive internode elongation the plant resembled a rosette.

The morphology of the mature plant differed from a normal sib in at least two ways: a pair of leaves appeared to be inserted at each node and those leaf pairs were spirally arranged along the stem. Modifications of the tassel branch insertions were observed also. While the insertion of a pair of leaves at a node could be interpreted in terms of a long and short internodal system (as reported by Weber and Weatherwax, MGCNL 40:49, 1966) we found no evidence in our specimen to support this interpretation. The leaves do overlap one another at the node but they appear to be inserted at the same level. No evidence of two nodes was found from 100 u thick sections through a node and its two leaves. We suggest, in the absence of critical data, that two leaves were initiated simultaneously from the apex. Direct observation of the developing apex would be necessary to confirm this.

No rosettes or plants with opposite leaves have been found among 70 seeds planted in the winter crop. Several hundred kernels remain from the self pollinations.

R. I. Greyson
D. B. Walden

UNIVERSITY OF WISCONSIN
Madison, Wisconsin

1. Nondisjunction of abnormal chromosome 10.

Relatively small populations involving different inbred W22 sublines homozygous for abnormal chromosome 10 have given numerous plants which combine the particular phenotypic characteristics of homozygous K-10 and trisomic-10 in this inbred. Plants showing the compound phenotype, however, are sterile. To obtain trisomic plants in a population not showing the abnormal chromosome 10 phenotype, $\underline{R}^{r}K/\underline{R}^{r}K$ plants were pollinated with $\underline{r}^{g}k/\underline{r}^{g}k$. Among 423 progeny, 77 were classified on the basis of plant type as trisomic-10. In reciprocal crosses with $\underline{r}^{g}\underline{r}^{g}$, each of the 16 presumed trisomics tested gave characteristic trisomic ratios. A similar test of the colored kernels from several of the $\underline{R}^{r}K/\underline{r}^{g}k$ progeny crossed to $\underline{r}^{g}k/\underline{r}^{g}k$ ♂♂, in contrast, gave 412 plants with none resembling trisomic-10. The observation of one trisomic among every five or six offspring of K-10/K-10 used as female prompted an investigation into the nature of the nondisjunction utilizing genetically marked chromosomes-10.

Matings of W22 $\frac{g\ R^{st}\ K}{+\ r^{r}\ K}$ ♀♀ X W22 $\frac{g\ r^{g}\ k}{g\ r^{g}\ k}$ ♂♂ gave 512 (21.97%) plants with phenotypic characteristics of trisomic-10 and 36 (1.5%) other atypical plants in a total population of 2,330. The frequency of chromosome 10 trisomics, about 22 per 100, is consistent with the previous experiment. Forty-five putative trisomic-10 plants, including somewhat more than the overall proportion of golden plants, were testcrossed as male parents on homozygous $\underline{g}\ \underline{r}^{g}$ k. All gave segregation ratios characteristic of trisomy for 10. As tested by the markers used, 16 had one each of the maternal noncrossover chromosomes ($\underline{g}\ \underline{R}^{st}$ and $\underline{+}\ \underline{r}^{r}$), 13 had both maternally derived chromosomes of one or the other parental type, 15 had one crossover chromosome, and one had two crossover chromosomes. Recombination between $\underline{g}$ and $\underline{R}$ was 22% in disomic plants and 19.7% for a random sample of trisomics.

A marked excess of stippled was observed among the initial kernel population, but 61.4% of the resulting plants produced red anthers indicating the presence of $\underline{r}^{r}$. The disturbed ratios are understandable in terms of the observed frequency of trisomics carrying both $\underline{R}^{st}$ and $\underline{r}^{r}$.

At what stage in the life cycle does the trisomic condition originate? Considering the known cytological properties of abnormal-10, nondisjunction is presumed to be meiotic. Clearly the stage is not postmeiotic, since the two maternally derived chromosomes need not be identical. The present experiment does not exclude the possibility of mitotic nondisjunction in the sporogenous tissues, and the data are not sufficiently extensive to establish whether a particular division of meiosis is involved. Further genetic studies involving a chromosome 10 marker close to the centromere are needed to clarify these points.

K. V. Satyanarayana
J. L. Kermicle

2. Nonrandom association between R-stippled (R^{st}) to self-colored (R^{sc}) mutation and recombination of linked markers in R^{st} R^{st} and R^{st} R^{nj} plants.

Mutation of the unstable spotting factor R-stippled to a form conditioning uniformly pigmented aleurone (self-colored, R^{sc}) is known to occur at several stages during the maize life cycle. The germinal mutation frequency (based on single, self-colored seed from R^{st} bearing female parents) varies, however, depending upon the R locus genotype (Ashman, Genetics 45:19 and 52:835). Two fairly distinct rates were observed: approximately 7×10^{-4} characteristic of R^{st} r^{g} heterozygotes, and about 20×10^{-4} observed for certain R^{st} r^{r} heterozygotes and R^{st} R^{st} homozygotes. Subsequent tests indicate that the genotype dependent variation may be stage limited to near meiosis, suggesting crossing over as a possible basis for the two-fold excess of mutations in the high frequency combinations. Study of the possible relationship between R^{sc} mutation and recombination in the g-20-R-6-M^{st} region was made in genotypes giving the higher mutation frequency, therefore, even though previous tests had shown these two events to be independent in low mutation rate situations of the R^{st} r type.

Of the 39 singly occurring self-colored seeds among 12,348 produced from g R^{st} +/+ R^{st} M^{st} ♀ X g r^{g} + ♂ matings, progeny of 22 proved self-colored, whereas 17 yielded the parental, stippled phenotype. Characterization of the 39 cases for g and M^{st} gives the following distributions:

Progeny class	Linked marker composition: Parental		Recombinant		Classification incomplete
	g +	+ M^{st}	g M^{st}	+ +	
R^{sc}	4	6	7	2	3
R^{st}	5	8	2	0	2

Among the 15 failing to prove germinally R^{sc}, two were recombinant. This small sample accords with an average g to M^{st} recombination value of approximately 25 per cent. Within the R^{sc} class, on the other hand, g and M^{st} assort nearly independently, with nine in the recombinant and ten in the parental group. The excess of R^{sc} mutations in the recombinant class indicates that the basic seed pigmenting determinant of R^{st} may be separable by crossing over from a second component responsible for the spotting action. Furthermore, since the parent was homozygous for stippled, such exchanges must be effectively unequal. Presumably the spotting component, but not necessarily that for seed pigmentation, lies within a duplicated region.

More critical information on the possible crossover origin of some R^{sc} mutations derives from R^{st} R^{nj} heterozygotes. R^{nj} (R-Navajo, kernel crown spot) has not been observed to give self-colored mutations, and also

differs from $\underline{R}^{st}$ in time of pigment formation. Three kinds of nonparental kernel phenotypes were observed among 68,883 seeds from $\underline{g}\ \underline{R}^{st}\ \underline{M}^{st}/+\ \underline{R}^{nj}\ +$ ♀ X $\underline{g}\ \underline{r}\ +$ ♂ matings: colorless, self-colored, and Navajo-stippled compound ($\underline{R}^{nj:st}$). The colorless type occurred infrequently and each proved to be either $\underline{R}^{nj}$ or $\underline{R}^{st}$ in progeny tests. As self-colored kernels were frequent, only those from a portion of the total population were chosen for progeny analysis. The results of testing 25 for germinal transmission and linked marker composition are given in the first two lines of the table below:

Phenotype		Effective kernel population	Linked marker composition			
			Parental		Recombinant	
Kernel	Progeny		$g\ M^{st}$	$+\ +$	$g\ +$	$+\ M^{st}$
$\underline{R}^{sc}$	$\underline{R}^{st}$	14,035	11	0	0	1
$\underline{R}^{sc}$	$\underline{R}^{sc}$	14,035	6	0	4	3
$\underline{R}^{nj:st}$	$\underline{R}^{nj:st}$	62,815	0	0	0	15

The fraction of self-colored kernel selections which proved germinal, about one half, and the frequency of this class, 18.5×10^{-4} (based on the number of $\underline{R}^{st}$ gametes), agree with the corresponding values obtained for $\underline{R}^{st}\ \underline{R}^{st}$. The distribution of linked markers also conforms to that predicted from the findings with $\underline{R}^{st}\ \underline{R}^{st}$. Four $\underline{R}^{sc}$ mutants were found to carry the $\underline{g}$ + combination whereas expectation based on independence of mutation from recombination predicts less than one. To permit interpretation of these four as separation of the two components postulated for $\underline{R}^{st}$ by single exchanges, the location of the basic seed pigmenting element should be proximal to the element suppressing its action.

The third nonparental phenotype, $\underline{R}^{nj:st}$, strongly resembles the case described by Brink (M.G.C.N.L. 34:122). The typical stippled pattern is confined to those regions of the kernel normally pigmented by $\underline{R}^{nj}$. Each of the 15 progeny groups recovered from 16 initial $\underline{R}^{nj:st}$ kernel selections yielded the compound phenotype and are found to correspond to $\underline{R}^{nj}$, rather than $\underline{R}^{st}$, in time of pigment formation. They therefore appear to combine the basic $\underline{R}^{nj}$ pigmenting property with the instability of stippled. Each proved to carry the proximal marker of the $\underline{R}^{nj}$ chromosome and the distal marker of the $\underline{R}^{st}$ homologue. That this marker combination is complementary to the principal recombinant class of $\underline{R}^{sc}$ mutants suggests that the two originate as reciprocal products of a single kind of recombinational event. Neither $\underline{R}^{sc}$ nor $\underline{R}^{st:nj}$ expresses the characteristic plant color effects of $\underline{R}^{nj}$, however, so either the two are not strictly complementary, or plant pigmentation is suppressed when brought into cis position with the inhibiting component of $\underline{R}^{st}$.

J. L. Kermicle

Addendum:

ILLINOIS STATE UNIVERSITY
Normal, Illinois

1. Further tests of distributive pairing in 2N+2 Zea mays.

A. Univalent frequencies in 2N+1 plants.
Anaphase I data from 2N+2 plants (plants containing two chromosomes in addition to the diploid complement) were reported by the author in the 1966 News Letter. The two extra chromosomes were found to go to the same pole as frequently as to opposite ones at anaphase I; therefore distributive pairing was not detected in these plants. Data are now available on the univalent frequencies in very closely related plants containing one extra chromosome. The data are presented in Table 1.

The frequency of cells containing univalents at diakinesis in 2N+1B, In4a/N and 2N+4, In4a/N/N plants was found to be 100 and 32.7 per cent respectively. The product of these two values (32.7 per cent) is the expected frequency of cells containing a univalent B and a univalent chromosome 4. In the remaining cells (67.3 per cent) the two additional chromosomes would be expected to disjoin independently giving equal numbers of 11-11 and 10-12 disjunctions. Thus, one-half of 67.3 per cent, or 33.65 per cent of the cells should be included in each class (11-11 and 10-12). If distributive pairing were 100 per cent efficient in maize, 66.35 % (32.7% + 33.65%) of the cells should have had 11-11 disjunctions, and 33.65% should have had 10-12 disjunctions. This obviously was not the case. It was calculated that the results were significantly different from those expected if efficiency of pairing were 50 per cent or greater.

B. Diakinesis in 2N+2 plants.
In order to determine whether two heterologous univalents would pair at diakinesis, plants containing two extra nonhomologous chromosomes were examined cytologically. The results are presented in Table 2. If distributive pairing occurred prior to diakinesis, one would expect to find association of the heterologous univalents as a pair at diakinesis in cells where neither extra chromosome was involved in a trivalent configuration. This obviously is not the case, for no cells were found which clearly contained 11 bivalents at this stage. Therefore, distributive pairing does not occur before diakinesis in these plants.

2N + 4 + 1B, In 4a/N/N plants in Table 2 were slightly different in origin from the plants reported in the 1966 News Letter, and for this reason, their univalent frequencies are not directly comparable.

C. Metaphase I studies.
In the stocks used, the chromosomes at metaphase I were not well separated on the equatorial plate. As a result, it was not possible to score positively the number of bivalents, trivalents, and univalents in many of the cells. However, when 2N+4+1B, In 4a/N/N plants were analyzed at metaphase I, one or more univalents were seen in essentially all of the cells. Therefore, nonhomologous univalents were rarely, if ever, associated at metaphase I. One cell was scored as having 11 bivalents, but the

Table 1

Frequency of trivalent and bivalent-univalent configurations at diakinesis in 2N+1 plants

Chromosome constitution	Number of plants	No. of cells with: 10 bivalents, 1 univalent	9 bivalents 1 trivalent	Total	% of cells with 10 bivalents, 1 univalent
2N+1B, In4a/N	1	176	1 questionable cell	177	99.4%
2N+4, In4a/N/N	4*	338	695	1033	32.72%
2N+6, In4a/N	4**	208	845	1053	19.75%

*Heterogeneity $X^2 = 0.72$ $.9 > P > .8$ DF = 3

**Heterogeneity $X^2 = 1.92$ $.7 > P > .5$ DF = 3

Table 2

Frequency of different pairing configurations at diakinesis in 2N+2 plants

Chromosome constitution	Number of plants	No. of cells with: 11 bivalents	10 bivalents 2 univalents	9 bivalents 1 trivalent 1 univalent	8 bivalents 2 trivalents	Total
2N+4+6, In4a/N/N	1	1 questionable cell	12	68	94	175
2N+4+1B, In4a/N/N	3	0	77	274	0	315

Table 3

Frequency of different pairing configurations at metaphase I in 2N+4+1B, In4a/N/N plants

No. of plants	No. of cells with: 11 bivalents	10 bivalents 2 univalents	9 bivalents 1 trivalent 1 univalent	1 univalent, otherwise unscorable	no univalent seen otherwise unscoreable	Total
3	1 questionable cell	144	110	403	14	672

classification was uncertain since it was of poor quality. The data are presented in Table 3.

Clearly, distributive pairing does not occur in these plants or it occurs with a very low frequency which is far lower than that found by Grell. These results are not compatible with those obtained by Michel (1966 News Letter and unpublished thesis). The reason is not known.

David Weber

2. Studies of the distribution of unpaired chromosomes in the progeny of plants hyperploid for a B and a ring chromosome.

An independent test of the distributive pairing hypothesis involved a study of the distribution of chromosomes in the progeny of plants containing two unpaired chromosomes. The two extra chromosomes were the B chromosome and the Wd ring.

Neither the B nor the ring chromosome affects the viability of the gametophyte or decreases the vigor of the sporophyte. Since both are found as univalents in essentially 100 per cent of the cells at diakinesis, the hyperploid plants provide an extremely efficient system for testing the distributive pairing hypothesis. The ring was detected genetically, and the B, cytologically (in root tip preparations).

Transmission of the ring is variable since it may be lost or structurally modified in somatic cells. Therefore, sectors may occur which contain no ring. Since ears often contain one or more ring-deficient sectors, absolute transmission frequencies of the ring in plants with and without one B chromosome are meaningless. B chromosomes, on the other hand, are not lost from somatic cells; their transmission is regular. Plants containing one Wd ring and one B were backcrossed as female parents to diploid plants.

If the ring and B chromosome pair and disjoin by distributive pairing, the frequency of B chromosomes would be much lower in the progeny with the ring than in plants without the ring. On the other hand, if the two chromosomes behaved independently, the frequency of B's should be the same in both classes.

The frequencies of B chromosomes in sibling plants with and without one ring chromosome are presented in the following table:

Frequency of B chromosomes in ring-containing and ring-deficient offspring from crosses of female parents possessing the Wd ring and a B chromosome by diploid males (pooled data from 2 plants)

Seed constitution	2 B's	1 B	0 B	Total	Per cent plants with 1 B chromosome
no ring	0	39	37	76	51.3%
ring present	1#	34	37	71 + 1#	48.6%
Total	1#	73	74	147 + 1#	

X^2 value between ring-containing and ringless plants + .330, .5>P>.3, DF = 1

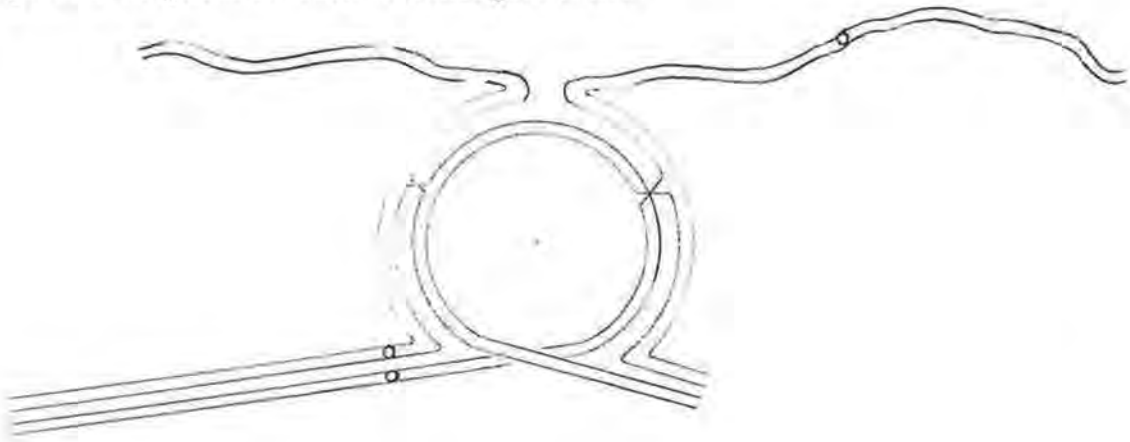
#This plant is not included in the totals and calculations since its origin is uncertain.

Differences in the values observed in the two classes of offspring were not statistically significant. Since both extra chromosomes should be found as univalents in essentially 100 per cent of the cells, this is a very sensitive test. The results are in agreement with the previous studies by this author, and favor the conclusion that distributive pairing does not occur in Zea mays.

David Weber

3. Evidence that recombination can involve both chromatids of one chromosome with chromatids of two differing chromosomes.

A very interesting configuration was observed in 2N+4+1B, In4a/N/N plants at anaphase I. Two cells were seen in which 3 chromosomes were joined by 2 bridges and 2 acentric fragments were released. The fragments were clearly smaller than B univalent chromosomes, and could not have been mistaken for them. Unless one hypothesizes chromosome breakage and reunion, the following pairing configuration and crossover positions would be required to produce such a configuration.



This exceptional anaphase demonstrates that recombination occurs at the four-strand stage (as earlier shown by single bridges and fragments in diploid plants containing a heterozygous paracentric inversion). It also shows that recombination can involve both chromatids of one chromosome with chromatids of two different chromosomes. Genetic evidence for the latter point comes from triploid Drosophila, but the author is unaware of any similar cytological demonstration of this point.

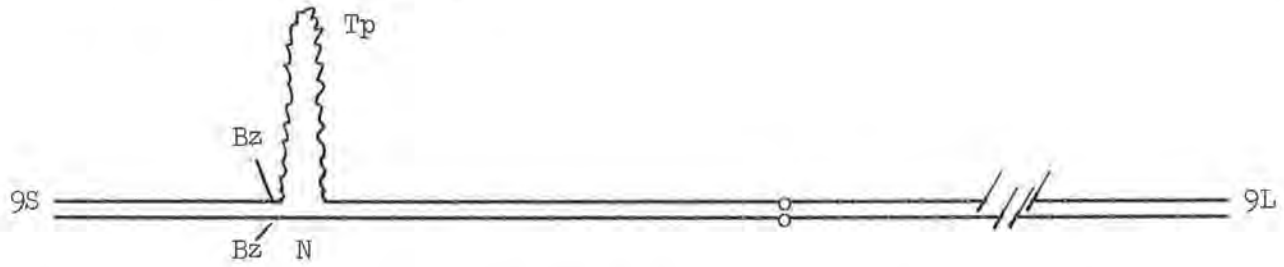
David Weber

4. On nonhomologous recombination.

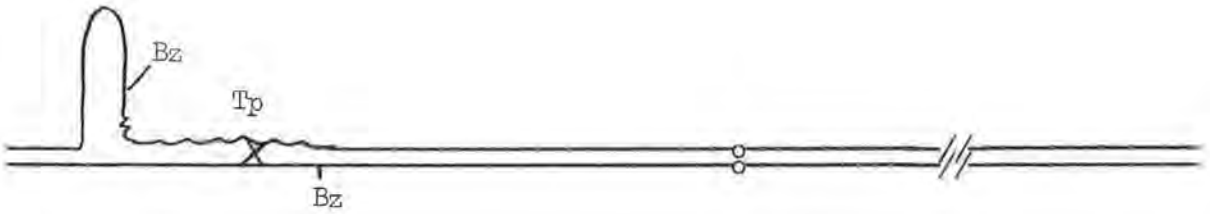
The present study was initiated because a genetic system in Zea mays was available in which a well-marked segment was frequently involved in nonhomologous pairing and in which an efficient test for recombination between nonhomologous segments could be made. Nonhomologous recombination is defined as recombination between dissimilar nucleotide sequences.

Frances Clark Beard isolated a transposition from 3S into 9L. The transposed segment is about 1/4 the length of 9S. The cytogenetic behavior of

this transposition has been intensively studied by Rhoades. The insertion point of the transposed segment in 9S is just proximal to the Bz locus, thus the following pairing configuration is expected in plants heterozygous for the transposition (Tp9/N9):



However, when pachytene in Tp9/N9 plants was examined, the buckle caused by the transposition was not found in a constant position, but the buckle could occur at essentially any position in 9S. In many meiocytes, no buckle was seen since the loop was completely contracted. This pairing behavior has been confirmed by the author. The variable position of the buckle indicates that extensive nonhomologous pairing has taken place. One of the known configurations resulting from nonhomologous pairing is diagrammed below:



If illegitimate recombination took place between the nonhomologously synapsed segments as indicated above, one of the crossover products would contain a deficiency for the Bz locus, and the other, a duplication. Thus, if these plants were crossed by a bz tester, the deficiencies caused by the exceptional nonhomologous recombinational event should be detected as bronze kernels on otherwise purple ears.

The following cross was made:

$$\frac{Bz\ Tp}{Bz\ N} \times \frac{bz\ N}{bz\ N}$$

No bronze kernels were obtained in a population of 327,000 kernels; thus nonhomologous recombination was not detected. Since one spontaneous mutation might be expected in a population of this size or larger, there would be little gained by a more extensive test.

Although the Bz data give no evidence for nonhomologous recombination, it is possible that such crossovers were present in the megaspore population

and that the deficiencies thus produced led to inviability of the female gametophyte. Data are available which indicate that such deficiencies are transmissible through the female gametes. These data will be presented in the 1968 News Letter.

Although deficiencies are transmitted much more readily through female than male gametes in plants, the reciprocal cross using a Tp9/N9 male parent was made to test the unlikely possibility that nonhomologous recombination is confined to microsporogenesis. From a population of 76,150 seeds, 9 bz kernels were obtained. Three of these seeds did not germinate. The remaining ones were all Bz/bz, therefore the phenotype expressed in the endosperm was not the same as that in the embryo, and loss of the Bz locus had occurred only in the endosperm. Therefore these were caused by a post meiotic event, and not by nonhomologous recombination during meiosis.

The Yg_2, Wx, and V_1 loci were examined in a similar manner. The results from all crosses are summarized in the following table:

Table 1
Tests for Nonhomologous Recombination in 9S

Maternal parent	Paternal parent	Population studied	Verified cases of nonhomologous recombination
Bz Tp/Bz N	bz N/bz N	327,000	0
bz N/bz N	Bz Tp/Bz N	76,150	0
yg N/yg N	Yg Tp/Yg N	10,180	0
Tp Wx/N Wx	N wx/N wx	14,472	0
Tp V/N V	N v/N v	22,300	0
N v/N v	Tp V/N V	25,220	0
Total		475,322	0

Deficiencies for the Yg locus have been shown by McClintock to be fully transmissible through the pollen side of plants; however, information on the other loci is not available. No cases were found where the recessive phenotype could be attributed to nonhomologous recombination between nonhomologously synapsed chromosomes. Since nonhomologous pairing in 9S is frequent in Tp9/N9 plants and since inviability of deficient gametes was ruled out for the Bz and Yg loci, it must be concluded that nonhomologous recombination does not occur in this material.

David Weber

III. REPORT ON MAIZE COOPERATIVE

Seed requests to the Maize Cooperative for 1966 were the largest on record. A total of 152 requests were received during 1966. This is a 28 per cent increase in the number of requests compared to 1965. About 84 per cent of the total requests were from the U.S. and 16 per cent from foreign countries. A total of 1,907 samples were supplied to fill these requests or a 28 per cent increase over 1965. The number of requests has been gradually increasing over the years and is expected to continue.

During the summer of 1966 seed increases were made on maize genetic stocks of chromosomes 5, 7, 8, 9, 10 and additional chromosomal testers that required increased seed supplies. In addition, stocks of the andromonoecious dwarfs $\underline{an}_1$, $\underline{d}_1$, $\underline{d}_2$, $\underline{d}_3$, and $\underline{d}_5$ were increased to meet the increasing number of seed requests for these stocks. About 120 reciprocal translocation stocks were increased at Urbana and by Dr. D. S. Robertson at Iowa State University. It will be necessary to grow a few previously grown translocations of Dr. E. G. Anderson's collection in 1967 in order to extract more desirable genotypes. Plans are to have a complete list of all reciprocal translocations in the collection available for the 1968 News Letter.

Work is also continuing on chromosome location of unplaced genes in the collection. Generally, several unplaced genes are located to chromosomes each year using A-B translocations and waxy-marked reciprocal translocation series. Work is in progress to more accurately map the location of these genes to a specific region of the chromosome.

Over a period of years we receive from maize geneticists and breeders mutant phenotypes of several different traits that could be allelic to other known loci. In 1966, 16 unknown endosperm mutants were tested for allelism with 8 known endosperm genes. The following table lists the results where the tests were positive.

Allele tested	bt_1	bt_2	bt_4	sh_1	sh_2	sh_4	sh_5	su_1
bt_{60-156}	-	-	-	-	+	-	-	-
*bt_{60-158}	-	+	-	-	-	-	-	-
bt_{C103}	+	-	-	-	-	-	-	-
su_{A-4583}	-	-	-	-	-	-	-	+
$bt_{65-1096}$	-	+	-	-	-	-	-	-
$bt_{65-1334}$	-	-	-	-	+	-	-	-
*sh_5	+	-	-	-	-	-	-	-

- = negative test
+ = positive test
*Also confirmed by Dr. O. E. Nelson, Purdue University.

Seeds of these different allelic sources are available upon request.

Over the past several years mutant traits located to chromosome have been sent to the coop for use as markers. These traits are usually observed for their desirability as a marker trait and to also determine if any other traits are present in the stocks before they are made available. The following stocks are being added to the stock list for 1967.

Gene symbol	Trait	Chromosome location	Description	Stock source
$bz_2^m;m$	Bronze mutable	1	Bronze aleurone in presence of other aleurone genes.	Neuffer
c_2	Colorless aleurone	4	Colorless aleurone in presence of $\underline{A_1 A_2 C_1 R}$	Coe
cl_1	Albino seedling	3	Paper-white albino	Robertson
rs_2	Rough leaf sheath	1	Leaf sheath has abnormal contorted growth.	Cornell collection
mn	Miniature seed	2	Seed size much reduced. Poor to fair germination of homozygotes.	Nelson
wt	White leaf tip	2	Tips of seedling leaves lack chlorophyll.	Sprague
ys_3	Yellow stripe leaf	3	Similar to $\underline{ys_1}$ in phenotype. Plant makes good growth.	Wright

The attached catalogue of stocks represents a listing of currently available genetic stocks. A more complete list of reciprocal translocations stocks is found 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 1

ad_1 an_1 bm_2

ad_1 bm_2

an_1 bm_2

as

br_1 Vg

br_2

bz_2^m;m

Kn

Kn Ts_6

lw_1

P^{CR}

P^{CW}

P^{MO}

P^{RR} ad_1 an_1

P^{RR} ad_1 bm_2

P^{RR} an_1 gs_1 bm_2

P^{RR} br_1 f_1 an_1 gs_1 bm_2

P^{VV}

P^{WR} bm_2

P^{WR} gs_1 bm_2

P^{WW} br_1 f_1 bm_2

P^{WW} br_1 f_1 an_1 gs_1 bm_2

P^{WW} hm br_1 f_1

rs_2

sr_1

Chromosome 1 (continued)

sr_1 P^{WR} an_1 bm_2

sr_1 P^{WR} bm_2

sr_1 P^{WR} an_1 gs_1 bm_2

sr_1 zb_4 P^{WW}

ts_2 P^{WW} br_1 bm_2

Ts_6

v_{19} bm_2

Vg

Vg an_1 bm_2

vp_5

vp_8

zb_4 ms_{17} P^{WW}

zb_4 P^{WW} bm_2

zb_4 P^{WW} br_1

zb_4 ts_2 P^{WW}

an_{6923}-bz_2 (apparent deficiency including an_1 and bz_2)

necrotic 8147-31

Chromosome 2

al lg_1 gl_2 B sk

al lg_1 gl_2 b sk

ba_2

fl_1

gl_{11}

Ht

Chromosome 2 (continued)

lg_1 gl_2 B

lg_1 gl_2 b

lg_1 gl_2 b fl_1 v_4

lg_1 gl_2 b fl_1 v_4 Ch

lg_1 gl_2 B gs_2

lg_1 gl_2 b gs_2 sk

lg_1 gl_2 b gs_2 v_4

lg_1 gl_2 b gs_2 v_4 Ch

lg_1 gl_2 B sk v_4

lg_1 gl_2 b sk v_4

lg_1 gl_2 b sk fl_1 v_4

lg_1 gl_2 B v_4

lg_1 gl_2 b v_4

lg_1 gl_2 b v_4 Ch

lg_1 gs_2 b v_4

w_3

w_3 Ch

ws_3 lg_1 gl_2 B

ws_3 lg_1 gl_2 b

ws_3 lg_1 gl_2 b fl_1 v_4

ws_3 lg_1 gl_2 B sk

ws_3 lg_1 gl_2 b sk

wt

Chromosome 3

A_1 ga_7; A_2 C R

A_1 sh_2; A_2 C R

Chromosome 3 (continued)

A^d-31; A_2 C R

A^d-31; A_2 C R Dt_1

A^d-31 sh_2; A_2 C R

a^P et; A_2 C R Dt_1

a_1; A_2 C R B Pl dt_1

a_1 et; A_2 C R Dt_1

a_1 sh_2; A_2 C R Dt_1

a_1 sh_2; A_2 C R dt_1

a_1^{st} sh_2; A_2 C R Dt_1

a_1^{st} et; A_2 C R Dt_1

ba_1

Cg

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

Chromosome 3 (continued)

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 na_1

ys_3

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

Chromosome 4 (continued)

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

Tu gl_3

v_8

Chromosome 5

a_2; A_1 C R

a_2 bm_1 bt_1 bv_1 pr; A_1 C R

a_2 bm_1 bt_1 pr; A_1 C R

a_2 bm_1 pr v_2; A_1 C R

a_2 bm_1 pr ys_1; A_1 C R

a_2 bt_1 pr; A_1 C R

a_2 bt_1 pr ys_1; A_1 C R

a_2 pr; A_1 C R

ae

bm_1 pr; A_1 A_2 C R

bm_1 pr v_2; A_1 A_2 C R

Chromosome 5 (continued)

bm_1 pr ys_1; A_1 A_2 C R

bm_1 pr ys_1 v_2; A_1 A_2 C R

bt_1 pr; A_1 A_2 C R

gl_5

gl_8

gl_{17} bt_1

gl_{17} v_2

lw_2

lw_3; lw_4

na_2

na_2 pr

pr; A_1 A_2 C R

pr ys_1; A_1 A_2 C R

v_3 pr; A_1 A_2 C R

v_{12}

vp_2 gl_8

vp_2 pr; A_1 A_2 C R

vp_7

vp_7 pr; A_1 A_2 C R

Primary trisomic 5

Chromosome 6

at = allele of $\underline{si_1}$

Bh

po Y_1 pl

po y_1 pl

Pt

Chromosome 6 (continued)

si_1

wi

y_1 l_{10}

Y_1 pb_4 pl

Y_1 pg_{11}; wx pg_{12}

y_1 pg_{11}; wx pg_{12}

y_1 Pl Bh

y_1 pl Bh

Y_1 Pl sm Pt

Y_1 Pl sm py; A_1 A_2 b P^{RR}

Y_1 pl su_2

y_1 pl su_2

y_1 Pl; seg w_1

l_{4920}

"male sterile-silky" = allele of $\underline{si_1}$

"orobanche" (seedling)

"ragged" (seedling)

"white 8896" (seedling)

Chromosome 7

bd

g_2

gl_1 ij bd

gl_1 sl

gl_1 Tp_1

Hs

Chromosome 7 (continued)

ij

in; pr A_1 A_2 C R

o_2

o_2 bd

o_2 gl_1 sl

o_2 ra_1 gl_1

o_2 ra_1 gl_1 ij

o_2 ra_1 gl_1 Tp

o_2 v_5 gl_1; seg ra_1

o_2 v_5 ra_1 gl_1

o_2 v_5 ra_1 gl_1 Hs

o_2 v_5 ra_1 gl_1 Tp_1

ra_1 gl_1 ij bd

Tp_1

vp_9 gl_1; wx

Chromosome 8

gl_g

v_{16} j_1

v_{16} j_1; l_1

v_{16} ms_8 j_1

"necrotic 6697" (seedling)

"sienna 7748" (seedling)

Chromosome 9

Bf_1

bm_4

bp Wx; p^{RR}

Chromosome 9 (continued)

C Ds wx

C sh_1 Wx; A_1 A_2 R

C sh_1 wx; A_1 A_2 R

c sh_1 wx; A_1 A_2 R

C wx; A_1 A_2 R

c Wx; A_1 A_2 R

c wx; A_1 A_2 R

Dt_1 (See chromosome 3 stocks)

gl_{15} Bf_1

gl_{15} bm_4

I Ds Wx

I wx; A_1 A_2 R B pl

K_9^L C sh_1 wx; A_1 A_2 R

l_6

l_7

ms_2

ms_2 sh_1; A_1 A_2 C R

sh_1 wx gl_{15}

sh_1 wx l_7

sh_1 wx v_1

wx Bf_1

wx Bf_1 bm_4

wx bk_2

wx bk_2 bm_4

wx d_3

wx l_6

Chromosome 9 (continued)

Wx pg_{12}; y_1 pg_{11}

wx pg_{12}; Y_1 pg_{11} pl

wx pg_{12}; y_1 pg_{11}

wx^a

yg_2 c sh_1 wx; A_1 A_2 R

yg_2 c sh_1 bz wx; A_1 A_2 R

yg_2 C sh_1 bz wx; A_1 A_2 R

Primary trisomic 9

Chromosome 10

a_3

bf_2

du_1

g_1

g_1 r^g; A_1 A_2 C

g_1 r^{ch}

g_1 r; A_1 A_2 C wx

g_1 R sr_2

g_1 r sr_2

gl_9

l_1

l_1; seg w_1

li g_1 R; A_1 A_2 C

li g_1 r; A_1 A_2 C

nl_1 g_1 R; A_1 A_2 C

Og R; A_1 A_2 C B Pl

oy "oil yellow"
(seedling and plant)

Chromosome 10 (continued)

r^r; A_1 A_2 C

r abnormal 10; A_1 A_2 C

R^g sr_2; A_1 A_2 C

r^r sr_2; A_1 A_2 C

r^g wx; A_1 A_2 C

R^r: Boone; A_1 A_2 C

R^{mb}; A_1 A_2 C

R^{nj}; A_1 A_2 C

R^{st}; A_1 A_2 C

v_{18}

w_2

w_2 l_1

zn

Primary trisomic 10

Unplaced genes

ct

el

gl_{12}

gl_{14}

gl_{16}

h

l_3

l_4

ms_6

ms_9

ms_{12}

Unplaced genes (continued)

ms_{13}

ms_{14}

rd

Rs_1

v_{13}

w_{11}

ws_1 ws_2

zb_1

zb_2

zb_3

"luteus 4923" (seedling)

"necrotic 8376" (seedling)

"white 8657" (seedling)

Multiple gene stocks

A_1 A_2 C R^r Pr B Pl

A_1 A_2 C R^g Pr B Pl

A_1 A_2 C R Pr

A_1 A_2 C R Pr wx

A_1 A_2 C R Pr wx gl_1

A_1 A_2 C R Pr wx y_1

A_1 A_2 C R pr

A_1 A_2 C R pr y_1 gl_1

A_1 A_2 C R pr y_1 wx

A_1 A_2 C R pr y_1 wx gl_1

A_1 A_2 c R Pr y_1 wx

A_1 A_2 C r Pr y_1 wx

bm_2 lg_1 a_1 su_1 pr y_1 gl_1 j_1 wx g_1

Multiple gene stocks (continued)

colored scutellum

lg_1 su_1 bm_2 y_1 gl_1 j_1

su_1 y_1 wx a_1 A_2 C R^g pr

y_1 wx gl_1

Popcorns

Amber Pearl

Argentine

Black Beauty

Hulless

Ladyfinger

Ohio Yellow

Red

South American

Strawberry

Supergold

Tom Thumb

White Rice

Exotics and Varieties

Black Mexican Sweet Corn (with B-chromosomes)

Black Mexican Sweet Corn (without B-chromosomes)

Gourdseed

Maiz chapolote

Papago Flour Corn

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

* gl_2 Inv 2a (also available with Ch)	2S.7; 2L.8
* wx^2 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
su_1 1-4a	1L.51; 4S.69
su_1 1-4d	1L.27; 4L.30
su_1 4-5j	4L.21; 5L.36
su_1 y 4-6a	4L.37; 6L.43
su_1 4-8a	4S.59; 8L.19
su_1 R 4-10b	4L.15; 10L.60
y 1-6c	1S.25; 6L.27
gl_2 2-3c	2S.46; 3S.52
gl_2 2-3 5304	2S.62; 3L.29
gl_2 2-6b	2S.69; 6L.49
gl_2, R 2-10b	2S.50; 10L.75
gl_1 6-7 4545	6L.25; 7S.73

*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 Hm
B-1b	1S.05	
B-3a	3L.1	
B-4a	4S.25	Proximal to su_1
B-7b	7L.3	Proximal to ra_1
B-9a	9L.5	Proximal to Bf_1
B-9b	9S.4	Between C and wx; close to wx
B-10a	10L.35	Proximal to g_1

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