MAIZE GENETICS COOPERATION

NEWS LETTER

40

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Department of Botany
Indiana University
Bloomington, Indiana

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I. FOREWORD

As has been true for the past eleven years, the task of editing, assembling, and supervising the Maize Genetics Cooperation News Letter has been in the efficient hands of Miss Ellen Dempsey. Her careful reading of the articles has lead to many contributors being spared the embarrassment of having published errors. All of us who profit from this exchange of research information owe her our thanks for an onerous task exceedingly well performed. Acknowledgement should be made of the voluntary assistance of Wayne Carlson, Earle Doerschug, John Mottinger, Reid Palmer, Karl Rinehart, and David Weber in proof reading.

If you will call to our attention any mistakes, irrespective of their source, which you find in your contributions we will be happy to issue an Errata.

It is most appropriate at this time to express our gratitude and deep appreciation of the admirable way that Dr. Earl Patterson has managed the Maize Genetics Stock Center at Illinois. As indicated in the Report on the Maize Cooperative, he will no longer be in charge of the Stock Center. It is no exaggeration to say that Earl has made a great personal sacrifice by devoting himself so wholeheartedly to an organization which exists for the benefit of us all. He deserves our highest commendation for his unselfish service. Responsibility for the maintenance of the Stock Center has been assigned to Dr. Robert Lambert of the Agronomy Department, University of Illinois. In the past he has been associated with the Stock Center and is qualified to take over from Earl. We wish him well.

M. M. Rhoades

II. REPORTS FROM COOPERATORS

BOSTON COLLEGE
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1. Further studies on T6-9t in maize.

As reported previously (M.N.L. 39: 5-6, 1965), a reciprocal translocation between the long arm of chromosome 6 and the short arm of chromosome 9 was found. In addition to the high frequency of chain configurations at diakinesis and low ovule sterility of the plants heterozygous for this T6-9t, it was further observed that the average pollen sterility of five plants was 15%, which is much lower than expected. This might indicate that the anaphase I disjunctions were not at random. It is likely that the alternate type of disjunction, leading to the production of fertile gametes, was favored against the adjacent types.

In the summer of 1965, progeny of the cross $\underline{Y}\ \underline{y}\ \underline{T}\ \underline{N} \times \underline{y}\ \underline{y}\ \underline{N}\ \underline{N}$ and its reciprocal was classified. The results are shown in Table 1. Among a total of 241 plants examined, 230 were parental types, while 11 were recombinants. Therefore, the recombination frequency is five per cent. The distance between the Y locus and the point of break of this translocation in the long arm of chromosome 6 is five crossover units. Whether the point of break is distal or proximal to the Y locus has not yet been determined. Data locating the point of break of this translocation in the short arm of chromosome 9 are incomplete. Further studies are in progress.

Table 1
Results of the cross $\underline{Y}\ \underline{y}\ \underline{T}\ \underline{N} \times \underline{y}\ \underline{y}\ \underline{N}\ \underline{N}$ and its reciprocal

	N y	T y	N Y	T Y
No. of Plants	114	2	9	116
X-over Plants		2	9	
Parental Plants	114			116
% of X-over		5		

Y. C. Ting
Hei-sook Park

2. The effect of X-rays on pollen fertility of maize.

In the summer of 1965, a study on the effect of X-rays on pollen fertility of maize was carried out. Freshly collected pollen was irradiated with X-rays at three different doses, 1500r, 3000r and 4500r. This treated pollen was applied on the silks of an inbred maize strain homozygous for gl_1. A total of 21 plants were fertilized with the rayed pollen; seven plants were fertilized with pollen rayed at a dose of 1500r, six plants with pollen rayed at a dose of 3000r, and eight plants with pollen rayed at a dose of 4500r. In addition, five plants were fertilized with non-irradiated pollen and these plants were maintained as control. During harvesting, conditions of the kernel sets of these plants were examined. It was found that as the radiation intensity on maize pollen increased, the per cent of kernel sets on the ears fertilized with this rayed pollen decreased. This decrease was about linearly proportional to the increase of radiation intensity. For instance, the kernel sets on the ears of the control were 95% of the total ovules produced, while the kernel sets on the ears fertilized with pollen rayed at 1500r were 65 per cent, the kernel sets with pollen rayed at 3000r were 20 per cent, and those with pollen rayed at 4500r were only five per cent. The results are shown in Table 2.

Table 2
Effect of X-rays on pollen fertility of maize
(based on % of kernel sets)

Dose (r)	% of kernel sets
1500	65
3000	20
4500	5
Control	95

Y. C. Ting

3. The inheritance of B-chromosomes.

Because of the study of mutagenic effects of B-chromosomes, an attempt was made to accumulate a large number of B's in individual maize plants. From the progeny of a selfed inbred maize plant, individuals possessing 3 B's were selected and self-fertilized in the summer of 1964. Bulked kernels

from three such plants were grown in the greenhouse in 1965. When the seedlings were three weeks old, samples of the root tips were collected and fixed in a 3:1 alcohol-acetic fixative. With the standard squash technique, B-chromosomes in the root tips of each plant were counted. Data were obtained as follows: among 40 plants, one had no B-chromosome; six plants, one B; nine plants, two B's; eight plants, three B's; seven plants, four B's; six plants, five B's; three plants, six B's. Therefore the distribution of B's among the plants of this small population follows, more or less, a standard modular form.

Y. C. Ting

4. Induced changes in number and structure of maize chromosomes by X-rays.

In the summer of 1964, maize pollen possessing B-chromosomes and other genetic markers was irradiated with X-rays at a dose of 1500r. The rayed pollen was crossed with an inbred maize strain having the factor Gp (good spreading pachytene chromosomes) and other known cytological markers to facilitate pachytene studies of the F_1 hybrids.

Kernels from the above hybrids were grown in the field in the summer of 1965. Up to the present, 126 plants were investigated cytologically. Among these plants, 57 were heterozygous for one translocation (some possible A-B translocation), four have one dicentric chromosome, one is monosomic, and three have a deficiency for one chromosome arm. Studies on the details of these alterations are in progress.

Y. C. Ting

Note: Certain phases of these experiments were carried out at Brookhaven National Laboratory, Upton, Long Island, New York. Dr. A. H. Sparrow's help in providing space and facilities is gratefully acknowledged.

BROOKHAVEN NATIONAL LABORATORY*
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Biology Department

1. Colored scutellum stocks to detect haploids and determine the distribution of a recessive gene in maize.

Maize geneticists have been interested for many years in inducing and detecting haploids. Haploids are of interest mainly because a completely homozygous diploid is produced when their chromosome complement is doubled, thereby obviating the several generations of inbreeding normally required to achieve uniformity. A considerable amount of the early studies on the genetics and application of haploid induction was done by Dr. S. S. Chase. Some of his first work dealt with the use of seedling markers to detect haploids. Some of this work is published in Genetics 34: 328-332, 1949 and in Heterosis, Chap. 25, pp. 389-399, 1952 and has recently been reviewed by Briggs (J. Heredity, in press).

The recent work of Coe and Sarkar (J. Heredity 55: 231-233, 1964) has shown that it is practical to detect haploids by scoring the dormant kernels. Their method uses stocks which are $\frac{C}{C}$ and which have colored scutella for the female parent. The male parent was a stock with a $\frac{C^I}{C^I}$ genotype. The diploid F_1 of such a cross should give a colorless endosperm and no scutellum color while the putative haploids should show color in the scutellum since the sperm nucleus, carrying C^I, does not fertilize the egg cell which would give rise to the embryo. In growing the kernels with colored scutella they reported 97% haploids from several types of crosses.

Chase and Nanda (Am. Soc. Agron. Abstr., p. 17, 1965) recently reported a similar procedure to detect haploids. Their method involved the use of a stock with the genotype $\frac{b\ pl\ A\ C\ R^{nj}}{}$:Cudu $\frac{pr\ P^{wr}}{}$ which imparts a purple embryo, visible in the dormant kernel. This stock is used as the male parent and can be crossed to any material as the female parent. The F_1 seeds that lack purple color in the embryo are selected and sown as putative haploids. By this technique approximately 90% of the kernels can be discarded before germination. This method, in contrast to that of Coe and Sarkar, can be used to extract haploids from commercial material.

As reported by Emerson, Beadle and Fraser (Cornell Univ. Agr. Expt. Sta. Mem. 180: 1-83, 1935) and Sprague (U.S. Dept. Agr. Tech. Bull. 292: 1-43, 1932) many genes are

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

needed to produce pigmentation in the scutellum. Five of these--S_1, S_2, S_3, S_4, and S_5--are concerned with the extension of aleurone pigment to the scutellum. If purple or red aleurone is present (in the phenotype A_1 A_2 C R) then scutellum color appears in the presence of: (1) the dominant allele S_1; (2) the recessive allele s_5; and (3) the dominant alleles of any two of the genes S_2, S_3, and S_4. The genes Pr and pr differentiate purple and red pigment in the scutellum as in the aleurone. It should be further noted that colored aleurone is required to produce scutellum pigmentation.

Chase has proposed that colored scutellum stocks be used to detect haploids in the dormant kernel and work has been initiated at Brookhaven to obtain data using such a method. This procedure of using colored scutellum stocks to detect haploids, as is true of the purple embryo marker technique, can be used to detect haploids in commercial material. Sib pollinations among the females can generally be detected since sibs will not have aleurone color, and in the stocks such as su_1 (sugary-1) and sh_2 (shrunken-2) they are readily detectable. To obtain scutellum color the genes A C R S_1 s_5 and at least two of the genes S_2, S_3, and S_4 are needed. Therefore, to produce scutellum color in a hybrid and to detect haploids by using colored scutellum stocks the recessive gene s_5 would need to be in the male as well as in the female. The critical gene is s_5 since the other genes needed to give scutellum color are dominant. Chase stated that scutellum stocks make excellent markers in certain stocks and that preliminary tests are necessary to determine their suitability in any given cross. One reason that preliminary crosses were necessary may have been because all of the female material that Chase used may not have been s_5/s_5. Therefore, it appears that by using the scutellum stocks as the male a survey of the frequency and distribution of a recessive gene could be made of various maize types, in addition to its use in detecting haploids.

Rhoades (see Principles of Genetics, 4th ed., Sinnott, Dunn and Dobzhansky, pp. 322-323, 1950) has self pollinated several varieties of open pollinated corn varieties. Many mutant genes were detected in this manner. Some of the genes found (white and yellow seedlings, defective endosperm, and germless and viviparous seeds) are lethal when homozygous, while others (virescent, pale green, and dwarf seedlings) may be classified as semi-lethals.

Bianchi and coworkers have carried on extensive studies, observing mutant genes in self pollinated progenies of Italian maize (MNL 38: 89-91, 1964 and earlier volumes). They have done allelism tests on some of the mutants obtained and have also classified the genes into seed,

seedling, and plant traits. Many different types have been discovered, some of which have been lethal and semilethal.

This work of Rhoades and Bianchi on inbreeding open-pollinated varieties of maize detects visible mutants directly and therefore some of the mutants may have been selected both naturally and artificially by man. In such studies the lethal genes are selected against naturally as albino seedlings, defective endosperm, germless and viviparous seed mutants. If any selection was made by man on mature plants certain morphological characters, e.g., stature mutants, may have been selected against. With the $\underline{s}_5$ gene such natural and artificial selection has probably not taken place. The reason for this is that this gene is not detectable by inbreeding and might not be considered as a visible in the usual sense. Therefore, the $\underline{s}_5$ gene may not have been selected naturally and quite certainly has not been selected for or against by man. That is, it probably has not been selected for or against in a conscious manner by man because the gene is detectable only by a special test cross. There is always the possibility that it may be linked with gametophyte factors which could bring about selection. Assuming that no gametophyte factors are involved it is quite probable that natural selection pressure would not be nearly as great on $\underline{s}_5$ as on an albino mutant; i.e., $\underline{s}_5$ is undoubtedly not a lethal. Also, $\underline{s}_5$ is probably not a detrimental gene. It therefore, seemed of interest to begin an assay of some maize material to obtain some information on the frequency and distribution of the $\underline{s}_5$ gene.

If the gene pr (in place of Pr) and the genes to give aleurone and scutellum color are present in a stock, red aleurone and scutellum color would be produced. By using such a stock as the male the frequency and distribution of the pr gene could be determined at the same time that the assay was being made for the $\underline{s}_5$ gene. That is, if the red scutellum stock was used as the male and the hybrid seed had red aleurone and red scutellum color the female stock would be pr. However, if the hybrid seed had a purple aleurone and purple scutellum the female would be Pr.

In order to obtain scutellum color the standard aleurone genes A C R are needed plus other factors discussed previously. If the inhibitor allele (C^I) of the C locus is present in the female no aleurone or scutellum color will be produced in the F_1. Therefore, this system of using scutellum stocks as the male to detect haploids and to assay a population for $\underline{s}_5$ will not be possible if C^I or any other aleurone color inhibitor gene(s) is present in the female material. However, by using the scutellum stocks as the male parent information on the distribution of such inhibitor genes can be obtained from the female material. Also, if aleurone color inhibitors are present, the purple embryo marker system of Chase and Nanda will not be usable.

For convenience the aleurone color inhibitor gene or genes will merely be considered as being a single gene throughout the remainder of this paper.

Scutellum color in maize has been known for many years (Sprague, U.S. Dept. Agr. Tech. Bull. 292: 1-43, 1932). Therefore, stocks with colored scutella are probably in various types of maize that represent various genetic backgrounds. It may therefore be worthwhile to survey these stocks for their ability to induce haploids. Coe (Am. Naturalist 93: 381-382, 1959) discovered a line of maize that produces 3.23% haploids. Prior to Coe's investigations, the highest frequency of haploids was reported by Chase (Genetics 34: 328-332, 1949) as 0.688%, with an average frequency of 0.111%. In order to perform such a survey colored scutellum stocks could be used as the male parent and crossed to a female parent that had been confirmed to be homozygous for the $\underline{s}_5$ gene. Also, the female parent cannot have any aleurone color inhibitor genes. By scoring the kernels from such a cross a survey could be made of various colored scutellum stocks to determine their ability to induce haploids.

The results of using colored scutellum stocks as the male on various types of maize are shown in Table 1. The third column, "No. seeds with colored aleurone and colorless scutella," gives the putative haploids in most cases. That is, the entries in this column should be haploid if the female parent is $\underline{s}_5/\underline{s}_5$ and if the female does not have an aleurone color inhibitor gene. The indication is that M14 has the required gene ($\underline{s}_5$) because most of the seeds have scutellum color. It therefore apparently is possible to detect haploids by using the scutellum stocks as the male in this cross.

In the Hayes White material, which is an open pollinated variety, one ear appears to be segregating for colored and colorless scutella. However, the number with colorless scutella are at such a frequency that they probably are not all haploids. This is confirmed since four of the seeds produced haploids (based on morphological criteria of the seedlings). The data do not fit a 3:1 ratio, but do fit a 13:3 ratio rather well. Various possibilities are being considered to explain such a ratio. The fact that the data fit a 13:3 ratio may not necessarily mean that two genes are segregating. That is, only one gene may be segregating, but the data deviate considerably from a 3:1 (12:4) ratio and appear to fit a 13:3 ratio. However, more data will be needed before a definitive conclusion can be made. Tentatively, it might be stated that $\underline{s}_5$ is segregating. Also, there is the possibility that some factor(s) may be segregating in the male. However, after harvest the kernels from the male parent were examined and all of them had scutellum color. Also kernels from another ear appear to be segregating for a color inhibitor gene in the female parent, because

Table 1
Results of Using Colored Scutellum Stocks as the Male and Crossing them with Various Females. Each Horizontal Line Represents an Individual Ear Except Where Noted.

Entries & (type)	No. seeds with colored aleurone & scutella	No. seeds with colored aleurone & colorless scutella	No. seeds with colorless aleurone & scutella	P value*	Haploids
M14 (dent)	318	3			
	257	20			
	319	13			
	300	6			
	251	7			
	290	4			
	404	18			1
	346	15			2
	202	5			
Hayes White (sweet, su)	314	6			2
	479	28			2
	278	66		.01 (3:1)	4
				.70 (13:3)	
	153	0			
	156	0	149	.70	
Illinichief† (sweet, sh_2)			3219		
Early Triumph (sweet, su)	368	33			2
	365	10			1
	271	4			
	37	0			
	28	1			
Tendercrisp (sweet, su)	205	29	158	.01	
	227	17	163	.01	
	170	11	190	.30	
	165	5	104	.01	
	137	17	120	.30	
	210	14	188	.30	1
	127	12	114	.50	
Minhybrid 250 (pop)			298		
			112		
			268		
			84		
			106		
Strawberry (pop)			154		
			55		
			69		
			83		

*Hypothesis of 1:1 ratio unless noted.
†Bulk of 9 ears.

a 1:1 ratio of seeds with colored aleurone and scutella to seeds with colorless aleurone and scutella was obtained.

Illinichief appears to carry an aleurone color inhibitor gene. This is a single cross hybrid and apparently both parents have the inhibitor. This material has recently been developed by a breeding program to replace the $\underline{su}_1$ gene with $\underline{sh}_2$. Therefore, it would be of interest to test the related single cross, i.e., Iochief, to determine if it carries an inhibitor or whether the inhibitor gene was added in addition to the $\underline{sh}_2$ gene. Early Triumph seems to carry the $\underline{s}_5$ gene in the homozygous condition, and therefore haploids should be obtainable from this material.

If one attempts to extract haploids from Tendercrisp only half of the material can be scored for haploids, i.e., the half with colored aleurone. The other half of the material has a colorless aleurone and therefore colorless scutella. This apparently is due to having an aleurone color inhibitor gene. Tendercrisp is presumably a single cross and one of the parents has an aleurone color inhibitor.

Minhybrid 250 apparently has an inhibitor gene since no aleurone color and hence no scutellum color was produced. The F_1 seed was segregating yellow ($\underline{Y}$) and white ($\underline{y}$) indicating that a cross rather than a sib was made. This is important to determine since the two lines that make Minhybrid 250 were derived from Japanese hulless. Japanese hulless has been reported to have a gametophyte factor by Nelson (Genetics 37: 101-124, 1952). The gametophyte factor may have been lost during the development of the lines; however, a low seed set was obtained in the cross.

Strawberry popcorn is not a very desirable source material from which to extract haploids or to survey for $\underline{s}_5$ since the seed has a red pericarp. However, by removing the pericarp no aleurone or scutellum color could be detected. This indicates that this material also has an aleurone color inhibitor gene. Strawberry popcorn, which is an open-pollinated variety, has a gametophyte factor and a low seed set was obtained in this cross also.

Based on morphological criteria of the seedlings, 15 haploids were obtained in this study (Table 1). By considering the seeds with colored aleurone this is a haploid frequency of 0.235%. To detect haploids by using colored scutellum stocks as the male on the various female stocks shown in Table 1 and considering only the kernels with colored aleurone, approximately 95% of the kernels can be discarded before germination.

This research has indicated that it may be feasible to use maize stocks with colored scutella to detect haploids. Also, stocks with colored scutella may be used to survey for the distribution of a recessive gene that is visible only by a particular cross.

Acknowledgement is made to William VonBergen of Seed Research Specialists, Inc., Ontario, Oregon for providing the Tendercrisp and Early Triumph seed.

Robert W. Briggs

2. Energy requirements and RBE for producing a cytogenetic phenomenon in maize by irradiating seeds with x rays and monoenergetic neutrons.

The frequency of occurrence of yellow-green (yg_2) sectors in seedling leaves that develop from irradiated Yg_2/yg_2 maize seeds was used as a criterion of radiation effect. The yg_2 phenomenon is due mainly to a break in chromosome 9 between the centromere and the Yg_2 locus, with loss of the Yg_2-containing segment. The dose-response curves for 250 kVp x rays (1420 to 14,250 rads) and for monoenergetic neutrons (0.43, 1.25, 1.80 and 14.7 MeV) were linear (or indistinguishable from linearity) and were independent of dose rate (with x rays from 10.3 to 1758 rads/min) thus indicating that breakage of the chromosome, with loss of Yg_2, may be due to a single charged particle. X-ray-induced yg_2 "mutation" rates were 16.4×10^{-7} and 8.3×10^{-7} per rad for cells of leaves 4 and 5, respectively. The "mutation" rates per rad for neutrons were dependent on the leaf scored and the neutron energies employed. For leaf 5 the range was from 3.9×10^{-5} (1.80 MeV) to 6.8×10^{-5} (0.43 MeV). The "effective volume" was assumed to be a sphere and, based on microdosimetric concepts, was computed to have a diameter of 1.35 μ in leaf 4 and 1.10 μ in leaf 5. The corresponding estimates arrived at by cytological methods were 1.52 μ and 1.38 μ, respectively. The results can be accounted for both relatively and absolutely on the assumption that the interphase chromosome is broken, to cause the occurrence of a yg_2 sector, when a single charged particle delivers an energy of approximately 93 KeV or more to a spherical region of the seed embryo cell nucleus that is approximately one micron in diameter but proportional to nuclear diameter.

The relative biological effectiveness of the neutron irradiations used, compared to 250 kVp x rays, ranged from 47 to 102.

Harold H. Smith
Harald H. Rossi

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1. Corn earworm resistance as affected by starchy (Su_1) and sugary (su_1) maize endosperm phenotypes.

In a continuing search for genetic factors contributing to resistance of corn to the corn earworm, Heliothis zea, the effect of the sugary (su_1) kernel phenotype as contrasted to the starchy (Su_1) phenotype, on similar or identical seed-parent backgrounds, was studied in replicated plots in 1964 and 1965. Other work has shown that husk tightness is important to resistance, but there has been much less evidence that other characters, including postulated chemical ones, play a very great role. The studies discussed here relate to earworm damage to ears on sweetcorn backgrounds, classified at fresh market harvest (some 15 to 20 days after pollination under Southern California conditions). Effects on ears carried to maturity, for seed, may be different.

In 1964, the sugary inbred, Purdue 39A, and a converted P39A homozygous for Su_1 were planted in randomized plots with 4 replications, and 20 top (upper) ears per replication were earbagged before any pollen shed in the early morning of the first day of silking. Each afternoon after pollen shedding had ceased, the bags were removed to allow egg-laying by the nocturnal earworm moth, which causes 98 to 100% natural infestation at Riverside; bags were replaced early each morning. On the 3rd and 5th days of silking the sugary ears were heavily hand-sib pollinated, while the homozygous starchy ears were open pollinated (since they would remain starchy anyway). Twenty days after silking the following data were obtained, expressed as earworm damage in inches measured downward from the tip of the ear (only ears filled to the tip were rated):

Inbred	A	B	C	D	Mean damage (inches)	Analysis of variance F test
P39A starchy	1.0	1.0	1.2	1.1	1.08	non-significant
P39A sugary	1.1	0.9	1.2	1.3	1.13	

The P39A starchy was a 4th backcross from a Su_1 line and it was highly similar to P39A itself in most characters, but average length of husk extension was 0.6 inch longer than in the P39A sugary. This difference should have had little effect on earworm resistance.

In 1965 three experiments were run, in which all the plants were F_1 hybrid sweet corn, thus eliminating genetic plant

and husk character variables. In the first experiment, using "F. M. Cross," replications and ear-bagging procedures were as in 1964 and natural earworm infestation was relied upon. Four replications were hand-pollinated by starchy pollen from a planting of the P39A starchy inbred, and four others were open pollinated by their own sugary pollen (with no starchy pollen nearby). The results of this test (below) indicated starchy kernels to be slightly the more susceptible.

F. M. Cross pollinated by	Replication A	B	C	D	Mean damage (inches)	F test
starchy (Su_1)	1.9	1.5	1.4	1.4	1.55	significant at 5%
sib (su_1)	1.4	1.3	1.3	1.1	1.28	

The second and third experiments were run with Golden Cross Bantam, T Strain, about 1 and 2 weeks later, but all ears, within each experiment, were hand-infested on the same day with three first or second instar earworm larvae, grown in culture. In both experiments the sugary ears averaged slightly but significantly more damage than the starchy ones. Since hand infestation on a single day should be more uniform than even the heavy natural infestation, the data suggest that the sugary kernel type does make for slightly more susceptibility during the first 15 to 20 days. It is also possible, but unlikely, that the starchy pollen used introduced other genes which affected endosperm attractiveness to the worm.

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L. D. Anderson

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1. Maternal effect and heterosis in maize.

Having obtained three luxuriant lines from the I_5 progeny of the same plant, after sibbing at the 4th generation, an experiment was performed to check the relative importance of the genetic and cytoplasmic determination of the observed heterosis.

The intraprogeny fertilization (sibbing) was performed on all the 4th generation progenies deriving from the same plant while the luxuriance phenomenon appeared only in all the plants of three progenies. No luxuriance has appeared in previous generations, neither in the remaining portion of the experiment after sibbing.

This fact and the care used in pollen harvesting and during fertilization eliminate the possible participation of strange pollen, the appearing of spontaneous mutants responsible for the luxuriance phenomenon, and the occurrence of technical errors.

Luxuriant plants were selfed. In the next generation selfing and crossing to the normal parent was performed, in order to obtain for each line one F_3 and two reciprocal backcrosses. Among the progeny the following characters were considered: (1) flowering date (2) number of branches in the tassel. All progenies were compared for the mean values and the variability.

Under the hypothesis that the observed phenomenon is completely dependent on additive genetic factors, the mean values and the variability estimates of the reciprocal crosses are expected to be similar, the genotype of the reciprocals being identical. The genotypes of reciprocals and selfed progenies are expected to differ in frequencies of homozygotes and heterozygotes and the mean values of backcrosses are expected to be closer to the mean value of the luxuriant genotype than that of the selfed progeny, while variation of backcrosses is expected to be larger than that of the selfed progenies.

From tables 1 and 2 it appears that the mean values of backcrosses involving all the lines used are different, therefore suggesting a maternal effect in the determination of the luxuriance phenomenon. Comparison between backcrosses and selfed lines suggests that there is a fair agreement between expected and observed values.

Table 1
Mean values for the "number of branches in the tassel" and results of comparisons performed using the Student t-test. [x]) Significance above the 0.05 P level; [xx]) Significance above the 0.01 P level

	line 32			line 88			line 96		
Normal parent	10.42			10.13			9.35		
Normal ♀ x F_2 ♂	13.26[x]	xx		15.55[x]	xx		20.24[x]		xx
F_2 ♀ x Normal ♂	13.98			16.59		xx	21.26		
Selfed F_3 progeny	14.05			13.53			20.24		

Table 2
Mean values for the "flowering date" and results of comparisons performed using the Student t-test. [x]) Significance above the 0.05 P level; [xx])Significance above the 0.01 P level

	line 32			line 88			line 96		
Normal parent	16.62			20.05			20.43		
Normal ♀ x F_2 ♂	13.49[x]	xx		14.61[x]	xx		16.68[x]	xx	
F_2 ♀ x Normal ♂	12.98		xx	13.86		xx	16.09		xx
Selfed F_3 progeny	14.54			16.60			15.26		

On the contrary the behaviour of variability estimates, given in terms of variances in tables 3 and 4, is found to be far from expectation, suggesting that a strong interaction effect takes place between genotypic and extra-nuclear factors.

Table 3
Variance estimates for the "number of branches in the tassel" and results of comparisons performed using the F ratio. [x])Significance above the 0.05 P level; [xx])Significance above the 0.01 P level

	line 32			line 88	line 96		
Normal parent	5.4070			8.5263	11.5753		
Normal ♀ x F_2 ♂	17.0085[xx]	xx		30.9458	25.6177	xx	
F_2 ♀ x Normal ♂	24.4437		xx	32.4826	23.9655		xx
Selfed F_3 progeny	56.7789			30.5313	46.0313		

Table 4
Variance estimates for the "flowering date" and results of comparisons performed using the F ratio. x) Significance above the 0.05 P level; xx)Significance above the 0.01 P level

	line 32			line 88	line 96		
Normal parent	3.9371	xx	xx	7.5476	8.4367	xx	xx
Normal ♀ x F_2 ♂	8.8558			19.5673	12.0128		
F_2 ♀ x Normal ♂	8.7360			19.3275	10.5126		
Selfed F_3 progeny	14.6826			15.4047	24.0438		

The data shown in the quoted tables suggest, moreover, that the maternal effect as detected through the reciprocal crosses may be underestimated as a consequence of the nucleo-cytoplasmic interaction.

The nature and the role of maternal factors in the heterotic phenomenon shown in our material will be considered in the continuation of our experiments. It is worthwhile to note that among the many hypotheses on the origin of the luxuriance phenomenon the importance of the interaction between genetic and non-genetic factors has been assumed by Jones, already in 1913; very recently Dhawan (1965, in press) was able to stress the importance of the extrachromosomal component of heterosis in crosses involving primitive types of maize.

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1. The present center of diversity of the genus Tripsacum

The widespread distribution of Tripsacum species in the Western Hemisphere suggests an ancient origin of the genus, but new species apparently are continuing to evolve among groups of populations in different habitats from near sea level to altitudes of about 2500 meters in Mexico, Guatemala and neighboring regions. Elsewhere throughout the range of the genus from Mexico northward to the northcentral and northeastern United States and from Central America southward in South America to southwestern Brazil and Paraguay populations are more uniform, discontinuities and habitat preferences are more apparent and species are more definitely delimited.

At the periphery of the present distribution of the genus diploid species are predominant and the taxonomic status of the limited number of known tetraploids is about as uncertain as elsewhere. In South America the diploid T. australe is widely distributed and an essentially glabrous, undescribed variant is well established on the western slopes of the Colombian Andes. Triploidy and tetraploidy are represented in South America by T. laxum, introduced for forage from Central America and the Caribbean about 35 years ago, and by tetraploid populations of uncertain affinities in Ecuador and Venezuela. In the United States a diploid form of T. dactyloides occurs from Texas northward to Kansas and there are tetraploids some of which may be autoploids of T. dactyloides and others that are sparsely distributed along the East Coast from southern Florida to Connecticut have the appearance of being relatively recent introductions from south of the Mexican border or the Caribbean. Four tetraploid species (T. lancelatum, pilosum, latifolium and laxum) have been described from Mexico and Guatemala, and in 1950 two very unlike diploid species (T. maizar and zopilotense) were described from the state of Guerrero, Mexico by Hernandez and Randolph (Ofic. Estud. Espec. de Mexico, Fol. Tec. 4).

From my field studies of 1963 and 1965 in collaboration with Professor Hernandez, other distinctive tetraploid populations in addition to those that have been given species names, and six additional localities for the diploid T. maizar as well as four reproductively isolated populations of T. zopilotense in the Canada del Zopilote and one near Tepic have been discovered. Mixed populations including types resembling T. maizar and morphologically variable tetraploids have been identified at Acahuazotla and Aguacate in the state of Guerrero and at Tepic and Oblatos Agua-Caliente in Jalisco. From these recent discoveries it has become increasingly obvious

that the present center of diversity of the genus Tripsacum is in southwestern Mexico and adjoining areas as previously suggested by Randolph and Hernandez (Genetics 35:668, 1950).

L. F. Randolph

2. Cytogenetics of speciation in Tripsacum.

There are many well known examples of species with the tetraploid number of chromosomes that apparently have arisen by hybridization of distantly related diploid species followed by chromosome doubling. Such alloploid species are essentially true-breeding because of the synaptic incompatibility of the chromosome sets of the parental species and lack of gene exchange between them at both the diploid and tetraploid levels. But the possibility that a series of tetraploid species might arise by chromosome doubling following the hybridization of two closely related but phenotypically very unlike diploid species having chromosomes sufficiently compatible to pair regularly and exchange genes freely seems not to have been generally recognized as a potentially significant evolutionary process. It is just these conditions, however, that appear to explain most satisfactorily the occurrence of extremely variable tetraploid populations of Tripsacum widely dispersed in Mexico and Central America, of which four types have been described as species (T. lanceolatum, laxum, pilosum and latifolium) and others appear to be equally deserving of specific or sub-specific status as they complete the process of acquiring adequate discontinuity and other essential attributes of definitive taxa.

There are only two diploid species of this region that combine most of the characteristics found among the tetraploid populations of this and neighboring areas: T. zopilotense and T. maizar. The former is a small, grass-like essentially glabrous plant with slender, sparsely branched culms usually less than a meter in height and with a single or rarely two terminal spikes, narrow flaccid leaves less than a cm. in width; staminate spikelets in pairs, one sessile. The latter is a robust very pubescent plant, corn-like in general appearance with thick culms branched at upper nodes, up to 4.5 meters in height; leaves 7-10 cm. wide; tassels with as many as 45-50 branches of which the staminate portion is much longer than the pistillate; staminate spikelets in pairs of which one is sessile the other pedicellate; a plant of rich moist soils, in sharp contrast to the habitat preference of T. zopilotense for the poorer soils of rocky, arid slopes. The pachytene chromosomes of T. maizar have few if any conspicuous knobs; those of T. zopilotense have numerous terminal and intercalary knobs. Although differing phenotypically in many traits these two species are cross-compatible and their chromosomes pair fairly regularly in the diploid F_1 hybrid (Prywer, Bolet. Bot Soc Mexico 28:11-18, 1960). Among the

natural tetraploids there is a low frequency of quadrivalent synapsis indicative of an auto-alloploid origin. Experimental verification of the hypothesis that the tetraploids did in fact originate as doubled hybrids of T. maizar and T. zopilotense or similar diploid species (Randolph and Hernandez, Genetics 35:686, 1950) has been undertaken by making the appropriate crosses to be followed by induced chromosome doubling of the diploid hybrids.

L. F. Randolph

3. Cytotaxonomic studies of Tripsacum in Mexico and Guatemala.

In 1963 field studies of Tripsacum populations were undertaken in Mexico and Guatemala and continued in 1965 to learn more about the interrelationships of the diploid and tetraploid species and to evaluate their taxonomic status. Included in these studies were populations from the state of Durango in northern Mexico and southward through Sinaloa, Nyarit, Jalisco and Guerrero on the west coast, eastward to Vera Cruz and southward into the states of Oaxaca and Chiapas. In Guatemala populations were studied from the rain forests of the Coban area, the San Antonio Huixta area of southwestern Guatemala, and the neighborhood of Jalapa in southeastern Guatemala. The type localities of the six species of Tripsacum described from these countries were visited. Utilizing appropriate techniques of cytogenetics and numerical taxonomy, measurements and other data were obtained for statistical analysis from 5 to 15 or more individuals selected as representative of more than 40 reproductively isolated populations. The size of the populations studied varied from a small number of clones in recently disturbed habitats to many hectares in undisturbed habitats of various kinds. The measurements included morphological characteristics of the culm, leaves, inflorescences, spikelets and the amount, kind and distribution of pubescence; also the percentage of good pollen, chromosome number and other features of taxonomic significance that were recorded totaled more than 20 items for each plant. Voucher herbarium specimens were preserved and live-plant collections were made for garden culture of individuals from which the measurements and other data had been recorded.

Preliminary evaluation of these data and the accompanying field observations indicated it is only at the diploid level that there are in the region studied good species as ordinarily defined. Among the tetraploid populations there is a unique array of phenotypes varying widely in combinations of morphological traits from extremes much like the assumed parental species, T. zopilotense and T. maizar, to intermediates including a wide range in combinations of the various contrasting traits of those two species or others

like them. Although habitat preferences were apparent with populations having plants with narrow leaves being restricted ordinarily to the more arid regions, those with broad leaves to humid areas and intermediates to localities with intermediate amounts of rainfall, morphological discontinuities between populations limited to these or other habitat preferences were not observed.

Among the populations studied there were noted various individual characteristics not present in either T. maizar or T. zopilotense as, for example, the soft lanulose-tomentose pubescence of T. australe noted in three geographically isolated tetraploid populations, essentially glabrous tetraploid types with narrow leaves and a general growth habit like that of T. dactyloides, and a diploid population from Chiapas having a growth habit in certain respects remarkably similar to that of Manisuris rugosa. Such characteristics might have originated as gene mutants in these particular populations or they could be segregants from hybrid combinations of species other than T. maizar and T. zopilotense. The distribution of T. australe and T. dactyloides, now apparently limited to South America and the United States, respectively, in earlier times might have included intermediate regions where they might have been sympatrically associated with T. maizar, T. zopilotense or similar species and participated with them in the origin of present day tetraploid populations. These possibilities need further study and it is essential to explore the possibility that relatively true-breeding, morphologically similar populations are in process of becoming established in contiguous areas with a geographical distribution adequate for their consideration as species or subspecies. Very much needed, also, are thorough karyotype analyses of the pachytene chromosomes in all existing diploid Tripsacum species and tetraploid populations from very different geographical areas. There is some indication that knob frequencies are variable in some of the diploid species, and this as well as other features of Tripsacum chromosome morphology need further study to clarify their usefulness in the study of natural relationships.

There is also need for ecological studies of Tripsacum, especially in Latin America where different types display an amazingly wide range of adaptation to differences in altitude, latitude, climatic and edaphic conditions. More needs to be known concerning modes of dispersal of the corneous Tripsacum "seeds" by migratory birds over long distances and over shorter distances by birds and other animals not actively migrating, and to a more limited extent by seeds floating in mountain streams, arroyas and drainage canals. The aggressive spread in recent decades of tetraploid populations into disturbed habitats bordering improved highways of Latin America is especially noteworthy. Various methods of sexual and asexual reproduction, the role of apomixis, polyembryony (c.f. Farquharson,

Indiana Acad. Sci Proc. 63: 80-82, 1954) and partheno-genesis in relation to the rare occurrence in tetraploid populations of atypical diploids and extremes of aneuploidy, and of their low percentages of viable seed produced, should be investigated more thoroughly. Various alleles affecting plant colors of Tripsacum apparently in much the same manner as in maize with respect to the well known A B Pl C R Pr series, are widely distributed at both the diploid and the tetraploid level from the equatorial region of South America northward in many localities of Central America, Mexico and the United States, suggesting that parallel mutation rather than "introgres-sion" is a simpler and more plausible explanation of the presence of tripsacoid traits in various unimproved races of maize.

L. F. Randolph

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1. Selection for different states of the R gene in pollen.

In Vol. 38 of MGCNL we reported the results of selecting the lightest and darkest aleurone phenotypes among kernels of selfed ears. Such kernels had three doses of R which had undergone paramutation with R^{st} for six generations. Plants from the lightest and darkest seed selections, when selfed, gave similar ear-mean pigment scores in the following generation.

In 1964 light and dark seed selections were made from testcross ears where paramutated R, introduced through pollen, was present in one dose. Testcrosses in 1965 show that those seeds which showed least pigment produced plants which still showed the least pigment in 1965 testcrosses. In Table 1 no overlap is found when comparing ear means of testcrosses from plants of lightest and darkest seed selections. It may be concluded that in testcrosses where paramutated R is introduced through the male, all kernel to kernel pigment differences may represent genetically different states of the R gene.

Table 1
1965 pigment scores showing persistence of light and dark phenotypes in testcrosses of plants grown from seeds selected from two different testcross ears of plants grown in 1964.

Dark Selections	Light Selections	Dark Selections	Light Selections
20.08	13.56	18.58	12.50
20.12	14.80	15.60	9.66
18.80	16.78	20.84	15.50
18.38	11.60	17.68	9.40
18.24	9.38	19.92	8.38
pooled $\bar{X}$ 19.12	13.22	18.52	11.09

Bernard C. Mikula
Dean Flightner

2. Genetic differences for R expression from tassels of a single plant.

In Vol. 39 of MGCNL polarized sectors were reported for R pigmentation. In corn grass background in RR^{st} heterozygotes, variation in paramutated R expression could be correlated with the day that pollen was collected from a single tassel.

Earliest collections which came from the upper part of the tassel tended to give the lightest pigment values. Seeds whose pigment had been scored in 1964 were grown out in 1965 to see if the differences in pigment scores persisted in the testcrosses of the following generation. Table 2 shows that the differences observed in 1964 are carried over into the results of 1965. It can be concluded that the pigment differences noted in different pollen samples represent different states of R pigmentation which can be carried over from one generation to another through the male gamete. Such pollen samples represent genetically distinct sectors in a tassel (or between tassels of a single plant) where the R gene is "more or less turned on". What is remarkable is that the partial "on-or-off" state can be transmitted so faithfully, that is, the darkest seeds still retain the dark phenotype in the following generation even though considerable reversion has taken place in paramutated R.

Table 2
Comparison of testcross scores of kernels in 1964 and progeny from these kernels in testcrosses of 1965.

Year	Testcross Pigment Scores			Pollen Source in 1964
*1964	6.28	8.90	11.06	Same plant, different tassels, same day
1965 pooled $\bar{X}$ (4 plants ea.)	13.38	17.16	18.88	
1964		6.14	13.80	Same plant, different tassels, different days
1965 pooled $\bar{X}$ (5 plants ea.)		14.62	19.07	
1964		3.34	5.28	Same tassel, different collections (5 days apart)
1965 pooled $\bar{X}$ (6 plants ea.)		4.77	10.08	
1964		6.58	6.94	Same plant, different tassels, same day
1965 pooled $\bar{X}$ (6 plants ea.)		6.13	6.55	

*scored seed source for plants grown in 1965

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3. Tassel mosaics (paramutation) from RR and Rr backgrounds.

In our reports above, differences in paramutated R expression, when R is introduced through pollen, are likely to represent different states of the R gene. Relatively large score differences have been found in tassel sectors and since these differences are carried over into the following generations, they are of genetic significance. It is possible to inquire whether such tassel mosaicism which attends paramutation is a peculiarity of the paramutagenic alleles (such as R^{st}) only. We find that tassel mosaics for R expression can be conditioned in RR and Rr backgrounds and thus all alleles of R can be considered paramutagenic, even R itself. This view has the value of permitting conceptual unity with respect to paramutation; paramutagenic alleles such as R^{st} can now be regarded as paramutagenic extremes in an allelic continuum where all alleles possess paramutagenic ability to one degree or another.

Table 3 shows scores from testcrosses of RR homozygotes. The pollen collected earliest on the tassel produced the darker phenotypes compared to those collections made four to seven days later. The same gradient is expressed in the data on Rr pollen collections where the last collections tend to be lighter than the first. Another point to be noted in the data is that slightly higher pigment values are recorded for the Rr combination. Brink and his students have already noted this difference in the earlier literature. The interesting point is that the difference in R expression becomes most marked in the last pollen collections; when the first pollen samples for RR and Rr testcrosses are compared a relatively slight difference is recorded. The data suggest, therefore, that the mosaic gradient for R expression is increased in single tassels of the RR combination compared to the Rr heterozygote. In terms of paramutation, R is somewhat more paramutagenic than r.

Table 3
Comparison of testcrosses from pollen samples taken from the same tassel. First and last pollen samples were separated by periods ranging from four to seven days apart.

Tassel Sample	Plant Number 1	2	3	4	5	6	7	Sample Pooled Mean
	RR Testcross Scores							
First Pollen Sample	20.94	20.68	21.06	20.30	21.48	20.98		20.91
Last Pollen Sample	19.60	19.76	20.24	19.42	20.48	20.04		19.92
	Rr Testcross Scores							
First Pollen Sample	21.12	20.80	21.36	20.94	20.94	21.24	21.16	21.08
Last Pollen Sample	20.12	20.60	21.22	19.72	20.80	20.76	20.98	20.60

4. Gene-modulating environments.

In inbred W22, the expression of the R gene can be altered by environmental conditions. The R gene, which conditions pigment in aleurone, is first sensitized by paramutation with R^{st} so that relatively small changes in phenotypic expression can be readily observed. Plants with paramutated R were grown for one month in growth chambers set for daily LD conditions of 12 hours light and 12 hours dark; other plants were given LL conditions (constant light). Plants were also given mixed treatments of LL-LD (two weeks constant light followed by two weeks of LD) and LD-LL (two weeks of LD followed by two weeks of LL). Growth chambers were held at 70°F for the month of seedling treatment. In early June, all plants were transplanted to field conditions for the remainder of the life cycle. Testcrosses of treated plants were made to colorless inbreds grown in the field; pigment in testcross ears was scored by methods outlined in our earlier reports (MGCNL 38, 39).

In Table 4 testcross pigment scores of treated plants show that the LD conditions during the first month of plant development produce paramutated R genes which condition more pigment; LL treatments show that less pigment is produced as a result of early environmental treatments. Mixed treatments LL-LD show pigment values close to those of LD-treated plants; LD-LL mixed treatments show pigment values close to the LL-treated plants. Mixed treatment scores suggest that the second two-week period of development determines the R expression of the testcrosses. Pigment differences between plants receiving LL and LD treatments during the second two-week period of development are highly significant, statistically.

Table 5 shows that plants grown in the field but which were given the above environmental treatments in 1964, still show the relative differences found between LL and LD conditions. In the report of Table 5 aleurone scores are based on phenotypes from three doses of paramutated R. Under these high R dosages, only relatively small differences can be observed but these differences made it possible to select the more sensitive level of paramutated R so that the large score-differences of Table 4 could be observed in 1965 testcrosses.

It was noted in the reports above that pigment score differences can be found in the testcrosses of different pollen samples from single tassels of the same plant. It can also be noted that while some of these differences can be quite small, the relative differences noted in one generation can be carried across into the testcrosses of the following generation. It was concluded that the phenotypic differences noted in each of the testcross kernels can be considered to represent different states of the paramutated R gene. Because of the differences in testcross scores in

Table 4
Testcross scores for R expressions from $\underline{RR}^{st}$ heterozygotes after environmental treatments during the first four weeks of seedling development. Scores represent ear means based on scores of 50 kernels per ear.

Environmental Treatments				
LD	LL	Field	LL-LD	LD-LL
12.64	8.72	8.58	13.08	7.62
11.90	6.66	10.38	12.96	5.54
16.58	10.48	12.36	13.78	8.64
16.14	4.48	7.72	12.80	7.86
12.60	7.66	11.84	13.52	7.64
14.44	10.68	14.06	13.36	10.30
14.82	10.96	11.96	13.04	8.06
14.76	8.20	13.74	12.64	8.44
pooled $\bar{X}$ 14.23	8.47	11.33	13.15	8.01

Table 5
Persistence of relative pigment differences associated with specific early plant environments.

1964 Treatment	1965 Treatment	Total Kernels Scored	% Kernels Fully Pigmented
LD	Field	*1299	77.5
LL	Field	1106	75.6
Field	Field	1072	76.7
LL-LD	Field	1571	74.9
LD-LL	Field	1430	68.1

*Scores based on self-pollinated ears. All kernels on each ear were scored according to the numbers of kernels showing full pigmentation over the crown of the kernel.

Table 4, one may conclude that the LL and LD environment can make significant contributions to the state of the paramutated R gene. One may no longer assume single gene expression to be immune from environmental influence from generation to generation.

One can only speculate about the mechanisms involved in this unusual behavior of R. One line of speculation which will offer experimental test possibilities is that R expression is internally regulated by specific growth substances. The LD and LL conditions may affect R expressions through internal balance of growth substances--at this point it may be useful to consider the model developed for insect larval development where hormonal control of chromosome puffing has been demonstrated. What appears novel in our situation with corn is the possibility that the differences observed are also carried over into the following generations.

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1. Development of an analytical procedure for use in genetic studies of fatty acid composition of corn oil.

Gas-liquid chromatography (GLC) has been extensively used for determining the fatty acid composition of oil of corn, flax, soybean, cotton, rape, safflower, castor bean, and other crops. Most reports indicate that the standard GLC procedure in common use requires from 15 to 25 minutes to complete the analysis of a single oil sample. Due to a lack of funds, time, and labor, development of an accurate and much more rapid method than the standard GLC procedure was necessary for studies of fatty acid composition of corn oil. The GLC procedure now in use requires about 3 minutes per sample for determination of five fatty acids (palmitic, stearic, oleic, linoleic, and linolenic). With aging of columns, the retention time of linolenic becomes shorter and sample analyses have been made in 2 minutes and 20 seconds.

A brief description of the rapid GLC procedure and some comments on past experience are as follows: The equipment consisted of an F & M Model 700 dual column chromatograph with flame ionization detectors and a Honeywell Electronik 16 recorder. Columns were 0.25 in. x 7.5 ft. packed with 15%, by weight, of stabilized diethylene glycol succinate (Analabs, Inc., Hamden, Conn.) coated on Anakrom AB 70/80 mesh solid support. Operating temperatures (C) were 235 (column), 265 to 275 (injector), and 290 (detector). Helium flow rate was 110 ml/min at 60 psi pressure. Attenuation was made for each peak in order to obtain maximum peak area for each fatty acid. Attenuation was usually 10^2 x 5 or 10 for palmitic, 10^2 x 1 or 2 for stearic, 10^2 x 10 or 20 for oleic and linoleic, and 10^2 x 1 for linolenic. Small samples (approximately 0.01 μl) were injected with a Hamilton No. 7101 1.0 μl syringe. Sample sizes may vary with different syringes; however, small samples were used so that attenuation was never higher than 10^2 x 10 for palmitic (preferably peak height no higher than 60 or 70 on the chart scale). Studies with sample sizes and the possibility of overloading the column or detector have shown that the present sample size may be increased several times without distortion of peak heights or of fatty acid composition. Our experience with another syringe (Hamilton No. 7001) has shown that a uniform sample size could not be obtained from sample to sample and that palmitic acid content was considerably over-estimated and, consequently, the other fatty acids were under-estimated. Detectors have been cleaned as frequently as once a day (a 15 min. job). Also, detectors have been used over a period of time without cleaning. The necessity for cleaning detectors is evident by the amount of baseline noise with attenuation set at 10^2 x 1.

Of course, a rapid GLC procedure is of no value unless the results are accurate and reliable. Confidence in the rapid procedure was obtained by comparing results with: (1) analysis of similar oil samples by others, (2) analysis of commercial corn oil, and (3) analysis of a reference standard with a known composition of the five major fatty acids found in corn oil. Over a period of time an average of 14 chromatograms of commercial corn oil gave the following results: 12.5% palmitic, 2.5% stearic, 28.7% oleic, 55.4% linoleic, and 1.0% linolenic. These results agree closely with the reported composition of commercial corn oil as found in the literature. Table 1 gives the results from analysis of a known standard containing 20% by weight each of five fatty acids. Comparisons were made with three column temperatures each at two helium flow rates. Time required for each procedure and recorder chart speed is also given in Table 1. The rapid procedure was as good as any of the other procedures.

Duplicate chromatograms of the same oil sample agree very closely as shown in Table 2 for 12 samples of oil. Fatty acid composition was determined by triangulation of peak areas. Only one measurement and calculation was made on each chromatogram. It is concluded that only one chromatogram per sample is necessary for genetic studies since differences (fatty acid composition) among the various oil samples are usually quite large. Duplicate chromatograms would be necessary if very small differences are to be determined.

Table 1
Average fatty acid composition of four chromatograms of a standard analyzed at three column temperatures each at two helium flow rates.

Recorder chart speed	Column temp.	Helium flow rate	Sample analysis time	Fatty acid composition (%)				
				16:0	18:0	18:1	18:2	18:3
in/min	C	ml/min	min					
0.5	180	55	25	19.6	20.1	20.7	20.2	19.4
0.5	180	110	16	20.2	20.2	20.5	20.1	19.0
0.5	200	55	15	20.1	20.1	20.6	20.4	18.9
1.0	200	110	9	19.8	20.5	20.6	20.0	19.0
1.5	235	55	4	20.5	20.2	21.6	19.9	17.9
3.0	235	110	3	19.7	20.1	20.7	20.2	19.4
Average				20.0	20.2	20.8	20.1	18.9
Standard error				0.21	0.16	0.13	0.12	0.21
Coefficient of variation (%)				2.12	1.63	1.24	1.15	2.21

Table 2
Examples of results obtained with duplicate chromatograms of 12 corn oil samples.

Sample No.		Fatty acid composition (%)				
		16:0	18:0	18:1	18:2	18:3
1501	A	12.46	2.67	23.05	58.30	3.51
	B	12.10	2.83	22.40	59.00	3.67
1502	A	12.76	1.47	24.33	60.69	0.75
	B	12.88	1.54	25.43	59.37	0.78
1503	A	14.74	1.78	13.81	67.15	2.52
	B	14.06	1.82	14.36	67.12	2.64
1504	A	15.15	1.01	15.76	66.56	1.52
	B	15.12	1.07	16.05	66.20	1.56
1505	A	16.22	2.49	24.23	54.09	2.98
	B	15.99	2.63	24.22	54.08	3.07
1506	A	15.32	1.60	30.45	51.85	0.78
	B	15.22	1.67	29.85	52.44	0.82
1507	A	19.17	1.58	15.28	60.94	3.03
	B	19.29	1.60	15.14	60.93	3.03
1508	A	18.43	1.11	19.54	58.78	2.13
	B	18.52	1.09	18.69	59.68	2.01
1509	A	14.88	1.82	26.26	54.03	3.00
	B	14.55	1.79	25.88	54.88	2.90
1510	A	14.99	1.53	26.74	55.82	0.92
	B	15.61	1.46	25.88	56.19	0.86
1511	A	19.33	1.85	12.91	62.65	3.27
	B	19.48	1.76	12.25	63.28	3.23
1512	A	18.85	1.16	15.44	62.35	2.20
	B	18.61	1.08	15.54	62.59	2.17

Variation is due to chromatographic equipment and, probably, mainly due to human errors in measurement of peak areas.

M. D. Jellum

2. Fatty acid composition of reciprocal crosses.

The fatty acid composition of a number of reciprocal crosses has been determined. The results for inbred lines and their reciprocal crosses are shown in Table 1. Averages are of nine kernels (fatty acid composition of oil) from each of two ears for each inbred and cross. Reciprocal crosses of GE295 and GE297 showed heterotic effect for palmitic acid. Oleic and linoleic acid composition of the reciprocal cross was similar to that of the inbred line used as the female parent. The reverse of this

(reciprocal cross similar to male parent) was shown for oleic and linoleic in reciprocal crosses of GEC314A and T61. Palmitic acid of GEC314A x T61 was higher than the high parent (GEC314A) and the reciprocal was lower than the low parent (T61). Fatty acid composition of reciprocal crosses of SC313 and GE297 were similar and close to the midparent value. Palmitic and stearic acid composition of the crosses were in the direction of the maternal parent value. GEC314A and Mp482 did not differ greatly in fatty acid composition. Reciprocal crosses were similar for oleic and linoleic composition and similar to the parent GEC314A. Therefore, dominance in the F_1 for high linoleic and low oleic was exhibited in this particular cross.

Fatty acid composition of reciprocal crosses are different in some cases but not in others. Whether differences between reciprocal crosses can be attributed to true maternal effects or to some type of cytoplasmic effect has not been determined.

Table 1
Average fatty acid composition of the oil from 18 individual kernels of parental inbred lines and their reciprocal crosses.

	Inbred No.	Fatty acid composition (%)				
		16:0	18:0	18:1	18:2	18:3
GE295	1	14.6	3.02	31.1	50.0	1.26
1 x 2		15.2	2.48	32.2	49.1	1.11
2 x 1		15.2	3.05	38.0	42.8	0.92
GE297	2	14.8	3.22	38.4	42.6	0.97
GEC314A	3	15.1	2.86	41.1	40.2	0.78
3 x 4		15.7	3.13	34.4	46.0	0.77
4 x 3		14.4	4.29	38.4	42.2	0.79
T61	4	14.9	3.72	34.0	46.4	0.91
SC313	5	13.1	0.97	22.9	62.3	0.82
5 x 2		13.9	1.89	32.8	50.5	0.90
2 x 5		14.5	2.50	32.4	49.7	0.95
GE297	2	14.8	3.22	38.4	42.6	0.97
GEC314A	3	15.1	2.86	41.1	40.2	0.78
3 x 7		16.2	2.76	40.9	39.5	0.69
7 x 3		15.1	2.72	40.4	41.0	0.76
Mp482	7	15.5	2.14	45.5	36.2	0.74

M. D. Jellum

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1. Inbred variation and hybrid performance.

Five to seven stocks of the same long-time inbred line for each of six inbreds were crossed to a common yellow or white single-cross tester. Comparisons at the same location of three-way testcrosses within the respective inbreds revealed significant differences between the hybrids in 9 out of 10 agronomic characters: yield, time of silking, ear height, plant height, stalk lodging, erect plants, ears per plot, dropped ears per plot, and leaf position. Grain quality was the only character in which no significant differences were obtained.

These results show a great amount of variation occurring within long-time inbreds maintained at different locations and in their hybrid progeny. Thus a hybrid produced with Inbred A from Texas may not give the same performance as the supposedly same hybrid produced with Inbred A from Georgia.

A. A. Fleming

2. Biochemical analyses used to differentiate lines of maize.

Biochemical analyses were used to differentiate stocks of the same long-time inbred line of maize in this experiment. Significant differences were found in the amounts of carbohydrates and amino acids in the stocks. The significance of these findings is to provide a biochemical tool for the geneticist to use in selection phases of breeding programs and in basic genetical work.

Seedlings of four stocks of the inbred CI 7 were compared biochemically for total carbohydrates, alcohol soluble sugars, and free amino acids. Apparently three sub-lines with different biotypes have evolved in the four stocks. In general, the stocks which had agronomic differences in a previous study also had biochemical differences in this study.

A. A. El-Eryani
A. A. Fleming

3. Effect of Phosfon on growth of maize.

Phosfon is a chemical height retardant on certain plants such as Easter lilies, chrysanthemums, lima beans, seedling Jonathan apple and several other plants. The effect of Phosfon-D on a white single-cross tall hybrid corn,

GA 151 X T 113, was studied in a greenhouse experiment. The five levels of Phosfon had a marked effect on early plant height and leaf coloration. A certain degree of recovery of height growth was observed except for some plants which were extremely stunted. At 60 days from the date of Phosfon application, there were no significant differences between the zero level and other levels of Phosfon. Chlorosis seemed to affect mostly the leaves which emerged within the first ten days from the date of application. At later dates all new leaves emerged with normal coloration. Levels of the chemical had no effects on the date of tasseling.

O. K. Hudson
A. A. El-Eryani
A. A. Fleming

4. Source of resistance to maize dwarf mosaic virus.

GA 209, a white inbred line, has given excellent ratings of resistance to dwarf mosaic virus in tests in Tennessee and Ohio. Small amounts of seed may be available for distribution.

A. A. Fleming

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1. Blotching gene on short arm of chromosome 4.

Earlier tests (MNL 31:60) have shown that one of the three genes involved in the blotching system, which causes blotches of color to appear in the aleurone layer when the genotype for the principal color factors is AA cc RR, is located on chromosome 4. Three-point backcross tests completed during the past season show that the Bh gene is located on the short arm of this chromosome, 42 cross-over units from Su and 50 units from Gl_3. This is one of the few genes so far located in this general region. The data are shown below:

Table 1
Three-point Tests of Linkages of Bh, Su, and Gl_3 on Chromosome 4.

Genotypes XY	Linkage Phase	Number of Individuals XY	Xy	xY	xy	Total	Recombinations Number	Percent
Su Bh	RB	439	593	631	454	2117	893	42.2
Su Gl_3	CB	180	149	138	200	667	287	43.0
Su Gl_3	RB	248	382	338	234	1202	482	40.1
Total						1869	769	41.1
Bh Gl_3	CB	287	303	299	313	1202	602	50.1
Bh Gl_3	RB	159	171	159	178	667	337	50.5
Total						1869	939	50.2

P. C. Mangelsdorf

2. Races of maize in Argentina.

This work has been started with a collection of maize ears from the highlands of Northwestern Argentina.

On the basis of the external morphology of the ear, a preliminary classification of the entire collection was made and it was possible to choose typical ears to represent the different races. The internal characters of the ear and kernels are being studied and the preliminary classification may have to be altered in some cases. Roughly there seem to be about 20 different races of indigenous corn in Northwestern Argentina. Almost half of them are related to races of Peru such as Confite Puntiagudo, Kculli, Confite Puneño, Chullpi, Uchuquilla, and most of the races show close relationship with Bolivian races.

Cytological material has been collected to obtain information on the knob numbers and their position at the pachytene stage for each race. However, it was found that most of these races showed a poor spreading of chromosomes at pachytene. Nevertheless, it has been possible to obtain the following frequencies of total number of knobs: 0 knobs, 35.4 per cent; 1 knob, 47.0 per cent; 2 knobs, 13.7 per cent; 3 knobs, 3.9 per cent; 4 or more knobs, 0.0 per cent. The percentage of knobless chromosomes is higher than that reported by Grobman et al. (1961) for the races of Peru, probably because the proportion of high-altitude races is larger than in the Peruvian studies.

Julián A. Cámara-Hernández

3. A preliminary report of meiosis in Tripsacum lanceolatum.

Cytological studies are being made on plants of Tripsacum from Mexico and Guatemala collected by Wilkes and Chaganti (MNL 39) and now maintained at the Fairchild Tropical Garden in Florida. A study of one of these, originally collected from Penjamo and identified as T. lanceolatum, has produced the following data: The pachytene chromosomes are differentiated into proximal deep staining heterochromatic and light staining distal euchromatic regions. The euchromatic regions are terminated by a knob or more often by a deeply stained chromomere. This species is a tetraploid and consequently the chromosomes are often associated in more than pairs. Usually the two sets of homologs that make up a quadrivalent are associated at the centromere. However, in a few cases association and partner exchange was observed in the euchromatic regions also.

At diakinesis and metaphase I, varying numbers of quadrivalents, trivalents, bivalents, and univalents were observed. Of the ten possible types of quadrivalents (Darlington, 1937), types 11, 12, 15, 16, 17, and 18 were encountered. The most frequent types, however, are types 11 (a chain of four) and 17 (a ring of four). The average quadrivalent frequency at diakinesis is 5.8. Both ring and rod types of bivalents are present and the mean number of bivalents per nucleus is 22.5. At metaphase I the chromosomes are pretty much crowded on the plate and the univalents were found scattered outside the plate. Several lagging chromosomes were observed at anaphase but these eventually reach the poles. The second division is quite regular and at the end of the second meiosis normal pollen tetrads are organized.

R. V. Tantravahi

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1. Maternal effect on oil content and fatty acid distribution.

Twelve agronomic inbreds were reciprocally crossed to produce 18 F_1's. The hybrids were analyzed by wide-line NMR for oil content and two were analyzed for fatty acid content by gas-liquid chromatography.

The conclusions that were drawn are:
a. Substantial maternal effects were observed.
b. Heterosis for total oil was observed in 15 of the 18 hybrids. The mean of the F_1 was higher than the mean of the high parent in 8 of the hybrids.
c. The genotype of the embryo primarily determines fatty acid distribution, i.e., the maternal sporophyte did not appear to have an appreciable effect.

Vernon Reich
D. E. Alexander

2. Accuracy of wide-line NMR analysis of oil in corn.

Samples of dried kernels are placed between pole pieces of a permanent magnet. A radio beam is interposed and the field strength simultaneously modified electromagnetically. Resonance for protons in the liquid phase in the kernels occurs in concert. Protons in solids resonate throughout the shift in field strength and are disregarded.

Accuracy of analysis was estimated by the standard deviation from the regression line of NMR signal on careful gravimetric analyses. The mean of two 30-second sweeps on 25 g. samples was found to fall within $\pm 0.12\%$ of the oil content 95% of the time. Approximately the same error, in terms of oil percentage, was found for single seeds.

D. E. Alexander
Luis Silvela S.
Floyd Collins
Ralph Rodgers

3. Application of wide-line NMR to breeding high oil corn.

NMR was used to non-destructively analyze 3,800 individual kernels coming from 38 ears of the third cycle of a high oil synthetic. The ten higher, the ten lower and ten intermediate oil kernels from each ear were planted and the resulting plants were self-pollinated. NMR analyses were made of oil content of each selfed ear.

The overall correlation coefficient of per cent oil of the parent kernels and per cent oil of the progenies was 0.857.

Comparisons of response to selection by classical recurrent selection for oil (destructive analysis and planting of remnant seed) and by a single kernel NMR-selection scheme suggests that progress should be about 2.25 times as rapid per generation by the latter scheme.

Luis Silvela S.
D. E. Alexander

4. Seed set in an autotetraploid maize synthetic.

Per cent seed set has been determined in autotetrapolid syn B each year since 1958. The synthetic was maintained by selecting approximately 200-300 well filled ears from agronomically desirable plants each year as parents of the next generation. Each year seed set was determined in a random sample of 30-40 ears by determining the actual number of kernels on the ear and estimating the potential number from the kernel row number and a count of the potential kernels in a typical row. Mean per cent seed set for each year was:

1958	1959	1960	1961	1962	1963	1964	1965
60	68	74	69	78	80	69	83

Seed set has increased each year except for 1961 and 1964. Nineteen sixty-four was an adverse year for seed set which may account for the low seed set in this year. Improvement in seed set was rapid through 1962 but has since been slow, indicating that this synthetic is becoming stabilized at the autotetraploid level.

J. W. Dudley
D. E. Alexander

5. Mutation rate of opaque-2.

Recent findings by Nelson, et al, (Science 1964) on the altered amino acid composition of opaque-2 endosperm proteins has stimulated the interest of corn breeders in the improvement of protein quality. Many breeders are presently converting inbred lines to the opaque-2 genotype. The backcross method is satisfactory for this conversion. However, if opaque-2 mutants could be obtained directly through spontaneous mutation, considerable time could be saved in developing opaque-2 versions of these lines.

With this in mind, an investigation of the mutation rate of the normal allele (O_2) to the opaque-2 allele (o_2) was conducted in 1965. The male sterile (T-sterile) versions of the lines B37, C103, M14, and W64a (Genotypes O_2/O_2) were used as female parents in an isolated crossing block.

The male parent was a homozygous opaque-2 stock. Any mutations of the normal allele to the opaque-2 allele can be detected in the F_1 seed, assuming no aberrant reproductive events. The following table summarizes preliminary results for three of the inbred lines. The results are based on the phenotypes of the F_1 kernels but verification of their genotypes will be made by crossing with a homozygous opaque-2 stock.

Table 1

	Inbred	Estimated No. Gametes Tested	No. Suspected Mutants	Rate
1.	C103	655,660	6	$.9 \times 10^{-5}$
2.	M14	1,043,077	6	$.6 \times 10^{-5}$
3.	W64a	1,118,665	2	$.2 \times 10^{-5}$

If these suspects are the result of mutation, then two conclusions are possible:

1. the mutation rate of $\underline{O}_2$ allele is comparable to that of other maize loci;
2. the mutational events of $\underline{O}_2 \longrightarrow \underline{o}_2$ are frequent enough to warrant closer scrutinizing of inbred seed by corn breeders for these events.

R. J. Lambert
D. E. Alexander
E. B. Patterson

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1. An investigation of abberant transmission associated with the etched locus in maize.

The mutant etched allele of chromosome three is sometimes associated with viability effects which in certain backgrounds cause an upset in progeny ratios (Rhoades, M.G.C.N.L., 1957). Rhoades (M.G.C.N.L., 1961) observed that the elimination of etched individuals, in response to a "zygotic semilethal" system, varies in different genetic backgrounds. The complete elimination of etched individuals was not reported in this study.

In the present study self-pollination of a plant of the A Et/a et genotype produced an ear totally devoid of etched kernels. The absence of etched kernels is demonstrated to be conditioned by the action of a previously unreported modifier, M^{et}, (modifier of etched). Individuals homozygous for the modifier are defined as full strain. Because of the close linkage (12 units) of the color factor A to the etched locus, self-pollination of A Et/a et (full strain) individuals produces ears having distorted colored to colorless ratios indicative of the complete elimination of all expected etched individuals (Table 1; Experiment 1). When these full-strain plants are used as pollen parents with colorless, etched testers (a et/a et), normal etched transmission is observed. The use of full-strain plants, as pistillate parents, as in the cross A Et/a et; M^{et} M^{et} X a et/a et; ++, produces ears that indicate the elimination of all normal etched kernels. Some (53%) of the ears resulting from this cross possess vestigial etched kernels in varying frequencies and sizes. The other 47%, although they possessed the same genetic background, were entirely devoid of these abortive etched kernels. These reduced kernels are viewed as etched individuals able to develop at least partially in this background. If these reduced kernels are ignored in the scoring of ears the data indicate the total elimination of etched individuals (Table 1; Experiment 2). The cross A Et/a et (full-strain) X a Et/a Et yields results that indicate that the system of aberrant etched transmission is based on the zygotic elimination of etched individuals (Table 1; Experiment 3).

All the kernels produced by a cross between M^{et} M^{et} (full-strain) plants and nonrelated plants (+/+) are defined as half-strain, $+M^{et}$. Self-pollination of any half-strain A Et/a et individual produces an ear having 50 per cent of the expected etched kernels (Table 1; Experiment 4). It is possible to demonstrate the survival of 50 per cent of the expected etched kernels as a result of the testcross A Et/a et(half-strain) X a et/a et(table 1; Experiment 5). When any half-strain individual of the A Et/a et

Table 1
Outline of experimental results.

Experiment	Model	Cross	Frequencies expected (A:a)	Kernels A	Kernels a	X^2	Probability
1.	No survival of et individuals	$\frac{A\ Et/a\ et}{Met\ Met}$; ⊗	0.92A: 0.08a	2887	225	2.51	≅0.12
2.	No survival of et individuals	$\frac{A\ Et/a\ et}{Met\ Met}$ X $\frac{a\ et/a\ et}{+/+}$	0.88A: 0.12a	2764	341	3.12	≅0.08
3.	1A:1a ratio expected	$\frac{A\ Et/a\ et}{Met\ Met}$ X $\frac{a\ Et/a\ Et}{+/+}$;	0.50A: 0.50a	2889	2777	2.14	≅0.16
4.	Survival of 50% of et individ's.	$\frac{A\ Et/a\ et}{Met\ /+}$; ⊗	0.82A: 0.12a	15810	3424	0.51	≅0.48
5.	Survival of 50% of et individ's.	$\frac{A\ Et/a\ et}{Met/+}$ X $\frac{a\ et/a\ et}{+/+}$;	0.63A: 0.37a	8704	5303	1.25	≅0.28
6.	Survival of 50% of et individ's.	$\frac{A\ Et/a\ et}{Met/+}$ X $\frac{A\ Et/a\ et}{Met\ Met}$;	0.82A: 0.18a	850	160	3.20	≅0.07
7.	No survival of et individuals	$\frac{A\ Et/a\ et}{Met\ Met}$ X $\frac{A\ Et/a\ et}{Met/+}$;	0.92A: 0.08a	1629	124	1.98	≅0.18

Table 2
Classification of ears produced by tests of F_2 individuals from self-pollinated half strain A Et/a et. Also shown is the chi-square value testing the fit of these data to a 1:2:1 ratio.

Full Strain ($M^{et}M^{et}$)	Half Strain ($+ M^{et}$)	Normal (++)	X^2(2 d.f.)	Probability range
8	15	7	0.066	.95-.99

Table 3
A résumé of the various crosses performed in the course of this investigation, the resulting genotypes in terms of the modifier M, and the corresponding effects on the survival of the mutant etched allele.

Cross	Zygote	Endosperm	Progeny
$M^{et}M^{et}$ ⊗	$M^{et}M^{et}$	$M^{et}M^{et}M^{et}$	No et kernels
++ X $M^{et}M^{et}$	$+ M^{et}$	$+ + M^{et}$	Normal et survival
$M^{et}M^{et}$ X ++	$M^{et} +$	$M^{et}M^{et} +$	No normal et (reduced only)
$+ M^{et}$ ⊗	$+ M^{et}$ $+ +$ $M^{et}M^{et}$ $M^{et} +$	$+ + M^{et}$ $+ + +$ $M^{et}M^{et}M^{et}$ $M^{et}M^{et} +$	50 per cent of the expected etched individuals survive
$+ M^{et}$ X ++	$+ +$ $M^{et} +$	$+ + +$ $M^{et}M^{et} +$	50 per cent of the expected etched individuals survive
++ X $M^{et} +$	$+ +$ $+ M^{et}$	$+ + +$ $+ + M^{et}$	Normal etched survival

++ = Nonrelated Tester; $M^{et}M^{et}$ = Full Strain; $+ M^{et}$ = Half Strain

genotype is used as a pollen parent with a nonrelated etched tester, normal phenotypic frequencies are observed.

If the modifier which conditions the zygotic elimination of etched individuals is independent of the etched locus, self-pollination of F_1 half-strain individuals should express this factor in a 1:2:1 (full-strain:half-strain:normal) ratio among the resulting kernels. This distribution was demonstrated by tests of the F_2 population. (Table 2).

Reciprocal crosses between full-strain and half-strain A Et/a et plants indicate the operation of an endosperm dosage phenomenon as the causal factor of etched elimination. The zygotic genotypes produced by these reciprocal crosses are the same. However, when full-strain plants were used as the maternal parent in these crosses no normal etched kernels were produced. But when half-strain plants were used as the pistillate parents, 50 per cent of the expected etched kernels were observed (Table 1; Experiment 6 and 7). The inequality in the results of these reciprocal crosses infers the existence of an endosperm dosage phenomenon.

An interesting relationship between the endosperm dosage of M^{et} and the survival of etched kernels can be illustrated for all the levels of aberrancy thus far reported. Table 3 presents a résumé of the zygotic and endosperm genotypes which were obtained as a consequence of these studies.

The genotypic relationships presented in Table 3 imply that endosperm dosage of the M^{et} factor causes the elimination of etched individuals. It is interesting to note that at any given level of aberrancy, where a nonrelated tester (++) is used as the maternal parent, normal phenotypic frequencies are produced on the resultant ears. These findings imply that under the conditions of this type of cross, the etched individuals survive because they never receive more than one dose of the modifier. The zygotic elimination of etched individuals is apparently based on the maternal contribution of the factor, M^{et}, to the products of double fertilization.

Developmental studies were undertaken to determine the histological basis of the zygotic elimination of etched individuals. Self-pollinated, full-strain ears of the A Et/a et genotype demonstrated a 3:1 ratio for full:reduced developing kernels as early as eight days after pollination. Both full and reduced kernels were fixed and sectioned at 8, 10, and 15 days after self-pollination. These sections demonstrated normal development for a given full-sized developing caryopsis. The reduced kernels, although they were obviously postzygotic, maintained a juvenile appearance consisting of only a proembryonic axis embedded in a nondifferentiated endosperm.

These studies, both histological and genetic, demonstrate that the aberrant transmission under investigation is due to the zygotic elimination of etched individuals which is conditioned by the action of an independent modifier.

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1. Inheritance of chlorotic lesion resistance to Helminthosporium turcicum in the Australian inbred NN14.

Chlorotic-lesion resistance to northern leaf blight (H. turcicum) has been described in previous communications from this laboratory. It has been found in numerous pop, sweet, white dent, yellow dent, and flint corns and in teosinte. Usually resistance is inherited as a single dominant gene in each source, although a slightly different form of chlorotic-lesion resistance is apparently recessive in inheritance.

Inbred NN14 is unique in that it contains two dominant genes for resistance. This hypothesis is supported by the following data from crosses involving several susceptible inbreds and NN14:

Cross	Greenhouse or Field Test	Observed Res.	Seg.	Susc.	Expected Ratio	X	P Value
NN14 x B14 F_2	Greenhouse	92		4	15:1	0.7111	0.30-0.50
NN14 x Syn A F_2	"	93		5	15:1	0.2204	0.50-0.70
(NN14 x Syn A) x 168	"	74		26	3:1	0.533	0.80-0.90
R168 x NN14 F_2	Field	95		9	15:1	1.0256	0.30-0.50
		Res.	Seg.	Susc.			
NN14 x B14 F_3	Greenhouse	58	59	7	2:8:1	0.4770	0.30-0.50

A. L. Hooker
K. M. S. Saxena

2. Apparent reversal of dominance of a gene in corn for resistance to Puccinia sorghi.

Necrotic flecks develop on seedlings of the resistant inbreds NN14 and M16 when inoculated with P. sorghi culture 901aba whereas small pustules surrounded by chlorotic margins develop when these inbreds are inoculated with culture 933a. Well developed pustules without chlorosis form on the susceptible inbreds B14 and R168 when inoculated with either culture.

On single crosses between the resistant and susceptible inbreds, necrotic flecks developed when the seedlings were inoculated with 901aba but well developed pustules formed when inoculated with 933a. On the basis of F_1 data, resistance was dominant over susceptibility to 901aba but recessive to 933a.

In the first experiment involving segregating plants, F_2 progeny were tested for reaction to the two cultures.

Cross	Rust Culture	Observed Ratio Res.	Observed Ratio Susc.	Expected Ratio	X^2	P Value
M16 x B14	901aba	76	23	3:1	0.16	0.50-0.70
M16 x B14	933a	15	64	1:3	1.52	0.10-0.25
NN14 x B14	901aba	100	32	3:1	0.04	0.80-0.90
NN14 x B14	933a	19	84	1:3	2.36	0.10-0.25

In the second experiment, individual F_2 seedlings from the cross NN14 x B14 were inoculated sequentially with cultures 901aba and 933a. All seedlings were inoculated at the three-leaf stage; the third leaf was covered with a thin paper envelope so that only the first and second leaves were exposed at inoculation with the first culture. Three days later, paper envelopes were removed and the plants were inoculated with the second culture. Half of the plants were inoculated with culture 901aba followed by 933a and the other half with 933a followed by 901aba.

In this experiment, 21 seedlings were resistant to both rust cultures and 36 seedlings were susceptible to both cultures. The remaining 61 seedlings were resistant to culture 901aba but susceptible to 933a. The observed ratio fits an expected 1:2:1 ratio ($X^2 = 3.949$, $P = 0.10\text{-}0.20$).

In the third experiment, F_2 plants were selfed and approximately 20 seedlings in each progeny were inoculated with each culture. The following data were obtained:

Cross	P. sorghi Culture	Observed Ratio Res.	Seg.	Susc.	X^2	P Value (1:2:1)
NN14 x B14	901aba	36	67	22	3.784	0.10-0.25
NN14 x B14	933a	36	67	22	3.784	0.10-0.25
M16 x B14	901aba	17	41	15	1.219	0.50-0.75
M16 x B14	933a	17	41	15	1.219	0.50-0.75

No progeny in the F_3 was uniformly resistant or susceptible to one culture and segregating for the other or uniformly resistant to one but susceptible to the other.

The dominant gene has previously been designated as Rp_3. Gene Rp_3 acts as a dominant in conferring resistance to culture 901aba of P. sorghi and as a resessive in conferring resistance to culture 933a. The apparent reversal of dominance may be accounted for on the basis of dosage effect of a single allele or on the basis of two alleles being closely linked.

A. L. Hooker
K. M. S. Saxena

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1. Preferential pairing in chromosome 10 structural heterozygotes.

Rhoades (1952, in Heterosis, Iowa State Press) has observed at diakinesis a high degree of preferential pairing of structurally alike homologs in chromosome 10 trisomes which were duplex or simplex for abnormal chromosome 10 (K10). Results indicating preferential pairing of chromosome 10 are reported here for duplexes (K10/K10/k10/k10) derived from K10-carrying asynaptic diploids crossed as females with an established tetraploid stock. The duplex heterozygotes were backcrossed to the tetraploid parent and the resulting progeny were scored for K10 in dividing root tip cells prepared by a modified Feulgen squash technique. The data obtained are presented below.

Duplex crossed as	#plants	# progeny with 0K10	1K10	2K10	total
male	2	11	74	6	91
female	5	9	93	13	115
No. observed		20	167	19	206
No. expected with random chromosome segregation		34.33	137.33	34.33	205.99
X^2		5.98	6.42	6.86	19.26

The somewhat reduced ability of K10-carrying pollen to compete with k10 pollen in fertilization may account for the slight excess of male backcross progeny in the 0K10 compared to the 2K10 class while the reverse imbalance noted in the female data might reflect the occurrence of preferential segregation of K10 (Rhoades, 1942, Genetics 27:395). Assuming that these two complicating factors would essentially cancel out each other, the male and female data were pooled for purposes of X^2 calculations.

The observed distribution of K10 among the progeny does not fit (X^2=19.26, P=0.005, df=2) that expected on the basis of random chromosome 10 pairing and disjunction in the duplex parents. Rather, the data suggest the occurrence of a high frequency of homomorphic bivalents (K10/K10 and k10/k10) leading to the excess of 1K10 progeny. Cytological data on chromosome 10 pairing relationships at diakinesis support this contention. It was found that homomorphic bivalents occurred in 195 (56.7%) among a total of 344 microsporocytes while heteromorphic bivalents occurred in only 43 cells (12.5%). Quadrivalents and trivalents plus univalents were found in 98 (28.5%) and 8 (2.3%) cells, respectively. If pairing and chiasma formation were random among the four chromosomes 10 in duplex heterozygotes, the frequency of homomorphic associations among total bivalents scored should be only 33.3% whereas it was actually found to be 81.9%. This represents considerable preferential pairing.

Undoubtedly preferential synapsis will render the factors of double reduction and numerical non-disjunction considerably lower in this case than the estimates of 2.7% and 2.6% for chromosome 4 (Catcheside, 1956, Heredity 10:205) so that their elimination from the above considerations is warranted.

A. J. Snope

2. <u>The effect of abnormal chromosome 10 on numerical non-disjunction</u>

Tetraploids carrying 0, 1, and 2 abnormal chromosomes 10 (K10) were derived from asynaptic diploids segregating for

K10 and crossed as females to established tetraploid stocks. The K10 constitution and chromosome number of the derived tetraploids were determined from examination of dividing root tip cells prepared by a modified Feulgen squash technique. The extent of numerical non-disjunction of chromosome 6 was determined by scoring the nucleolar constitution of quartets of balanced 40-chromosome plants. Quartets with 3 or 4 spores containing 2 nucleoli each were scored as having arisen from 2-by-2 anaphase I disjunction. Quartets with 2 spores containing 3 nucleoli each (or 3 and 2 nucleoli) and separated by the anaphase I division plane from 2 spores containing 1 nucleolus each were scored as having arisen from 3-by-1 disjunction. Numerical non-disjunction of chromosome 6 is recorded below as per cent of 3:1 quartets.

Chromosome 6 non-disjunction	0K10			1K10			2K10		
	A*	B	C	A	B	C	A	B	C
#plants	4	2	3	3	2	2	1	2	1
#quartets	618	520	473	517	364	364	360	229	327
%3:1 quartets	4.9	5.0	7.0	9.3	7.2	12.1	11.4	11.7	15.3
overall quad. freq. at metaphase I									
tot. #homologues	1540	1150	1340	1260	1090	1080	980	1010	810
quad. frequency	.853	.853	.893	.873	.853	.880	.895	.848	.874
X% quads. with free ends	41.7	46.3	42.6	42.3	35.7	40.6	37.2	36.2	38.8

*lines A and B had the same diploid but different tetraploid parentage while line C consisted of backcross progeny of 2K10 plants from line B

It is apparent that a striking increase in chromosome 6 non-disjunction is associated with the presence of K10 in all lines, especially in C. At least three explanations could be offered for this phenomenon: (1) K10 causes a significant increase in chromosome 6 quadrivalent frequency thereby increasing the possibility of numerical non-disjunction; (2) K10 alterations in chiasma frequency result in an increase in those quadrivalent configurations which lead to the more irregularly disjoining linear and indifferent centromere co-orientations (see Darlington, 1931, Jour. Gen. 24:65); (3) K10-induced neocentric activity overtakes true centromere activity resulting in greater disjunctive irregularity.

That explanation (1) is unlikely is suggested by the above data demonstrating that K10 has little or no effect on overall quadrivalent frequency as determined from whole-cell scoring. Furthermore, preliminary data reveal no difference in chromosome 6 quadrivalent frequency at diakinesis (.943 versus .938) between 0K10 and 2K10 plants from line C.

If possibility (2) were tenable one might expect that the presence of K10 would result in an increase in those quadrivalents with free ends, that is, those configurations with one or more chromosome arms not involved in a chiasma. However, data reported in the above table show that such quadrivalent types scored at diakinesis actually decrease in the presence of K10.

Possibility (3) postulates a relationship between K10-induced non-disjunction and knob constitution. This prediction seems to be fulfilled at least for T6-9b/N heterozygotes. Dempsey (MNL 33:55, and personal communication) has obtained data which indicate, first of all, a substantial K10-induced increase in 3 to 1 segregation in these translocation heterozygotes and secondly, a greater increase in 3 to 1 segregation in those heterozygotes with two chromosome 9 knobs than in those with only one. Pachytene analysis of several line B plants in the present study revealed that chromosome 6 was quadriplex for one knob in the long arm and duplex for another more distal knob.

Limited data (Carlson, personal communication) suggest that non-disjunction may also be increased by K10 in T5-9c/N heterozygotes. Non-disjunction of chromosome 10 itself has been found to occur in K10 carrying diploids (Emmerling, 1959, Jour. Hered. 49:203; Ashman, 1964, MNL 38:122) and has been attributed by Emmerling to neocentric activity.

A. J. Snope

3. The effect of abnormal chromosome 10 on female fertility in autotetraploids.

Autotetraploid sterility in maize, as well as in other plants, has been attributed to both genetic and cytological causes although its precise nature remains unresolved. In a new approach to this problem (suggested by Dr. Rhoades) abnormal 10 (K10) was introduced into three tetraploid lines A, B, and C, as described in the above report, to investigate the possibility of correlating K10-induced neocentric activity, or increases in crossing over and chiasma frequency, with effects on fertility. Forty-chromosome tetraploids were pollinated daily until fresh silks no longer appeared. Developed kernel and ovule counts were made on the resulting ears from which the tip and butt ends had been removed and fertility is expressed below as the per cent of ovules which successfully developed into mature kernels.

	0K10			1K10			2K10		
line	A	B	C	A	B	C	A	B	B
#ears	6	2	4	7	4	5	-	9	2
tot. # ovules	2631	607	1188	2887	1610	2239	-	3434	633
% successful ovules	72.3	74.7	76.8	73.0	73.9	65.1**	-	70.7	54.8**

No significant differences in female fertility between K1O-carrying plants and k1O controls are noted for lines A and B although larger populations may be required to detect small differences that may exist. In line C, however, there was significantly greater ovule abortion in K1O-carrying plants than in k1O controls.

Although K1O was found to increase the recovery of chromosome 3 and 9 genetic recombinants in lines A and B, respectively, its presence was not accompanied by significant changes in chiasma frequency, as determined from the metaphase I frequencies of bivalents, trivalents plus univalents, and quadrivalents, in any of the three lines. It is thus not possible to correlate K1O effects on female fertility with changes in chromosome pairing relationships at meiosis.

However, in view of the previous report, it may be suggested that an increase in female sterility in the presence of K1O in line C reflects an increase in gametic aneuploidy resulting from K1O-accentuated numerical non-disjunction. The lack of a detectable effect of K1O on fertility in lines A and B could be a reflection of the differences between line C and lines A and B in overall chromosome knob constitution. That is, if non-disjunction is correlated with neocentric activity (see above report) then plants with more chromosome knobs should exhibit more non-disjunction. On this basis, there should be a greater number of knobs in line C than in line A or B plants to account for the differential effect of K1O on fertility. Because of the lack of complete information on knob constitution in the three lines, it will be necessary to await further experimentation designed to adequately test the hypothesis of a relationship between knob constitution, non-disjunction, and K1O-accentuated sterility.

A. J. Snope

4. A plant with opposite leaves.

During the summer of 1964, twin plants from one seed were observed which apparently bore two leaves at each node. The leaves were inserted opposite each other, and ear shoots also appeared in pairs inserted opposite each other. Both members of the uppermost ear shoot pair were fertile. It seemed highly possible that this trait might be inherited since two plants germinated from the same seed, and both possessed this trait.

However, when the plant was selfed, no opposite-leaved offspring were obtained in a population of 100 plants.

A careful morphological examination revealed that there were two meristematic areas at each apparent node. This indicates that the plant actually had alternating long and extremely short internodes.

David Weber

5. A test for distributive pairing.

From genetic data, Rhoda Grell (PNAS 48:165-72) hypothesized the following sequence of events in meiosis: (1) Exchange pairing (synapsis between homologous loci prior to exchange), (2) exchange, (3) Distributive pairing (crossover elements remain associated, non-crossover elements pair with one another. Pairing at this time may involve non-homologous elements), (4) disjunction.

The above scheme is based entirely on genetic data; therefore it would be highly desirable to study the process at the cytological level in order to determine the stage at which each of the hypothesized events occurs. Since cytological preparations of meiotic cells in Drosophila are not suitable, studies with analogous situations in maize were undertaken.

Two chromosomes in addition to the diploid complement were incorporated into plants and disjunction of the chromosomes at anaphase was studied. Two different situations were studied and are described below.

A. Trisome 4 plants containing one B chromosome. Plants were synthesized which contained 2 normal chromosomes 4 plus one chromosome 4 which carried inversion 4a. In addition, these plants contained a B chromosome. These plants were made heterozygous for inversion 4a in order to increase the percentage of cells which would have one of the chromosomes 4 as a univalent (see MNL., 34:55-56). This is an analogous situation to that reported by Grell where she studied disjunction of a chromosome which was mostly heterochromatic (the Y chromosome) from a chromosome II carrying inversions.

If there were no interaction between the extra chromosomes at anaphase I, there should be equal numbers of sporocytes with eleven chromosomes going to each pole and sporocytes with twelve chromosomes going to one pole and 10 going to the other. Many cells would also be expected to contain univalents on the metaphase plate which are undergoing equational division. However, if the two extra chromosomes interact and disjoin from each other, there should be an excess of the class with eleven going to each pole. The following data were obtained from a plant of the above constitution:

Anaphase I Disjunction of Chromosomes (Number of chromosomes going to each pole)			
11-11	12-10	One or Two Lagging Univalents	Not able to score
94	94	84	30

Since there is no excess of the 11-11 class, it can clearly be seen that there is no tendency for the two extra chromosomes to disjoin from each other. Scoring was done at very early anaphase; therefore the frequency of sporocytes with one or two univalents is probably somewhat high.

Because of the apparent lack of homology between A and B chromosomes, it was felt that this might not be a completely valid test; therefore, the following situation was also studied:

B. Double trisomic plants.
Five plants were studied which were trisomic for chromosome 6, and contained two normal chromosomes 4 plus one chromosome 4 containing inversion 4a. Data on disjunction of chromosomes at anaphase I are presented below:

Anaphase Disjunction of Chromosomes (Number of chromosomes going to each pole)				
11-11	12-10	10-11 + 1 Lagging Univalent	10-10 + 2 Lagging Univalents	Not able to score
202	217	219	44	129

In these plants, there is an excess of the 10-12 class, which is not in agreement with what would be expected on Grell's hypothesis. Therefore, one must conclude that there is apparently little or no distributive pairing in maize under the conditions studied. Further tests with additional combinations are in progress.

These data appear to be in conflict with those found by Michel (see the Minnesota report in this newsletter). However they may be reconciled if (a) pairing at diakinesis does not necessarily result in disjunction of the non-homologously paired chromosomes from each other at anaphase or (b) the chromosomes analyzed in Michel's study contained small regions of homology, resulting in pairing. Further work will be necessary to resolve these differences.

David Weber

6. Conversion at the B locus.

It was reported in the 1963 Newsletter that a light-colored plant had appeared in the selfed progeny from a plant presumed homozygous for B Pl. This was originally thought to represent a convertor allele at the Pl locus. Evidence now indicates that it is the B locus which is involved. Preliminary indication came from crosses with dilute purple (b Pl) and sun red (B pl) stocks. When

crossed to B, convertor plants heterozygous for b segregated in a 1:1 ratio for light and intense purple. A parallel cross with Pl, using pl heterozygotes, gave no segregation. Linkage data which implicates the B locus were obtained in the following manner. The convertor (Ws Lg Gl B') was crossed to a chromosome 2 tester (ws_3 lg_1 gl_2 b). Since a chromosome 2 tester stock carrying B was not available, these F_1 plants were crossed to Ws Lg Gl B and the progeny were first classified phenotypically as either intense purple (B/b) or light (B'/B') and then progeny-tested for the segregation of the chromosome 2 markers, ws_3, lg_1, and gl_2.

	Intense purple		Light purple	
n.c.o.	ws lg gl	316	Ws Lg Gl	330
c.o. I	Ws lg gl	42	ws Lg Gl	44
c.o. II	Ws Lg gl	66	ws lg Gl	70
c.o. III	Ws Lg Gl	77	ws lg gl	125
c.o. I-II	ws Lg gl	1	Ws lg Gl	0
c.o. I-III	ws Lg Gl	2	Ws lg gl	10
c.o. II-III	ws lg Gl	7	Ws Lg gl	6

Σ = 1096
ws-lg = 9.04%
lg-gl = 14.79%
gl-b = 20.73%

Certain stocks were reported to be nonresponsive to convertor action since offspring grown in Florida during the winter of 1963 were quite dark. Both plant color and cob color were affected. Seeds from the same ears grown in the field in Indiana the following summer gave plants which were light in color. Subsequent crosses of these plants to B have yielded only light-colored progeny. The Florida effect must have been due to some environmental modification of phenotype and has not reoccurred. All B alleles tested so far have been converted. Tests of some B alleles derived from South American lines are now being made.

The original light-colored stock has been maintained for five generations through selfing and back-crossing to B. All progeny have been light purple. The F_5 self when crossed to B yields only light-colored offspring.

Both in behavior and in phenotype this system parallels that described by Coe (P.N.A.S. 45: 828). The two convertors are phenotypically indistinguishable in the field.

Dorothy Stroup

7. The E_4 esterase.

The E_4 esterase in maize migrates toward the positive electrode in starch gel electrophoresis at pH 8.5. There are five different alleles of the gene responsible for the production of the E_4 esterase. Four of the alleles are distinguishable by the relative rates of migration of the enzymes which they produce in electrophoresis. The fifth form is a null gene which produces no active E_4 esterase. In the roots of seven day seedlings, each of the four active alleles produces a series of enzyme bands. In each case, the slowest moving band of the series is the most intense, with each faster moving band having a lower intensity than the band below it. Each of the four alleles is distinguished by the position of the slowest moving band of the series. Diagram 1 shows the relative position of each enzyme series in starch gel electrophoresis.

When samples of root extracts from seedlings with different genotypes are run side by side on the same piece of filter paper, it is observed that the bands produced by the different alleles correspond. That is, the slowest moving band produced by allele $\underline{E_4}^F$ migrates to the same position as the second band produced by allele $\underline{E_4}^E$; the slowest band produced by allele $\underline{E_4}^E$ migrates to a position identical to the second band of the $\underline{E_4}^D$ series, etc. This correspondence holds for all four series.

In heterozygotes between any two of the four active alleles, the enzyme produced by each allele migrates to the same position as that found in the homozygote. For example, a $\underline{E_4}^C/\underline{E_4}^D$ heterozygote produces an enzyme series in which the bands migrating to the C and D positions are intense, with the faster moving bands being less concentrated. A $\underline{E_4}^C/\underline{E_4}^E$ heterozygote produces a series in which the bands migrating to the C and E positions are intense with the other bands being less concentrated. There is no evidence of hybridization by dimer formation in the $\underline{E_4}$ series.

The question arises as to the nature of the differences between the enzyme bands which cause them to migrate to different positions in starch gel electrophoresis. There are several possibilities which could explain the differential migration rate. One possibility is that the differences in migration rate could be due to significant differences in molecular weight, as would occur if the different bands were to represent different degrees of enzyme polymerization. The differences in migration rate could also be due to differences in charge between enzyme forms in the different bands. This charge difference could be due either to differences in charged side groups associated with the enzyme molecule or to differences in net charge of the amino acids in the polypeptide. Two lines of evidence support the theory that the differences in migration rate are due to differences in charge rather than to significant differences in molecular weight.

The first line of evidence comes from experiments in which extracts from roots of seven day seedlings with genotype $\underline{E}_4^D/\underline{E}_4^D$ were run in electrophoresis using starch gels of different concentration. One set of starch gels contained 10.5 grams of starch per 100 milliliters of buffer while the other set contained 15 grams of starch per 100 milliliters of buffer. If the different bands were to migrate to different positions due to differences in the degree of polymerization of each band, one would expect that the larger molecules would be relatively more hindered in their movement in starch gels than would smaller molecules and thus, a change in concentration of the starch gel would affect the movement of the larger molecules more extremely than the smaller molecules. Measurements were taken of the movement of each band with respect to the origin. The results are shown in table 1. The results are expressed as ratios of the movement of the bands which migrate to the D and E positions (see diagrom 1) as compared to the movement of the band which migrates to the F position for each sample. The table shows that there is no significant difference in the rates of movement of the bands migrating to positions D and E for each sample in the two types of starch gel. Thus, the evidence indicates that there is no significant difference in the molecular weight of the enzymes of the different bands.

The second line of evidence comes from experiments in which samples were run in gels that were made with buffer that was lower in pH than the pH 8.5 buffer normally used to make starch gels. If the differences in the migration rates of the bands are due to differences in charge, then one should be able to lower the pH of the gels to a point where it is below the isoelectric point of the slower moving bands but still above the isoelectric point of the faster moving bands. At such a pH, one should be able to obtain movement of some of the bands toward the cathode while others are still moving to the anode. Samples from seedlings with genotypes $\underline{E}_4^E/\underline{E}_4^E$ and $\underline{E}_4^F/\underline{E}_4^F$ were run on gels with three different pH ranges, pH 6.5, pH 6.0 and pH 5.5. With the pH 6.5 gels, all bands from the F seedlings still migrated in the direction of the anode. However, the lowest band from the E seedlings migrated slightly to the cathodal side of the origin. At pH 6.0, all bands from the F seedlings still migrated to the anode. The lowest band from the E seedlings migrated further from the origin in the direction of the cathode. At pH 5.5, the lowest band from the F seedlings migrated slightly to the cathodal side of the origin. The lowest band from the E seedlings migrated still further in the direction of the cathode and the second band also migrated slightly to the cathodal side of the origin. Thus, by lowering the pH of the starch gels, it was possible to divide the series of bands produced by a single allele, with some migrating to the cathode, while others migrated to the anode. Therefore, the evidence indicates that the bands of a series differ in charge.

Diagram 1
Enzyme Series Produced by the Alleles of the E_4 Esterase

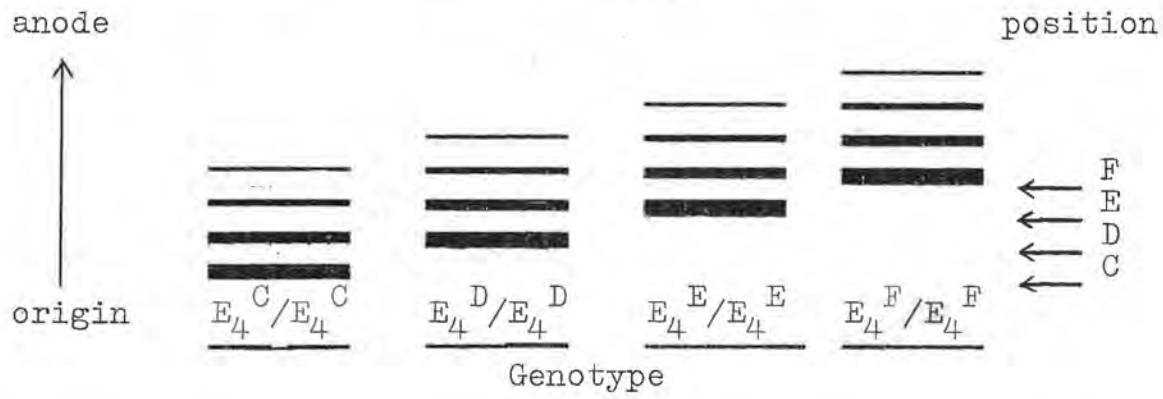


Table 1
Esterase Migration in Starch Gels of Different Concentration

Set 1 (gel conc. = 10.5 gm./100 ml.)			Set 2 (gel conc. = 15 gm./100 ml.)		
Sample	Band 1	Band 2	Sample	Band 1	Band 2
1	.76	.87	1	.78	.90
2	.77	.87	2	.75	.87
3	.76	.88	3	.74	.87
4	.75	.89	4	.75	.88
5	.77	.89	5	.75	.88
6	.77	.88	6	.77	.90
Average	.76	.88	Average	.76	.88

Evidence supporting the theory that the charge differences are due to differences in charged side groups comes from experiments with glyceraldehyde. When extracts of seedlings of each of the four genotypes ($\underline{E_4}^C/\underline{E_4}^C$, $\underline{E_4}^D/\underline{E_4}^D$, $\underline{E_4}^E/\underline{E_4}^E$, $\underline{E_4}^F/\underline{E_4}^F$) are incubated for 24 hours in the presence of glyceraldehyde and then run in electrophoresis, it is found in each case that the series of enzyme bands is converted to a single more acidic band. In all four cases, the converted bands move to the same position in the starch gel. Thus, glyceraldehyde eliminates the charge differences between the various enzyme bands while maintaining their esterase activity. These results favor the theory that the charge differences are located in side groups attached to the enzyme molecules, since it seems unlikely that glyceraldehyde would cause breakage of the peptide linkages between amino acids in the enzyme molecules, resulting in the loss of charged amino acids. It is interesting to note that glyceraldehyde also has an effect on another, non-allelic esterase in maize, the $\underline{E_1}$ esterase. In this series, charge differences between enzymes in the series are also eliminated by glyceraldehyde treatment. In this case, it has been demonstrated (Schwartz, Genetics 52: 1295-1302, 1965) that glyceraldehyde exerts its effect on the esterase molecule by causing a change in the net charge of the molecule rather than by causing dissociation of the molecule into smaller subunits.

John W. Harris

8. Maize DNA composition: analysis of plants with and without B-chromosomes.

DNA was extracted from etiolated maize seedlings by the following modified Marmur technique. Plants were ground in liquid nitrogen to a fine powder in a mortar. The powder was added to an equal weight of NaCl-EDTA solution (0.15 M NaCl + 0.1 M EDTA, pH 8.0). Sodium lauryl sulfate (25% in H_2O) was added to a final concentration of 2% and the suspension was lysed at 60°C for 10 minutes. After the solution cooled to room temperature, 5 M NaCl was added with rapid stirring to a final concentration of 1.4 M NaCl. The suspension was centrifuged at 3000 x g for 5 minutes. The supernatant was filtered through silk, layered with ice-cold ETOH and the DNA was wound out on a glass rod and dissolved in dilute saline citrate (DSC) (0.015 M NaCl + 0.0015 M sodium citrate). The solution was brought to standard saline citrate concentration (SSC) (0.15 M NaCl + 0.015 M sodium citrate) using concentrated saline citrate (CSC) (1.5 M NaCl + 0.15 M sodium citrate). The DNA solution was deproteinized three times by shaking 15 minutes with an equal volume of chloroform-isoamyl alcohol (24:1, v/v, layering the aqueous phase with ice-cold ETOH, and winding out the DNA. In all cases, the DNA was dissolved in DSC and brought to SSC with CSC (all steps must be carried out using a minimum volume of saline citrate). RNase (5mg/ml in H_2O: heated for 10

minutes at 70°C) was added to a concentration of 50 µgm/ml and incubated for 30 minutes at 37°C. Deproteinization was repeated until free of protein. After the last deproteinization the DNA was dissolved in DSC and 1/10th volume acetate-EDTA (3.0 M sodium acetate + 0.001 M EDTA, pH 7.0) was added while stirring. The DNA was wound onto the stirring rod upon dropwise addition of 0.54 volume of isopropyl alcohol. After washing the DNA progressively in 70, 80, and 95% ETOH to remove the acetate, it was dissolved in SSC and stored over chloroform. This technique was obtained from Dr. Gene Williams, Botany Dept., I. U. The amount of DNA was calculated by absorption at 260 mµ assuming 1.0 O.D. unit equals 45 µgm of DNA. In runs with the marker DNA present (Figures 1 & 2) 2 µgm of the sample DNA and 1.5 µgm of the marker DNA were dissolved in 0.80 ml of SSC and added to 1.0300 gm of CsCl (optical grade obtained from The Ealing Corp., Cambridge, Mass., Cat. #90-495). This gives the sample a density of approximately 1.71 g/cm^3. In the run shown in Figure 3, 4.2 µgm of DNA were used. All samples were centrifuged in a Beckman Model E Analytical Ultracentrifuge at 44,770 rpm at 25°C. After 20-22 hours photographs were taken using UV optics and the developed photograph was scanned on a Beckman Analytrol Densitometer. The marker DNA was Aerobacter aerogenes which has a buoyant density of 1.715 gm/cm^3 ** and a G-C content of 56%. Heat denaturation studies of maize were carried out on a Beckman DU equipped with a high temperature cell.

Fig. 1

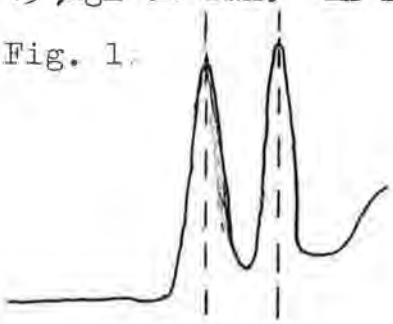
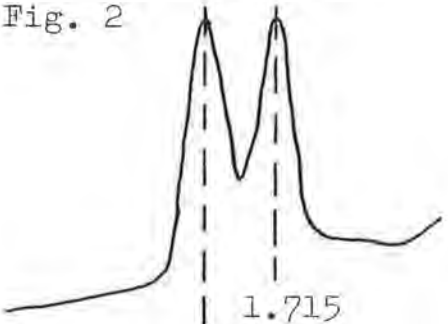
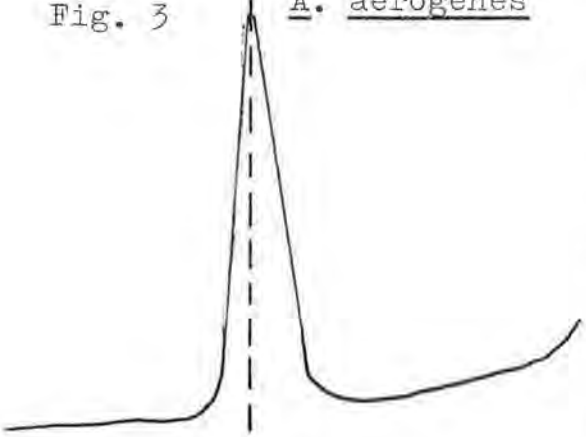


Fig. 2



1.715
A. aerogenes

Fig. 3



1.7015
maize

Figure 1 shows the densitometer tracing of the photograph produced by centrifugation of DNA extracted from Black Mexican Inbred Line with no B-chromosomes present. Figure 2 is the tracing from a run using another line with an average of 4.8 B-chromosomes per diploid genome. Both of these runs used Aerobacter as the marker DNA. It can be seen that both samples of maize DNA band at the same place in relation to the marker DNA. The density of the maize DNA calculated from the marker density is 1.7015. This

**(determined in relation to E coli at 1.710 gm/cm^3)

corresponds to 42% G-C content (Ifft et. al., 1961, J. Phys. Chem. 65: 1138-1145).

Figure 3 is the tracing of a run when excess DNA from plants with an average of 4.8 B-chromosomes per diploid genome was used in order to note any minor amounts of DNA of a different density. As can be seen from this curve, no significant minor peaks are present. All centrifuge runs reported banded at the same point in relation to the reference markers in the centrifuge.

This study indicates that the B-chromosome DNA has an overall G-C content of 42%, the same as the DNA from the A-chromosomes. Heat denaturation studies of plants with B-chromosomes give results consistent with this base-ratio. Dr. Norman Sansing at The University of Georgia has analyzed the DNA from a single cross hybrid of maize using CsCl centrifugation, heat denaturation, and enzymatic hydrolysis and subsequent column chromatography. He determined a G-C content of 42% for this stock.

Note: van Schaik and Pitout in this MNL have reported that they find differences in base-ratio for three different stocks studied. Their base-ratio determination for the inbred agrees with those reported here, but their determinations for the other lines do not. Differences in extraction and analysis procedures exist and at this time no definite conclusions can be stated.

Karl Rinehart

9. Loss of dominant markers in single chromosomes.

In the 1964 field planting and in the 1965 greenhouse crop, crosses of $\frac{A_1\ Sh_2}{A_1\ Sh_2}$ male parents on $\frac{a_1\ sh_2}{a_1\ sh_2}$ silks gave a few $\frac{a\ sh}{a\ sh}$ seeds. Many and perhaps all of these had colored embryos. A large number of crosses were made in 1965 involving the same $\frac{A_1\ Sh_2}{A_1\ Sh_2}$ stock as male parent with female parents homozygous for recessive genes on several different chromosomes (su, pr, r, wx, c, a_1). The resulting ears showed a low frequency of the mutant present in the female parent. Although these kernels must be tested further, it is evident in several cases that contamination is not the explanation. For example, the a sh kernels on ears resulting from a cross of a sh dt females with $\frac{A\ Sh}{A\ Sh}$, $\frac{Dt}{Dt}$ males were also Dt. In crosses of r wx females with $\frac{R}{R}$ $\frac{Wx}{Wx}$ males, colorless kernels were found which were Wx in phenotype. A few colorless waxy kernels also were found and these are probably contaminants. Kernels with small sectors of mutant tissue have also been observed but the frequency of fractional deficiencies is much less than that of whole kernel losses in the "high loss" ears. Preliminary observations indicate the highest rate of loss occurred for markers on chromosome 3.

Two sib plants arising by self pollination of an $\underline{A}_1$ $\underline{A}_1$ $\underline{A}_2$ $\underline{A}_2$ $\underline{C}$ $\underline{C}$ $\underline{R}$ $\underline{R}$ plant in the "high loss" stock showed quite different behavior when used as males in crosses with an $\underline{a}_1$ tester. The seeds were classified for whole losses of $\underline{A}_1$ and mosaics; the latter class is not as well defined since some kernels were small or had loose pericarp making it difficult to score small sectors. The results are presented below:

Male parent	A	A-a mosaics	a Dt
27342-19	364	5	45
	77	1	2
	252	0	20
	469	9	40
	380	11	27
	320	3	27
Total	1862	29	161

Male parent	A	A-a mosaics	a Dt
27342-27	378	3	1
	329	5	1
	427	5	1
	309	3	0
	410	7	0
	244	0	0
Total	2097	23	3

It is evident that there is considerable variation in this phenomenon from plant to plant, but that it is fairly consistent for any one male parent. This would indicate that the loss phenomenon has a genetic basis which will be the subject of further investigation.

A few of the exceptional a kernels were planted. They gave rise to A plants and on backcrossing showed typical 1 A: 1 a ratios. It was considered possible that the a kernels with A embryos arose by nondisjunction of chromosome 3 in the mitosis giving rise to two sperm cells. This is apparently not the case since the A plants did not exhibit trisomic ratios for A:a.

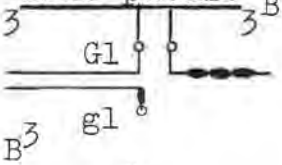
M. M. Rhoades
Ellen Dempsey

10. Cytological location of gl_6.

In the MNL 29:48, backcross data were presented involving TB-3a heterozygotes of $\underline{Gl}_6/\underline{Gl}_6$ constitution as male parents

on gl_6 females. The observation of gl individuals in the progeny was taken to indicate that gl is located distal to the breakpoint of TB-3a in the long arm of chromosome 3 (3L.1). These tests have not been considered entirely critical because hypoploid offspring often have an abnormal phenotype which might have been confused with gl even though the plants carried the Gl allele.

Recently this objection was eliminated by the following crosses:

Female parent	Male parent 	Gl	gl	Σ	%gl
a_1 gl_6		354	84	438	19.2
a_1 sh_2 Gl_6	"	331	0	331	0

The same male parents were used in crosses on the gl and Gl testers. In both tests a kernels were found on the ears and in the second cross these were also sh. Although hypoploids must be present in both progenies, gl plants were observed only in the backcross to gl females. Therefore, it is believed that the test is a legitimate one and that the Gl locus falls distal to .1 in the long arm of chromosome 3.

M. M. Rhoades
Ellen Dempsey

11. Recombination values in homozygous duplication and homozygous deficient plants.

In the Maize News Letter for 1960 I reported that crossing over in the Sh-Wx region was no greater in plants homozygous for a piece of 3L inserted into chromosome 9 than it was in plants homozygous for structurally normal chromosomes 9. The inserted piece of 3L was located between the Bz and Wx loci so, in the physically larger segment of chromatin between the Sh and Wx genes, one might expect to find higher recombination values. The crossover value of 17% found in Dp Dp plants is certainly no greater than the standard distance for this region and appears to be similar to the frequency found in control plants. Following publication of the preliminary report a considerable body of testcross data have been accumulated on crossing over in Dp Dp plants. They are listed below:

Female parent		Recombination percentages C-Sh	Sh-Wx	Coincidence value	Population
C Sh Dp Wx / c sh Dp wx	N3 N3	7.5	16.2	1.1	2175
C Sh Dp Wx / c sh Dp wx	Df3 Df3	8.1	15.9	0.8	2233
		Yg-C	C-Wx		
Yg C Dp wx / yg c Dp Wx	N3 N3	28.0	16.7	0.7	3728
		(1)	(2)	(3)	(1-2) (1-3) (2-3)	
		Yg-C	C-Sh	Sh-Wx		
Yg c sh Dp wx / yg C Sh Dp Wx	N3 N3	25.9	7.5	15.2	0.1 0.8 0.9	1333

Coincidence values are low for double exchanges in the studied regions of chromosome 9 in plants with structurally normal chromosomes 9. A remarkable feature of the Dp Dp data is the increase in number of double exchanges. This is particularly striking in those doubles where one exchange is in the Sh-Wx region. Although no increase in crossing over occurred in this extended segment, its physically longer length reduced the interference distance so that the probability of a second exchange taking place in either the Yg-C or C-Sh region was not markedly reduced. A second feature of the Dp Dp data is the enhanced crossover values for the Yg-C and C-Sh regions. In the latter region the recombination values are twice that normally found. The data on chromosome 9 indicate that no recombination occurs in the segment of 3L inserted into chromosome 9. The question as to whether or not recombination took place in this segment when part of a normal chromosome 3 was answered by the following data.

Female parent		Recombination percentages Gl-Lg	Lg-A	Coincidence
Gl Lg Df a / gl lg Df A	Dp9 Dp9	25.1	31.3	0.7

The deleted segment of 3L was originally located between the Lg and A loci so in homozygous Df3 Df3 plants these two genes are separated by a smaller segment of chromatin than they are in N3 N3 plants. A lower percentage of crossing over might be anticipated but the observed amount is not less than that occurring in closely related plants with normal 3's.

It may be concluded that recombination does not take place in the segment of 3L involved in the transposition either in N3 N3 chromosomes or in Dp9 Dp9 bivalents. That it is not genetically inert is evidenced by the abortion of both N9 Df3 megaspores and microspores.

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1. Paramutagenic action of the C locus.

A paramutagenic gene c^{IP} (provisional designation), has been found in a strain which was the product of a cross between Euchlaena mexicana x Zea. This cross was backcrossed 10 times with the recurrent homozygous strain of genotype: a a, C C, r r, gl gl, ij ij. The action of the c^{IP} gene produces mutations of the alleles C and c and the mutational sequence is C→c and c→c^{IP} (induced inhibitor). The paramutation shows extensive areas in the ear of somatic mosaicism. The mutated genes are more unstable than the standard.

Experimental data:
(1) In the cross: $c^{IP}/C(\rightarrow c)$ x C/C the following data were obtained:

1389 Colored Kernels Pr
1414 Colorless Kernels

Data from the reciprocal cross were as follows:

808 Colored Kernels Pr
688 Colorless Kernels

In the preceding data the mutation of C→c was not detectable due to the dominant effect of the C/C parent.
(2) In the cross $c^{IP}/C(\rightarrow c)$ x c/c (tester) the following data were obtained:

426 Colored Kernels Pr
896 Colorless Kernels

The mutation C→c was detected by an excess of colorless kernels.
(3) Allelomorphism.

In the crosses c^{IP}/c x c/c (tester) 18 ears with all colorless kernels were obtained. By selfing the genotype, c^{IP}/c, 8 ears with all colorless kernels were obtained.

(4) In the crosses $\underline{c}^{IP}/\underline{c}$ x $\underline{C}/\underline{C}$ (tester $\underline{A}$ $\underline{C}$ $\underline{R}$ $\underline{B}$ $\underline{Pl}$ $\underline{Pr}$) the following data were obtained:

600 Colored Kernels $\underline{Pr}$
1031 Colorless Kernels

The mutation $\underline{c} \rightarrow \underline{c}^{IP}$ was detected by an excess of colorless kernels, frequently expressed in extensive areas in the ear of somatic mosaicism.

(5) The following data were obtained on localization of the induced unstable gene, $\underline{c}^{IP}$, with the marker $\underline{sh}$, and on expression of mosaicism on the ear:

$$\frac{\underline{c}^{IP}\ (\text{induced})\quad \underline{sh}}{\underline{C}\qquad\qquad \underline{Sh}} \times \frac{\underline{C}\quad \underline{sh}}{\underline{C}\quad \underline{sh}}$$

lateral right half of the ear:	$\underline{Pr}$ $\underline{Sh}$	48
	$\underline{Pr}$ $\underline{sh}$	9
	colorless $\underline{Sh}$	0
	colorless $\underline{sh}$	25
lateral left half of the ear:	$\underline{Pr}$ $\underline{Sh}$	52
	$\underline{Pr}$ $\underline{sh}$	47
	colorless $\underline{Sh}$	0
	colorless $\underline{sh}$	4

In other ears of the same origin, these phenomena of mutation and mosaicism with variable expression were also observed.

Hypothesis:
The phenomenon could be due to an excess of replicated sub-units (#) and (*) of the gene $\underline{c}^{IP}$ (this gene was created in maize by teosinte). These sub-units (#) and (*) could remain transitorily free or attached with variable frequency to the locus $\underline{C}$. $\underline{C}$ + (#) = $\underline{c}$; $\underline{c}$ + (*) = $\underline{c}^{IP}$. This hypothesis could account for the contiguous phenomena of paramutation, mosaicism, mendelian segregation and genetic instability.

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1. Instability at A_2 and C_1.

In tests to uncover instability at the A_2 and C_1 loci twenty-two newly induced and independent changes have been confirmed in a population of over 10 million gametes. These unstable loci representing a wide spectrum of states (time and frequency of the mutation event) were found in a_1^m lines containing the En system and will be tested to: (1) identify the controlling-element system involved, (2) determine the state of each, and (3) compare the state induced with the state of the original a_1 mutable. Although there are differences in states between lines (resulting in identifiable patterns), the patterns of mutants isolated within lines are strikingly uniform. This suggests that the transposable element is the determinant for the pattern phenotype.

Peter A. Peterson

2. Phase variation of regulatory elements.

Two particular phenotypes represented by reciprocal patterns of mutability in the aleurone are due to the modification of activity of the regulatory elements (Enhancers, En) governing mutability at the a_1^m locus. The one, $En^{(flow)}$, is active at the base of the kernel but inactive at the crown, while the other, $En^{(crown)}$, is active at the crown of the kernel but inactive at the base. Mutability is found only where En is active. It is hypothesized that here the regulatory elements "switch on" and "switch off" (phase variation) during development of the endosperm.

Peter A. Peterson

3. Linkage and control of mutability of w^m_{13} - a white seedling mutable.

A white mutable seedling, w^m_{13}, was found among the progeny of some pg^m lines. The states of w^m_{13} mutability, like those of pg^m, vary from very early to very late. Stable forms have been isolated. w^m_{13} is located on chromosome 3, 28-30 units from a_1 and near lg_2. Its exact location with reference to lg_2 is under investigation.

In order to determine whether the mutability of w^m_{13} is related to the En system, crosses were made with the En tester - $a_1^{m(r)}$. From the cross, $a_1^{m(r)}/a_1$ sh x w^m_{13}

$\underline{a}_1$ $\underline{sh}/\underline{a}_1$ $^{m(r)}$ $\underline{Sh}$, (non-variegated $\underline{Sh}$ x variegated $\underline{Sh}$) variegated and non-variegated non-shrunken ($\underline{Sh}$), and non-variegated shrunken ($\underline{sh}$) kernels were selected, and plants obtained from these were selfed in order to test the presence of $\underline{w}^m$ 13.

Three sets of progeny (1, 2 and 3) of three crosses.

	progeny of $\underline{w}^m$ 13	in selfed progeny absent	present
variegated $\underline{Sh}$	1	5	17
	2	2	19
	3	0	13
non-variegated $\underline{Sh}$	1	21	0
	2	22	0
	3	14	0
non-variegated $\underline{sh}$	1	8	8
	2	4	19
	3	6	14

The data indicate that $\underline{En}$ is part of or closely linked to $\underline{w}^m$ 13. Most of the variegated $\underline{Sh}$ progeny are associated with $\underline{w}^m$ 13; some, however, are without $\underline{w}^m$ 13. This indicates that $\underline{En}$ is separable from $\underline{w}^m$ 13, except that the same result could be obtained from the mutation of $\underline{w}^m$ 13 to $\underline{W}$ 13 (green). Distribution of progeny types in the non-variegated $\underline{Sh}$ class supports the indication of a close relationship between $\underline{w}^m$ 13 and $\underline{En}$. If $\underline{En}$ were separable from $\underline{w}^m$ 13, $\underline{w}^m$ 13 would be expected to occur in a ratio reciprocal to that of the variegated $\underline{sh}$ class. None were found. Results obtained and listed under the heading non-variegated $\underline{sh}$, show linkage of $\underline{w}^m$ 13 with $\underline{a}_1$ $\underline{sh}$. The non-$\underline{w}^m$ 13 progeny arise from crossovers between $\underline{sh}$ and $\underline{w}^m$ 13 which is near $\underline{lg}_2$. $\underline{En}$ is either part of the $\underline{w}^m$ 13 complex or it is closely linked to $\underline{w}^m$ 13. This relationship is now being tested further.

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1. Linkage studies involving the $\underline{a}_2$-$\underline{bt}_1$ region of chromosome five.

For the past several years we have undertaken a rather intensive crossover study of the region from $\underline{a}_2$-$\underline{bt}_1$ in

chromosome five. These studies have involved the loci $\underline{a}_2$, $\underline{vp}_2$, $\underline{bm}_1$ and $\underline{bt}_1$. Plants of the constitution $\underline{a}_2 \pm \pm \pm / \underline{a}_2$ $\underline{vp}_2 \pm \pm$ were crossed to $\underline{A}_2 \underline{A}_2 \pm \pm \underline{bm}_1 \underline{bm}_1 \underline{bt}_1 \underline{bt}_1$ plants to produce F_1 seeds of the genotypes $\underline{A}_2 \pm \underline{bm}_1 \underline{bt}_1 / \underline{a}_2 \underline{vp}_2 \pm \pm$ and $\underline{A}_2 \pm \underline{bm}_1 \underline{bt}_1 / \underline{a}_2 \pm \pm \pm$. Also, plants of the constitution $\underline{A}_2 \pm \pm \pm / \underline{A}_2 \underline{vp}_2 \pm \pm$ were crossed to $\underline{a}_2 \underline{a}_2 \pm \pm \underline{bm}_1 \underline{bm}_1 \underline{bt}_1 \underline{bt}_1$ plants to produce $\underline{a}_2 \pm \underline{bm}_1 \underline{bt}_1 / \underline{A}_2 \underline{vp}_2 \pm \pm$ and $\underline{a}_2 \pm \underline{bm}_1 \underline{bt}_1 / \underline{A}_2 \pm \pm \pm$ F_1 seeds. The F_1 plants were used as females in an isolation plot in which the male parents were $\underline{a}_2 \underline{a}_2 \pm \pm \underline{bm}_1 \underline{bm}_1 \underline{bt}_1 \underline{bt}_1$. These testcross ears were then classified for crossovers in the $\underline{a}_2$-$\underline{bt}_1$ region. The number of rows on each testcross ear was determined and multiplied by the number of seeds per row to give an estimate of the total seeds per ear. Then the number of crossover seeds on each ear was determined. These crossover seeds were saved for further testing. The crossover data from these testcross ears are given in Table 1.

Table 1
Summary of crossover data for the $\underline{a}_2$-$\underline{bt}_1$ region.

Genotype of F_1	Crossover classes: $\underline{A}_2 \pm$	$\underline{a}_2 \underline{bt}_1$	Totals	% C. O.
$\frac{\underline{A}_2 \quad \pm \quad \underline{bm}_1 \ \underline{bt}_1}{\underline{a}_2 \ (\pm \text{ or } \underline{vp}_2) \ \pm \ \pm}$	5,700	5,417	269,518	4.12%
	$\underline{a}_2 \pm$	$\underline{A}_2 \underline{bt}_1$		
$\frac{\underline{a}_2 \quad \pm \quad \underline{bm}_1 \ \underline{bt}_1}{\underline{A}_2 \ (\pm \text{ or } \underline{vp}_2) \ \pm \ \pm}$	9,401	7,911	330,136	5.24%
	15,101	13,328	599,654	5.05%

There is a consistent deficiency in the $\underline{bt}_1$ class in these data. This perhaps is the result of abortive development of $\underline{bt}_1$ seeds or the tendency of $\underline{bt}_1$ seeds to mold, thus hindering their color classification.

In order to determine the $\underline{vp}_2$ and $\underline{bm}_1$ constitution of the non-purple crossovers from the two classes of testcross ears, plants from the non-purple crossover seeds were grown in an isolated plot, detasseled, and open pollinated by plants known to be heterozygous for $\underline{vp}_2$. The results of these crosses are given in Tables 2, 3 and 4.

Table 2

$\underline{vp}_2$ and $\underline{bm}_1$ constitutions of non-purple $\frac{\underline{a}_2 \; \underline{A}_2 \; \pm \; \underline{bm}_1 \; \underline{bt}_1}{\underline{a}_2 \; \underline{a}_2 \; (\pm \text{ or } \underline{vp}_2) \; \pm \; \pm}$ crossovers from crosses of $\underline{a}_2 \; \underline{a}_2 \; \underline{bt}_1 \; \underline{bt}_1 \times \underline{a}_2 \; \underline{a}_2 \; \pm \; \pm$

$\underline{bm}_1 \; \underline{bm}_1 \; \underline{bt}_1 \; \underline{bt}_1$.

	Genotypes of crossovers				
	$\underline{a}_2 \pm \underline{bm}_1$ Region 1	$\underline{a}_2 \; \underline{vp}_2 \; \underline{bm}_1$ Region 2	$\underline{a}_2 \; \underline{vp}_2 \pm$ Region 3	$\underline{a}_2 \pm \pm$**	Totals
Observed numbers	787	77	5	8	877
Corrected value*	355	77	5	0	437
% Corrected Data	81.2	17.6	1.1		
Total % C. O. for regions 1, 2 and 3 = .0505 (Total C. O. $\underline{a}_2$-$\underline{bt}_1$ from Table 13 x line 3	4.10	0.89	0.06		

*This correction is necessary since only ½ of the F_1 plants carried $\underline{vp}_2$. Thus, calculations are made on basis of that half that came from heterozygous $\underline{vp}_2$ plants.

**A crossover class involving region 3 of non-$\underline{vp}_2$ F_1 plants.

Table 3

vp_2 and bm_1 constitutions of non-purple $\frac{a_2}{A_2} \frac{a_2}{(+ \text{ or } vp_2)} \frac{+}{+} \frac{bt_1}{+}$ cross-overs from crosses of $\frac{a_2}{bm_1} \frac{+}{bt_1}$ x $\frac{a_2}{bm_1} \frac{a_2}{bm_1} \frac{+}{bt_1} \frac{+}{bt_1}$.

	Genotypes of crossovers			
	$\frac{a_2}{+} \frac{vp_2}{+}$ Region 1	$\frac{a_2}{+} \frac{+}{+}$ Region 2	$\frac{a_2}{bm_1} \frac{+}{+}$ Region 3	Totals
Observed numbers	505	430	15	950
Corrected values*	505	156	12	673
% Corrected Data	75.0	23.2	1.8	
Total % C. O. for regions 1, 2 and 3 = .0505 (Total C. O. a_2-bt_1 from Table 1) x line 3	3.79	1.17	0.09	

*This correction is necessary since only ½ of the F_1 plants carried vp_2. Thus, calculations are made on basis of that half that came from heterozygous vp_2 plants.

Table 4

Totals for C. O. regions 1, 2 and 3 (a_2 (1) vp_2 (2) bm_1 (3) bt_1).

	C. O. Region 1	C. O. Region 2	C. O. Region 3	Total
Sum of corrected values from tables 2 and 3	860	233	17	1,110
%	77.5	21.0	1.5	
Total % C. O. for regions 1, 2 and 3 = .0505 (Total C. O. a_2-bt_1 from Table 1) x line 2	3.91	1.06	0.08	

The data used in making these calculations are based on selected crossover seeds from the a_2-bt_1 region, a distance of 5 crossover units. They are, therefore, equivalent of testing 22,200 (20 x 1,110) unselected gametes from 2 four point test crosses, and indicate the following linkage map: a_2 - 3.91 - vp_2 - 1.06 - bm_1 - 0.08 - bt_1.

Donald S. Robertson

2. Genetic and biochemical studies of cl_1 and its modifiers.

In the Maize Genetics Cooperation News Letter of 1963 (37:74-76) the results of allele tests were reported that suggested the dominant Cl^2_M, Cl^3_M, and Cl^4_M modifiers of the albino seedling phenotype of the white endosperm-albino seedling mutant cl_1 were allelic. Since then more extensive data have been collected and the dominant modifier Cl^5_M which was found in our genetic stocks was also tested for allelism. The data reported in Table 1 lends further support to the conclusion that all known modifiers of cl_1 are allelic. Such modifiers seem to be rather widespread in corn lines. The original Cl^2_M and Cl^3_M modifiers were found in the inbreds T1 and C106 and Cl^4_M in inbred CI31A. In crosses to transfer cl_1 into the inbreds M14 and W22 they also were found to carry modifiers of cl_1. These modifiers are being tested for allelism with the others. The inbreds OH43 and N25 seem to be devoid of cl_1 modifiers as do some, if not all, lines of Tama flint.

The modifier locus has not been determined as yet. Early attempts to locate it were hampered by the presence of modifiers in the series of translocations which were being used as linkage testers. However, we now have a series of waxy chromosome-nine translocations converted to M14 and this series has been crossed to cl_1 devoid of modifiers. If the M14 modifier turns out to be allelic to the other modifiers, it is hoped that analysis of F_2 progeny of this series of translocation crosses will reveal the location of the modifier locus.

Table 1
Summary of data from allele tests of Cl^2_M, Cl^3_M, Cl^4_M and Cl^5_M

F_1 Cross									# F_2 seedlings	# albino	Conclusions
cl_p cl_p	Cl^4_M	Cl^4_M	x	cl_1	cl_1	Cl^3_M	Cl^3_M		6119	0	Allelic
cl_1 cl_1	Cl^3_M	Cl^3_M	x	W_{7716}	W_{7716}	Cl^5_M	Cl^5_M		2842	0	Allelic
cl_1 cl_1	Cl^3_M	Cl^3_M	x	Cl_1	cl_1	Cl^2_M	Cl^2_M		13,571	0	Allelic
Cl_1 cl_1	Cl^2_M	Cl^2_M	x	cl_p	cl_p	Cl^4_M	Cl^4_M		9045	0	Allelic
cl_p cl_p	Cl^4_M	Cl^4_M	x	W_{7716}	W_{7716}	Cl^5_M	Cl^5_M		1810	0	Allelic
Cl_1 cl_1	Cl^2_M	Cl^2_M	x	W_{7716}	W_{7716}	Cl^5_M	Cl^5_M		1724	0	Allelic

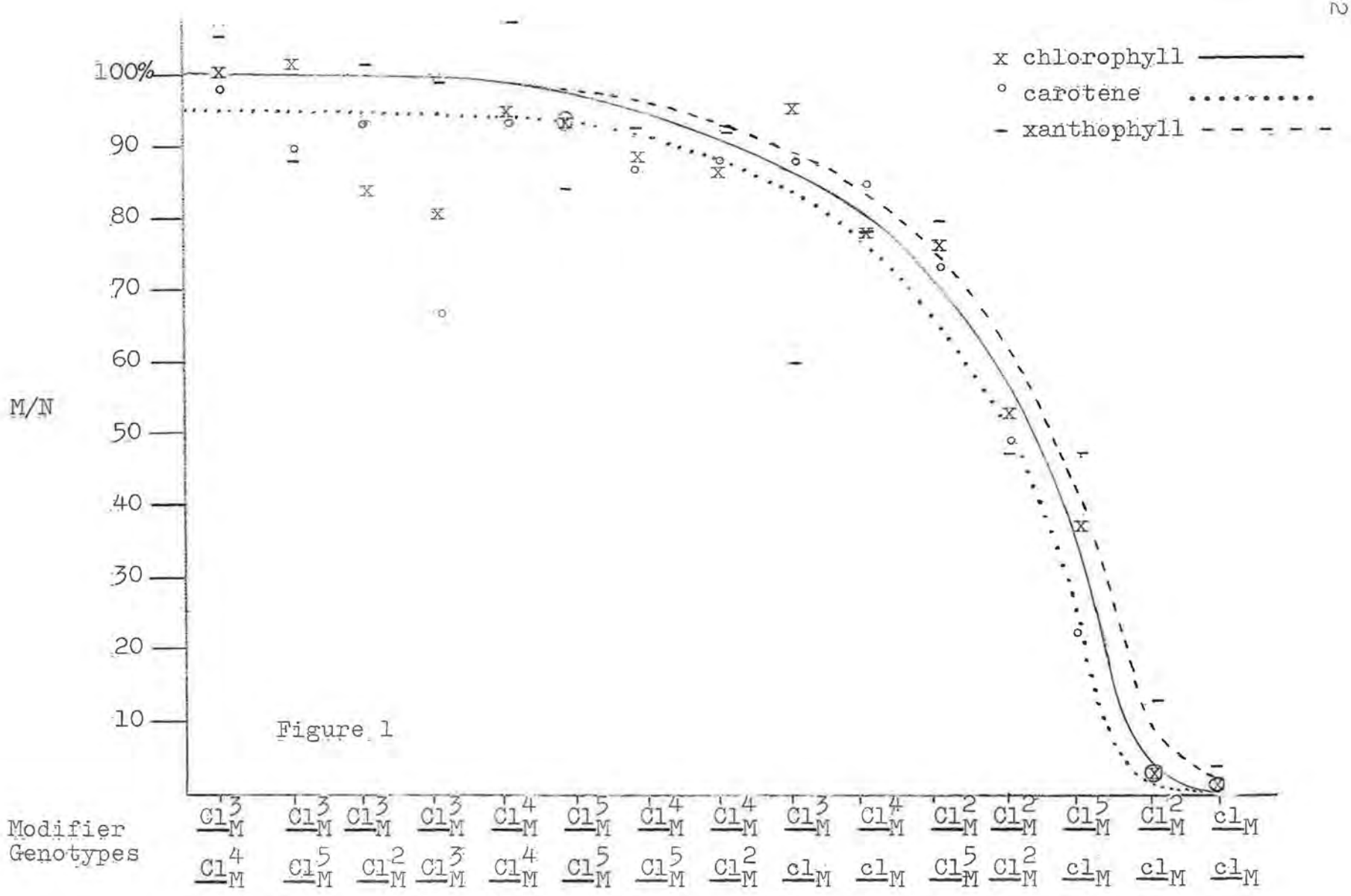
The allele tests of the modifiers resulted in stocks that were heterozygous for the various modifier alleles. These along with the various homozygotes and stocks which were heterozygous for the modifier and the recessive allele at this locus were analyzed for their ability to synthesize plastid pigments in the seedling stage.

Tests established that $\underline{cl}_1$ and its alleles $\underline{w}_{7716}$ and $\underline{cl}_p$ in the absence of modifiers were able to produce normal or near normal amounts of protochlorophyllide in the dark and to convert this to chlorophyll in the light (Table 2). On further exposure to light the chlorophyll is destroyed in the absence of carotenoid pigments. In this regard these mutants are similar to other white-albino mutants. This observation suggests that the genetic lesion at the $\underline{cl}_1$ locus primarily involves carotenoid synthesis and that chlorophyll is only secondarily involved. Tests of the four homozygous suppressed phenotypes ($\underline{cl}_1$ $\underline{cl}_1$ $\underline{Cl}^2_M$ $\underline{Cl}^2_M$, $\underline{cl}_1$ $\underline{cl}_1$ $\underline{Cl}^3_M$ $\underline{Cl}^3_M$, $\underline{cl}_p$ $\underline{cl}_p$ $\underline{Cl}^4_M$ $\underline{Cl}^4_M$ and $\underline{w}_{7716}$ $\underline{w}_{7716}$ $\underline{Cl}^5_M$ $\underline{Cl}^5_M$) also establish that they possessed normal or above normal ability to make this pigment (Table 2). Since tests of effect of homozygous modifiers on the chlorophyll synthesizing system would seem to indicate that they have the ability to produce this pigment (Table 2), any variation in pigment concentration in light grown seedlings must be due to the effect of the modifiers on carotenoid synthesis.

Figure 1 indicates the percentage of plastid pigments (chlorophyll, carotene and xanthophyll) that mutant seedlings have when compared to their normal siblings from the same ear. The data are expressed in this form since the various genotypes were not in a homogeneous background and there is considerable variation in pigment level among normals of the various lines tested. The genotypes are arranged along the abscissa in descending order, with those giving the closest approximation to normal on the left. The determination of how closely a given mutant approximates normal was largely subjective. In making this judgment, visual comparison of normal and mutant plants from the same segregating ear was made using such criteria as plant height at maturity, date of flowering and differences in plant pigmentation obvious to the eye. Such judgments are easier to make in hybrid material that produced the plants heterozygous for two different modifiers since these populations tended to be more uniform. This was also true for homozygous $\underline{cl}^4_M$ plants which were in an inbred background. However, for stocks like $\underline{Cl}^3_M$ $\underline{Cl}^3_M$ and $\underline{Cl}^5_M$ $\underline{Cl}^5_M$ differences were more difficult to determine accurately because the progeny of the self pollination that produced them showed considerably more variation in both the normal and mutant individuals. This is particularly true for the $\underline{Cl}^2_M$ $\underline{Cl}^2_M$ line which for the most part has been perpetuated in the homozygous condition

Table 2
The formation of protochlorophyllide and chlorophyll in normal and mutant seedlings from self-pollinated ears of plants carrying $\underline{cl}_1$ alleles with and without modifiers.

Genotype of self-pollinated plant	Seedlings tested	O. D. 630 mu.	Dark Grown Seedlings: Proto-chlorophyllide mg./gm.	Proto-chlorophyllide Mutant ÷ Normal	O. D. 667 mu.	Chlorophyll mg./gm.	Chlorophyll Mutant ÷ Normal
$\underline{Cl}_1\ \underline{cl}_1\ \underline{cl}_M\ \underline{cl}_M$	N	.063	.00106	3.3	0.0	0.0	--
	M	.102	.00348				
$\underline{W}_{7716}\ \underline{w}_{7716}\ \underline{cl}_M\ \underline{cl}_M$	N	.037	.00144	0.5	0.0	0.0	--
	M	.055	.00074				
$\underline{Cl}_p\ \underline{cl}_p\ \underline{cl}_M\ \underline{cl}_M$	N	.053	.00182	1.9	0.0	0.0	--
	M	.079	.00350				
$\underline{Cl}_1\ \underline{cl}_1\ \underline{Cl}^2_M\ \underline{Cl}^2_M$	N	.062	.00240	1.1	0.0	0.0	--
	M	.078	.00254				
$\underline{W}_{7716}\ \underline{w}_{7716}\ \underline{Cl}^5_M\ \underline{Cl}^5_M$	N	.070	.00272	0.7	0.0	0.0	--
	M	.056	.00185				
$\underline{Cl}_p\ \underline{cl}_p\ \underline{Cl}^4_M\ \underline{Cl}^4_M$	N	.052	.00187	1.3	0.0	0.0	--
	M	.063	.00240				
$\underline{Cl}_1\ \underline{cl}_1\ \underline{Cl}^3_M\ \underline{Cl}^3_M$	N	.047	.00153	1.4	0.0	0.0	--
	M	.064	.00216				
	Seedlings exposed to 1 min. of light at 1000 ft. c. and harvested after 1 hour of dark						
$\underline{Cl}_1\ \underline{cl}_1\ \underline{cl}_M\ \underline{cl}_M$	N	.030	.00091	1.9	.036	.00112	3.9
	M	.058	.00170		.146	.00440	
$\underline{W}_{7716}\ \underline{w}_{7716}\ \underline{cl}_M\ \underline{cl}_M$	N	.024	.00080	2.0	.024	.00082	3.9
	M	.056	.00162		.107	.00319	
$\underline{Cl}_p\ \underline{cl}_p\ \underline{cl}_M\ \underline{cl}_M$	N	.047	.00185	0.8	.012	.00049	5.7
	M	.044	.00150		.078	.00276	
$\underline{Cl}_1\ \underline{cl}_1\ \underline{Cl}^2_M\ \underline{Cl}^2_M$	N	.045	.00139	0.9	.023	.00073	2.2
	M	.037	.00121		.048	.00161	
$\underline{W}_{7716}\ \underline{w}_{7716}\ \underline{Cl}^5_M\ \underline{Cl}^5_M$	N	.037	.00140	0.9	.011	.00042	1.3
	M	.042	.00131		.017	.00055	
$\underline{Cl}_p\ \underline{cl}_p\ \underline{Cl}^4_M\ \underline{Cl}^4_M$	N	.038	.00111	1.6	.022	.00066	2.4
	M	.043	.00177		.037	.00157	
$\underline{Cl}_1\ \underline{cl}_1\ \underline{Cl}^3_M\ \underline{Cl}^3_M$	N	.033	.00091	2.0	.010	.00028	4.8
	M	.056	.00182		.040	.00135	


x chlorophyll
° carotene
– xanthophyll
100%
90
80
70
60
50
40
30
20
10
M/N
Modifier Genotypes
Cl^3_M Cl^4_M
Cl^3_M Cl^5_M
Cl^3_M Cl^2_M
Cl^3_M Cl^3_M
Cl^4_M Cl^4_M
Cl^5_M Cl^5_M
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Cl^4_M Cl^2_M
Cl^3_M cl_M
Cl^4_M cl_M
Cl^2_M Cl^5_M
Cl^2_M Cl^2_M
Cl^5_M cl_M
Cl^2_M cl_M
cl_M cl_M

Figure 1

so that we have had very little opportunity to make accurate comparisons between normals and mutants from a given ear. Those that have been made would indicate that mutants are slightly less vigorous than normal mature plants. However, the chemical data would suggest that the phenotype of this genotype should fall below that of $\underline{Cl^3_M}\ \underline{cl_M}$ plants. It is obvious from visual observation that this cannot be the case since even to the casual observer mature $\underline{Cl^3_M}\ \underline{cl^3_M}$ are decidedly pale-green plants with a tendency to have white sheaths and zebra striping while $\underline{Cl^3_M}\ \underline{Cl^3_M}$ plants are a definite dark green and closely approximate normals. The explanation for the low values for $\underline{Cl^3_M}\ \underline{Cl^3_M}$ could be due to an increased effeciency of the modifiers as the plants mature so that the seedling values do not accurately reflect performance in mature plants. However, this is not observed to be the case for the other genotypes. Perhaps the low value for $\underline{Cl^3_M}\ \underline{Cl^3_M}$ is due to some peculiarity in the particular background of the material used for these determinations which came from lines of rather low vigor due to several generations of inbreeding. We are in the process of crossing this gene out to inbreds that do not possess modifiers and re-extracting what we hope to be a more vigorous $\underline{Cl_1}\ \underline{cl_1}\ \underline{Cl^3_M}\ \underline{Cl^3_M}$ line for further pigment tests.

The outstanding characteristic of Figure 1 is that the levels of the three plastid pigments vary together. Since it is known that both the albino mutants and the modified genotypes have a chlorophyll producing mechanism that, as far as has been tested, appears to be normal, it is strongly suggestive that the marked parallelism between chlorophyll content and the carotene and xanthophyll levels is dependent on the amount of one or both of the latter two pigments that can be produced under the influence of a given modifier. This is just what would be expected if carotene is acting here to protect chlorophyll from photodestruction. At low levels of carotene productions only small amounts of chlorophyll can be protected; at higher carotene levels more chlorophyll is protected. These results are in agreement with those of other workers that suggest that one of the functions of colored carotenoids is to protect chlorophyll from photo-auto-oxidation.

Marilyn Bachmann
I. C. Anderson
D. S. Robertson

3. Electron microscopy studies of plastid development in mutants at the white endosperm - albino seedling w_3 locus.

This past year we have begun an electron microscopy study of plastid development in normal and mutant plant material. In these studies seedlings were grown for 10 - 14 days in the dark at 26.6°C. (80°F.). Others were grown under

normal day-night conditions with a light intensity of 2000 ft. candles. Samples were taken from secondary leaves of dark grown plants and fixed in the dark, after which the plants were exposed to 2000 ft. candles of light and sampled at intervals up to 24 hours. Tissue was fixed with either 4% $KMnO_4$ or 3% Glutaraldehyde post-fixed with 1% Osmium tetroxide dehydrated in an alcohol series, embedded in Epon 812 and sectioned on an LKB ultramicrotome with a diamond knife. Sections were stained with Uranyl acetate in methanol and examined under the electron microscope.

A good portion of the year was devoted to perfecting techniques and to determining normal plastid development. This was determined by studying both dark grown tissue at intervals when exposed to illumination up to twenty-four hours and by sectioning tissue from the apical meristem.

Following these preliminary studies, work has been, and is at present, mainly concerned with the structural development of the chloroplast of the albino w_3 and its pale green pastel$_{8686}$ allele as compared with the normal dark green chloroplast. The albino w_3 is capable of some chlorophyll production but lacks colored carotenoids so that its chlorophyll breaks down in the light. This mutant when grown in the dark shows a structurally organized prolamellar body similar to that found in the dark grown normal chloroplast. In dark grown normals exposed to light this prolamellar body undergoes a breakdown or disorganization and an increase in lamellar membranes. However, after 1-4 hours of illumination the membranes of the albino begin to break down and become disorganized. This disorganization of lamellar membranes of mutants continues on further illumination up to 24 hours with no formation of grana as observed in normals. In addition, the albino plastids contain numerous starch grains, even in dark grown tissue in contrast to the normal, where starch was not seen until after 24 hours of light. The pale green (pas_{8686}), which is presently being studied, shows some lamellar organization after 24 hours of illumination, but its "grana" unlike the normal which have short stacks of membranes, are long lamellar aggregates, sometimes loosely arranged. The developing normal chloroplast, by 24 hours, has numerous well developed lamellae and stacks of grana throughout the plastid.

Further work is planned with these mutants. The above studies were carried out at 26.6°C. However, the phenotypic expression of pas_{8686} mutant is strongly influenced by temperature. Grown at 22°C. it has only about 11.1% as much chlorophyll and 7.9% as much carotene as normals, while grown at 37°C. it produces 59.6% as much chlorophyll and 61.4% as much carotene. The effect of these temperature differences on the development of plastid structures in pas_{8686} and the F_1 between pas_{8686} and w_3 will be studied in the future.

Further studies on other white endosperm-albino mutants are planned as well as studies on other pigment deficient mutants (e.g., luteus, pale greens, virescents, etc.).

Marilyn Bachmann

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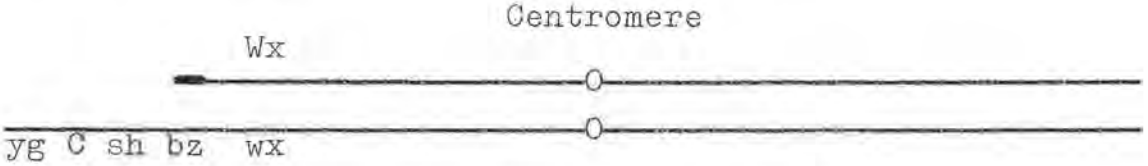
1. Very low cross-over rate between wx and the breakage point of TB-9b.

The position of the waxy locus has been indicated at about 2/5 of the length of the short arm of chromosome 9 taken from the centromere (McClintock). The breakage point of TB-9b has also been given as .4 of the arm from the centromere (Roman).

Since the wx locus is not uncovered by the TB-9b it should be inferred that the cytological distance between wx and the breakage point of such a translocation is quite negligible. Genetical data suggest that the cross-over distance is also very tiny indeed.

Crossing of TB-9b on a multiple tester of chromosome 9 (yg C sh bz wx) permits the easy identification of the hypoploid individuals of the following constitution:



When these plants are backcrossed to the multiple tester, the kernels obtained turned out to be of the following type:

Wx	wx	Total	% of Wx
13	6053	6066	0.21

Obviously the rate of crossing-over between Wx and the break point could be evaluated also on the basis of pollen grains produced by such hypoploid plants. Provided that the Wx bearing chromosome, because of the terminal deficiency, leads to pollen abortion, normally filled pollen grains possessing the dominant factor should originate only from crossing-over between Wx and the break point.

Staining of the pollen produced by the hypoploid type plant with iodine-potassium iodide solution permitted the following classification:

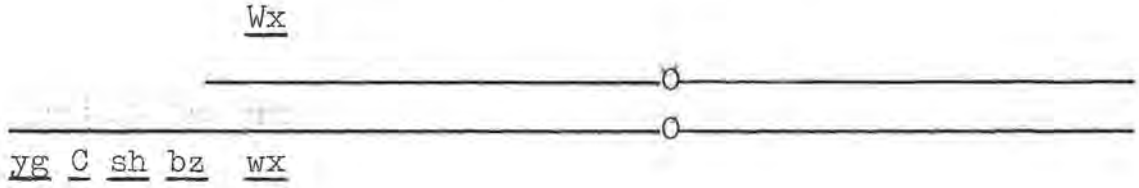
Normally filled grains		Deficient grains
Wx - type (blue staining)	wx-type (brown staining)	%
371 (0.23%)	163,236	47.7 (11,350 empty grains in a total of 23,813)

The rate of the cross-over type is of the same order of magnitude not only in the two tests, as expected, but also is of the order of size of the rate of the intracistron recombination within the locus Wx and of the (intergenic) crossing-over between A_1 and Sh_2. This situation may be of use in the study of some phenomena.

A. Bianchi
B. Borghi

2. Reversion of chromosome 9 markers in normal and hypoploid maize.

The multiple recessive tester for chromosome 9 markers yg_2 C sh bz wx has been fertilized with pollen produced by plants of the following hypoploid constitution (produced in the progeny of the TB-9b stock on the same multiple recessive tester):



Other plants of the multiple tester have been self- and/or sib-pollinated. The kernels obtained in the two types of crosses have been analyzed as to endosperm and seedling traits in order to detect possible reversion events. The results of the scoring have been as follows:

Total no. of kernels	Reversion for C		Reversion for sh		Reversion for bz		Total no. of seedlings	Reversion for yg	
	no.	x10^{-5}	no.	x10^{-5}	no.	x10^{-5}		no.	x10^{-5}
				from normal x hypoploid					
6,066	0	0	0	0	0	0	5,156	0	0.0
				from normally diploid plants					
70,190	2	2.85	0	0	1	1.42	48,000	0	0.0

The reversion rate of the genes considered, on the basis of these preliminary data, indicates that the hypoploid condition, where no opportunity for normal pairing and crossing-over is given, is accompanied, as in other species (Saccharomyces, e.g.) for certain mutants (supposed to be due to base losses and insertions in DNA), by lower reversion rates than in the normally diploid condition which has been postulated to favour such reversion by means of recombination phenomena. This finding is at variance with what has been obtained by Bianchi and Tomassini (Mutation Research, 2 : 352, 1965) for the waxy character on pollen grains with a much larger statistical basis, where no difference has been detected between the two chromosome conditions. However, obviously, more data are needed to confirm or to disprove the differential behavior of the markers yg, C, sh, and bz as compared with that of the wx locus.

Moreover, the figures of the normal x hypoploid combination are not directly comparable to those from the normally diploid plants, because in the former case the paternal chromosome has not undergone regular pairing and crossing-over, as contrasted, obviously, to the seed parent chromosome which, in this respect, has undergone the same meiotic processes as the chromosomes of the diploids.

A. Bianchi
B. Borghi

3. A three point test for an endosperm trait in chromosome 7.

Data reported in the last MNL issue indicated that a "collapsed endosperm" (cl) mutant uncovered by TB-7 showed 8-9% crossover with gl_1.

An F_2 of the mutant with a tester marked with o and gl_1 produced the following data:

Gene pair	Phase	A B	A b	a B	a b	Recombination % ± st. error
o cl	R	101	56	33	1	16.1 ± 4.5
o gl	C	146	11	9	25	13 ± 1.8
cl gl	R	98	36	57	0	very low

From these data it appears that the mutant is likely to be located distally to $\underline{gl_1}$.

B. Borghi
F. Salamini

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1. Analysis of variation of growth rate of maize root tips cultured in vitro.

The technique of root cultures in vitro appears to be a useful tool for the study of the genetic control of continuous variation, because it offers the possibility of a rigorous control of the environmental factors. This technique offers the possibility of carrying out experiments for studying the effects of the gene action which control continuous variation at the biochemical level (Ottaviano and Zannini, 1965).

The main purpose of this work is to study the genetic control of variation of growth rate of maize root tips in order to see if this material is suitable for genetic biometrical studies. In this experiment five inbred parents and all their possible F_1's, reciprocals included, have been considered. The biometrical analyses are those of diallel crosses as indicated by Jinks and Hayman (1953), Hayman (1954) and Jinks (1954).

The growth rate, expressed as weight after two weeks, has been studied on two different media: 1) Standard (Ottaviano and Zannini, 1965) and 2) standard with nicotinamide. For each genotype four repetitions on both media have been accomplished. The whole experiment has been completely randomized.

Table 1
Hayman Analysis

Items	S S	D F	M S	F	P
a	362.3354	4	90.5838	7.0161	<0.001 ***
b_1	56.0211	1	56.0211	4.3391	0.01-0.05*
b_2	17.8075	4	4.4519	<1	>0.20
b_3	115.6640	5	23.1328	1.9066	>0.05
b	189.4926	10	18.9493	1.4677	>0.05
c	231.8063	4	57.9516	4.4886	<0.001 ***
d	234.6017	6	39.1003	3.0285	0.01-0.001**
t	1018.2360	24			
N	0.6751	1	0.6751	<1	
Na	11.7900	4	2.9475	<1	
Nb_1	0.0341	1	0.0341	<1	
Nb_2	20.2012	4	5.0503	<1	
Nb_3	45.2350	5	9.0470	<1	
Nb	65.4703	10	6.5470	<1	
Nc	22.5512	4	5.6378	<1	
Nd	21.1386	6	3.5231	<1	
Nt	121.9501	24			
Total	1140.8612	49			
Residual	2065.7280	160	12.9108		

* : P 0.05 ** : P 0.01 *** : P 0.001

The results are as follows:

(1) The factorial analysis (Jinks and Broadhurst, 1963) shows that the variability of the character is genetically controlled. The differences between the genetical contributions of the five parents are significant ($P<0.05$) whether we consider the variance between male or the variance between female array means.

(2) Hayman analysis (table 1) shows that:
   (i) there is significant additive variation (item a);
   (ii) there is significant directional dominance variation (item b_1); since the overall F_1 mean is higher than the parental one, this means that dominance increases the growth rate;
   (iii) there are significant differences between reciprocal crosses (items c and d);
   (iv) there is no evidence of an effect of nicotinamide (items N).

(3) More information has been obtained by analyzing the regressions Wr/Vr and Wr'/Wr (Jinks and Hayman, 1963; Jinks 1954 and Hayman, 1958). This analysis (figure 1) carried out on the experiment on standard medium shows that:
   (i) There is significant complete dominance (regression Wr'/Wr significant, $P<0.05$);
   (ii) There is no evidence of interallelic interaction.

The same analysis carried out on the data from the experiment with the second medium (Standard + nicotinamide) gives a strong indication of interallelic interaction. The comparison of parental means with F_1 means indicates that the amount of heterosis is increased from 8.69 to 14.41. However, a complete repetition of the experiment is needed in order to strengthen these nicotinamide effects.

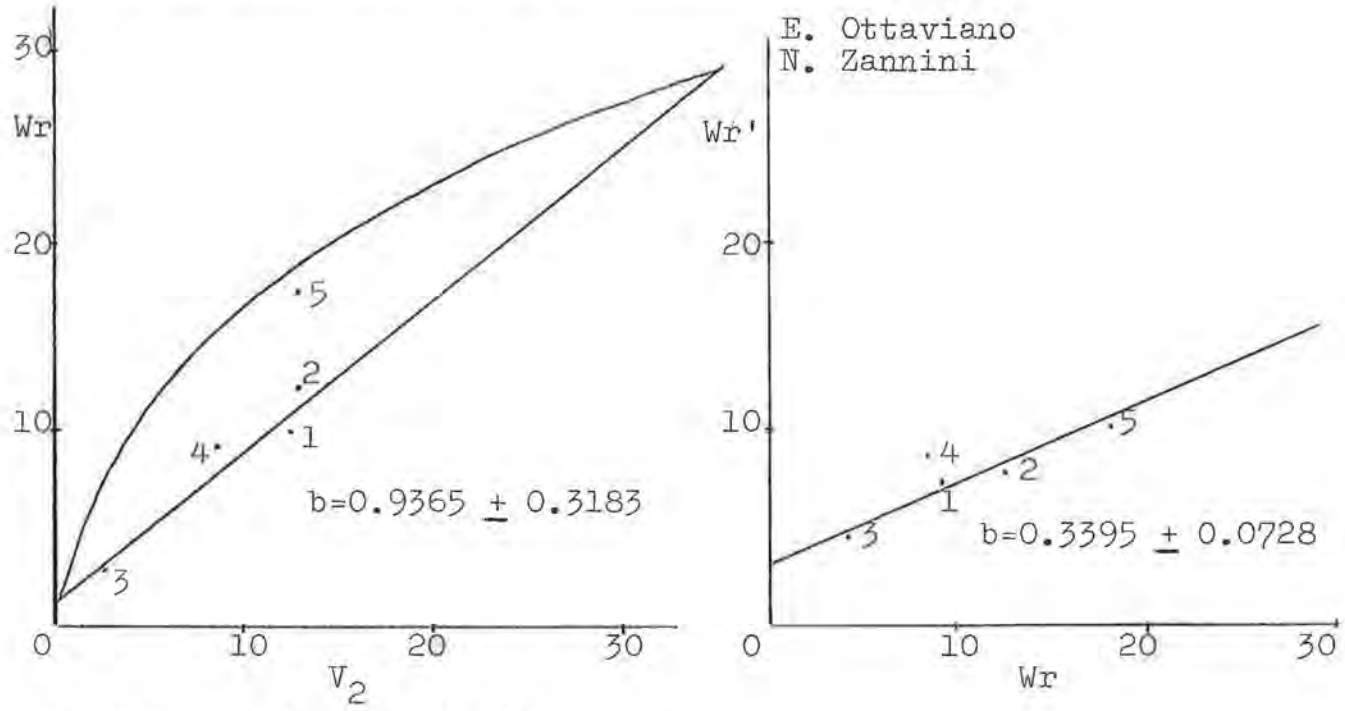

Fig. 1. Wr/Vr and Wr'/Wr graphs

2. A study of transmission of a B^4 derived from a TB-4a stock.

As Roman first showed (1947), TB-A translocations in maize are a useful tool in studying the specificity of different chromosome segments. Bianchi, Bellini and Ottaviano (1961) studied the influence of the TB-4a translocation on the endosperm development. They found that kernels were heavier when the endosperm was hyperploid for the segment B^4. It was suggested that the hyperploid endosperm condition could be fixed by transferring the B^4 into a normal line where it would undergo normal disjunction. Accordingly, four inbred lines and their hybrids were pollinated with the TB-4a stock. Because of B^4 non-disjunction the resulting progeny ears carried three different classes of kernels, in regard to B^4:

(1) hyperploid embryo and hypoploid endosperm
(2) hypoploid embryo and hyperploid endosperm
(3) both embryo and endosperm normal

Kernels of class 1 have then been selected using as criteria of selection both different weight and scutellum color markers. The hyperploid plants obtained were selfed, after collecting part of the tassel from each of them, during the stages of meiosis. The results of the study of the pachytene are summarized as follows:

Probably vital genotypes	Expected ratio		Observed frequencies	Expected frequencies	Observed ratio
$4,4,B^4$	1	1	5	5.3	1
$4,4,B^4,B^4$	1	2	0	10.6	0
$4,4,B^4,B^4,B^4$	1				
$4,4^B,B^4$	1				
$4,4^B,B^4,B^4$	2	5	29	26.5	5.8
$4,4^B,B^4,B^4,B^4$	2				
$4^B,4^B,B^4,B^4$	1	2	16	10.6	3.2
$4^B,4^B,B^4,B^4,B^4$	1				
4,4	0		3	0	0.6
Total		10	53	53	

From the table it appears that genotypes $(4,4,B^4,B^4)$ and $(4,4,B^4,B^4,B^4)$ were not found, while normal genotypes (4,4), which were not expected were found. The finding suggests that the B^4 may be lost before or during both male and

female gametogenesis. Cytological observations of microsporocytes in plants with $(4,4,B^4)$ genotype, show homologous pairing at pachytene, in the region of the short arm of chromosome 4, between the chromosome 4 and the B^4, in the typical way of trivalents, and also non-homologous pairing in the same region. The B^4 may undergo partial or complete autosyndesis. During diakinesis the B^4 is often observed close to a bivalent, presumably the chromosome 4. At metaphase I the univalent B^4 is outside the equatorial plate in about 30% of the cells, while at metaphase II the B^4 shows the same behavior in about 20% of the cells. During anaphase I the univalent B^4 undergoes division in about 30% of the cells, but often at late anaphase or at beginning telophase. Both telophase I and II show micronuclei. These micronuclei at telophase I are presumably the result of lagging of the univalent B^4. Those observed at telophase II are thought to derive from the B^4 that divided at the previous division.

The $(4,4,B^4)$ plants, once selfed, yielded kernels of the following constitution (observations were made on the plants obtained from them):

Genotypes	Expected ratio	Observed frequencies	Expected frequencies	Observed ratio
4,4	1	67	22	1
$4,4,B^4$	2	21	44	0.3
$4,4,B^4,B^4$	1	0	22	0
Total		88	88	

These data indicate that: (1)The $(4,4,B^4,B^4)$ class, expected in ¼ of the progeny, was not found. (2) The (4,4) class largely exceeded the expected ½.

These observations suggest that: (1) Meiosis is an obstacle for the transmission of the B^4 in the normal genotypes examined. (2) Presumably the few pollen grains carrying the B^4 that escaped the meiotic barrier are then selected against, when in competition with normal pollen grains.

A. Ghidoni

3. The "smoky" derivative of R^{st}.

In the 1965 News Letter it was reported that following introduction of Mp into an R^{st} stock, several ears were observed carrying kernels with abnormal spotting patterns among the

standard stippled kernels. One of them has a very fine spotting pattern and has been called "smoky," symbolized $\underline{R}^{st}$ (sky).

Chromatographic comparison of pigment extracts of homozygous $\underline{R}^{st}/\underline{R}^{st}$ and $\underline{R}^{st}(sky)/\underline{R}^{st}(sky)$ seeds does not disclose any qualitative difference between their anthocyanin content. The smoky derivatives are strongly paramutagenic.

When $\underline{R}^{st}(sky)/\underline{r}^{g}$ is crossed with $\underline{r}^{g}/\underline{r}^{g}$, some of the resulting ears show, besides the expected colorless kernels (genotypically $\underline{r}^{g}/\underline{r}^{g}$) two kinds of smoky, darker and lighter, often in equal frequency. While the former breed true in successive generations, the lighter segregate again, when crossed with $\underline{r}^{g}/\underline{r}^{g}$, for darker and lighter smoky, in a ratio of 1:1.

Similar results seem to indicate that the lighter smoky phenotype results from the interaction of $\underline{R}^{st}(sky)$ with a Modifier of the smoky expression that assorts independently of $\underline{R}^{st}(sky)$.

Giuseppe Gavazzi

4. Chromatographic and spectrometric analysis of root and seed pigments.

Pigments are extracted from roots and seeds of a W22 $\underline{A}_1$, $\underline{A}_2$, $\underline{C}_1$, $\underline{C}_2$, $\underline{Pr}$, R stock carrying one of the following $\underline{R}$ combinations: $\underline{R}^{st}/\underline{R}^{r}$, $\underline{r}^{g}/\underline{R}^{r}$, $\underline{r}^{g}/\underline{R}^{r}$.

The extracting solvent used is a 0.1% concentrated hydrochloric acid in 95% ethanol (v/v) solution. The pigment extracts are concentrated under vacuum and then chromatographed with the ascending method on Whatman paper #1. Two solvent systems have been used:
(1) n-butanol, acetic acid, water (4:1:5)
(2) ethyl acetate, t-butanol, acetic acid, water (3:4:1:3).
Both seed and root extracts are separated into three red bands that turn blue when exposed to ammonia vapours. They represent three different anthocyanins. An additional yellow component appears in chromatograms of root extracts.

The absorbance spectra of the four components chromatographically separated are then determined spectrometrically. In Table 1 the Rf values and the absorption peaks (λ max.) of the four components are reported and in Fig. 1 their absorption spectra, after chromatographic separation, are indicated. It appears, from the graphs, that the three anthocyanins chromatographically separated have slightly different peaks of absorbance and are present in quite a different proportion. Their concentration increases from compound 1 up to the third in band three.

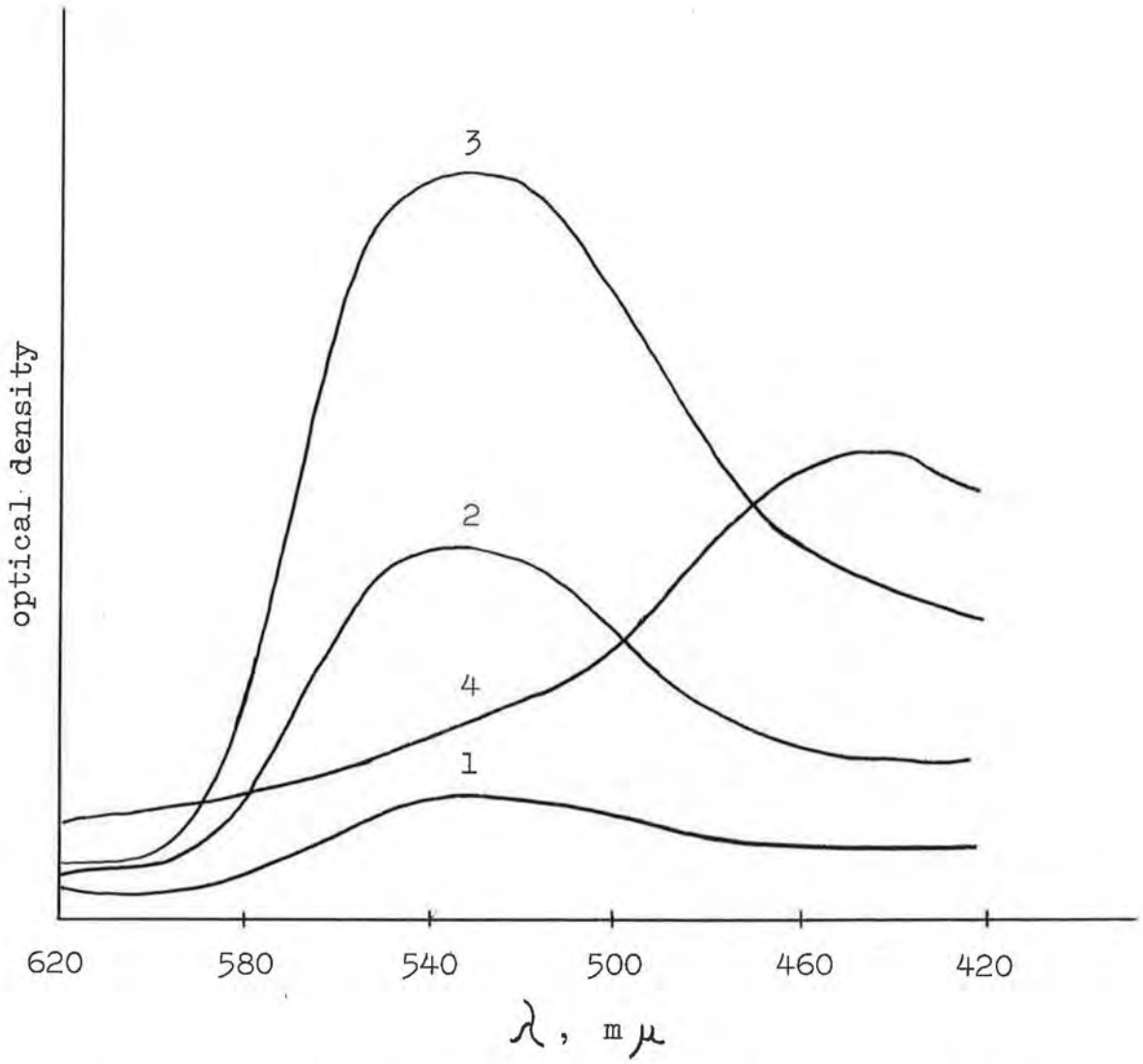

Fig. 1. Absorbance Spectra of the Root Pigments.

Table 1
Chromatographic and spectrometric identification of root pigments.

Compound	RF (1)	RF (2)	λ max (mμ)
1	0.28	0.18	535
2	0.36	0.25	533
3	0.45	0.37	530
4	0.71	0.58	448

The anthocyanins of seeds carrying other R alleles, have been chromatographed with the same procedure and they are all separated into three bands with the same Rf values reported in Table 1 for the first 3 compounds.

Compounds 2 and 3 have been tentatively classified, according to their Rf and λ max. as cyanidin-3-monoglucoside and pelargonidin-3-monoglucoside. Compound 1, present in much lower proportion, is still unknown.

The identity of pigment constitution of paramutable R and R' seeds seems to suggest that the phenotypic difference between the two rests only upon a difference in level of production of anthocyanins without a concomitant alteration in their single constituents.

Giusseppe Gavazzi

5. A test for the association of paramutation with roots.

Plant and seed pigments are controlled by the two subunits of the R gene, respectively symbolized P and S. If paramutation is not confined to the S component of the R locus but affects the R locus as a whole, it should also be possible to observe its expression in sporophytic tissues.

We compared the anthocyanin content of R^{st}/R^{r} with that of r^{g}/R^{r} roots with the intent of disclosing the existence of paramutation in sporophytic tissues. The former carry a paramutagenic allele (R^{st}) and a paramutable R, i.e. an R allele sensitive to the paramutagenic action of R^{st}; while the latter carry the same R^{r} allele in a heterozygote with r^{g}, i.e. an allele incapable of inducing paramutation. These roots derive from seeds obtained by parallel crosses of R^{st}/R^{st} and r^{g}/r^{g} plants with the same pollen parent R^{r}/R^{r}.

If paramutation takes place in roots, we expect to observe a decrease of pigment in R^{st}/R^{r} roots when compared to that of r^{g}/R^{r} roots. The determination of anthocyanin

Table 2
Comparison of mean anthocyanin content of:
I. $\underline{R}^{st}\ \underline{R}^{r}$ and $\underline{r}^{g}\ \underline{R}^{r}$ roots II. $\underline{r}^{g}\ \underline{R}^{r}$ and $\underline{r}^{g}\ \underline{R}^{r'}$ roots

Genotype selected	Pedigree	No. roots tested	Mean Score*	t value	P
	I. Comparison of pigment content of $\underline{R}^{st}\ \underline{R}^{r}$ roots with that of $\underline{r}^{g}\ \underline{R}^{r}$ roots				
	A - 7 days old roots				
$r^{g}R^{r}$	g 818 x g 830-3,-4,-6	100	0.28		
$R^{st}\ R^{r}$	g 780 x g 830-3,-4,-6	100	0.27	0.19	0.05
	B - 12 days old roots				
$r^{g}\ R^{r}$	g 818 x g 830-4	25	0.21		
$R^{st}\ R^{r}$	g 780 x g 830-4	25	0.24	1.36	0.05
	C - 9 days old roots (pieces)				
$r^{g}\ R^{r}$	g 818 x g 830-3,-5	60	0.39		
$R^{st}\ R^{r}$	g 780 x g 830-3,-5	60	0.41	0.35	0.05
	II. Comparison of pigment content of $\underline{r}^{g}\ \underline{R}^{r}$ roots with that of $\underline{r}^{g}\ \underline{R}^{r'}$ roots				
	D - 9 days old roots (pieces)				
$r^{g}\ R^{r}$	g 944 x g 940 - a	35	0.24	0.29	0.05
$r^{g}\ R^{r'}$	g 944 x g 940 - b	40	0.28		

*expressed as mean O.D. at 530 m

content is based on the spectrometric reading of the optical density of the pigment extracts of the roots.

The data of Table 2 (Part I) indicate that R^{st}/R^{r} and r^{g}/R^{r} roots do not differ significantly in their pigmenting potential level. The failure to observe a decrease in pigmenting level in $R^{st}R^{r}$ roots could be due to the insufficient time, in terms of cell generations, given to the roots before testing the paramutagenic effect of R^{st} upon R^{r}. It could be that at least one generation of R^{st}/R^{r} heterozygosity is required before paramutation becomes phenotypically manifest. Accordingly, the comparison of pigment concentration has been extended to r^{g} $R^{r'}$ and $r^{g}R^{r}$ control roots (Table 2, Part II). However, also in this case, when the pigment potential of $R^{r'}$ r^{g} roots is compared to that of $R^{r}r^{g}$ roots, no decrease in the level of anthocyanin is observed in the former.

The lack of reduction in pigment concentration of $R^{r'}$ roots suggests that the R component, controlling pigment formation in roots, is either insensitive or less sensitive than the S component to the paramutagenic action of R^{st}. The differential sensitivity of the two R sub-units to the R^{st} action is here considered as an indication that the R locus as a whole is not involved in paramutation.

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1. Location of Ga_8 in chromosome 9 linkage group.

Preliminary data for a close linkage relationship between a gametophyte factor and the waxy locus have been presented both by Schwartz and by Bianchi, in previous issues of MNL. However, the question whether the Ga factor was between the wx locus and the centromere or placed distally to the wx locus remained unanswered.

Some data from backcrossing plants heterozygous for Ga_8, as well as for wx and bz, on a multiple tester for chromosome 9 are as follows:

Ear No. 65-174	Kernel types Bz	bz	Wx	wx	Total No. of kernels	% of bz	% of Wx	Cumulative %
/169-4	-	-	13	56	69	-	18.8	-
/169-18	244	20	51	213	264	7.5	19.3	26.8
/170-22	-	-	35	219	254	-	15.9	-
/170-22	297	59	80	276	356	16.6	22.5	39.1
/171-6	166	36	26	176	202	17.8	12.9	30.7
/171-44	164	8	25	147	172	4.6	14.5	19.1
/171-52	298	9	55	252	307	2.9	17.9	20.8
/172-1	339	28	79	288	367	9.1	21.5	30.6
/172-20	294	33	64	263	327	10.1	19.6	29.7
/172-40	205	28	52	181	233	12.0	22.3	34.3
Total	2007	221	480	2071	2228 2551	9.9	18.8	28.7 (standard about 25)

Such data suggest that $\underline{Ga}_8$ is placed closer to bz than to wx and that it is located between the two markers.

The $\underline{Ga}_8$ factor of the pollinator parent used in these backcrosses is the allele present in the stock originally obtained from Dr. Schwartz. In such a strain (Ga wx/ga Wx) the Ga - wx distance calculated on the basis of the frequency (17.4%) of the class segregating 25% of waxy kernels is approximately 22.8%.

These data, as well as others of a different nature, suggest that the Schwartz factor is different from that reported by Bianchi, which, on the basis of previous results, appeared identical or allelic to the former one.

A. Bianchi
M. R. Parlavecchio

2. Linkage relationships for some mutants.

For some of the recently detected mutants in Italian cultivars linkage relationships with well-known markers have turned up.

A shrunken type, known to be "uncovered" by TB-4, should be placed close to $\underline{su_1}$, although the unreliability of the classification of the double recessive makes it difficult to measure exactly the intensity of the linkage (which, however, should be close). Data involving $\underline{gl_3}$ and $\underline{gl_4}$ are presented in the following table (all data derived from F_2 from repulsion phase):

Gene pair	A B	Ab	aB	ab	Recombination % ± st. error	
$\underline{gl_3}$-shrunken type	2445	690	1041	82	32.5 ± 0.9	(1)
$\underline{gl_4}$-shrunken type	2248	818	940	26	18.7 ± 1.0	(2)

(1) The $\underline{gl_3}$-$\underline{su_1}$ distance, from data based on these ears, is 35.7 ± 0.6.

(2) The $\underline{gl_4}$-$\underline{su_1}$ distance, from data based on these ears, is 17.8 ± .4.

These values together with those reported in the table suggest close linkage between $\underline{su_1}$ and the shrunken factor.

Four mutants exhibiting a japonica phenotype recognizable in the seedling stage (in the background used) yielded the following linkage relationships (F_2 data; repulsion phase).

Gene pair	A B	A b	a B	a b	Recombination % ± st. error	
$\underline{su_1}$- jap. type	1122	401	309	46	37.8 ± 1.3	(1)
$\underline{gl_3}$- " "	190	83	77	1	12.0 ± 3.5	(1)
$\underline{y}$ - " "	10845	5532	5466	0	very low	(2)
$\underline{su_2}$- " "	8675	3626	2928	291	30.5 ± 0.5	(2)
$\underline{y}$ - " "	683	280	329	18	8.1 ± 1.8	
$\underline{gl_1}$- " "	254	143	114	0	low	

(1) The $\underline{su_1}$ - $\underline{gl_3}$ distance in these experiments turned out to be 41.0 ± 2.4.

(2) The $\underline{y}$ - $\underline{su_2}$ distance in these experiments turned out to be 30.0 ± 0.3.

Other chlorophyll mutants show indication of linkage with genetic markers, as follows (F_2 data; repulsion phase).

Gene pair	A B	A b	a B	ab	Recombination % ± st. error	
su$_1$ - virescent-type	909	366	195	55	43.5 ± 1.4	(1)
gl$_3$ - " "	264	109	94	0	low	(1)
gl$_1$ - *yg*-type	731	425	282	109	44.2 ± 1.4	
gl$_1$ - *f*-type	542	161	191	40	45.1 ± 1.7	(2)
o$_2$ - " "	169	57	63	4	29.1 ± 3.6	(2)
wx - *v*-type	584	336	248	5	13.0 ± 1.9	

(1) the *su*$_1$ - *gl*$_3$ distance in these experiments turned out to be 42.0 ± 2.1.

(2) The *o*$_2$ - *gl*$_1$ distance in these experiments turned out to be 14.2 ± 1.5.

C. Lorenzoni
M. Pozzi

3. Balanced lethal systems and physiological responses.

Two balanced lethal systems, based on defective caryopsis traits, have been recently described in maize - teosinte derivatives:

$\frac{De^{t1}\ de^{t2}}{de^{t1}\ De^{t2}}$ and $\frac{De^{t13}\ de^{t25}}{de^{t13}\ De^{t25}}$. The crossover percentage between the factors in both cases is about 14 so as to permit recovering of the normal genotype (ear segregating no defective) in about one case out of one hundred.

The double heterozygous plants have been compared with the normal ones for the field and laboratory traits, as follows:

Character	First system		Second system	
	double heterozygous	normal genotype	double heterozygous	normal genotype
Pollen shedding time (in days, from July 8th)	-	-	9.38 ± 1.49	7.53 ± .95
	19.40 ± 0.41	18.63 ± 0.39	17.29 ± 0.78	15.24 ± .85
Kernel No./ear	121.1 ± 9.3	151.8 ± 6.5	-	-
Kernel weight in g (defectives excluded)	.215 ± 0.33	.204 ± 0.43	-	-
Root growth in mm(1) after days 5	23.0 ± 0.7	24.2 ± 0.6	-	-
10	30.6 ± 0.9	32.5 ± 0.9	-	-
15	35.0 ± 1.3	36.3 ± 1.3	-	-
20	36.0 ± 1.7	38.5 ± 1.8	-	-

(1) Root-types of about 7 mm were cut and grown on artificial medium.

These preliminary results suggest a general delay in the growth rate of the double heterozygotes which produced ears with a lower number of kernels. Their larger size is obviously related to the presence of about 50% of defective caryopses.

C. Lorenzoni
M. Pozzi

MACDONALD COLLEGE OF McGILL UNIVERSITY
Province of Quebec, Canada

1. A test for Spm control of mosaic pericarp.

Mosaic (P^{mm}), one of the unstable alleles at the first chromosome pericarp locus, does not activate Ds and Barclay and Brink (PNAS 40:1118-1126, 1965) have inferred that the instability is not controlled by Mp or Ac. The mosaic pattern appears in a wider variety of patterns than variegated pericarp and it frequently changes from one unstable state to another. The instability controlled by Spm-En at other loci is so similar in many respects to mosaic, that it seems reasonable to suspect that an Spm-like element might regulate the P^{mm} allele.

To test this hypothesis an a_1^{m-1} P^{ww} stock lacking Spm but in which gene action is under the control of the Spm system was crossed as a male with six different geographical collections of P^{mm}. The mosaic lines were fourth generation backcrosses to inbred A171 (P^{ww}), and so the mosaic ears were homozygous A_1A_1 and heterozygous P^{mm}/P^{ww}. The F_1's which were A_1/a_1^{m-1} and either P^{mm}/P^{ww} or P^{ww}/P^{ww} were backcrossed to a_1^{m-1} P^{ww}. It was expected that ½ the ears would be mosaic and ½ colorless pericarp and on each ear ½ the kernels would be a_1^{m-1}.

The a_1^{m-1} kernels without Spm show pale aleurone pigmentation over all and with Spm they have deep spots on a colorless background. The presence of spotted kernels on the backcross ears would indicate response of a_1^{m-1} to Spm-like control. The ears were accordingly scored for pericarp color and the presence or absence of spotted aleurone. The results are shown in Table 1.

The data are confusing at best. One family, 2547, consisted of three ears with mosaic pericarp and approximately ½ spotted kernels, but also three mosaic ears without spots and one apparently P^{ww} ear with many spotted kernels. A sister family, 2548, with the same P^{mm} allele contained no spotted kernels. Ten other families segregating mosaic ears essentially did not show spotted kernels. Four of these ten families, however, each contained a single deeply spotted kernel. These single kernels could be contaminants from an Spm-carrying stock, but I am inclined to doubt it for (1) my usual pollination technique does not show this level of contamination, and (2) I have only a few known Spm-carrying stocks and these were widely separated from the mosaic stocks, which themselves were distributed over a considerable area interspersed with other corn.

The test does not give clear evidence that Spm controls gene action at the P^{mm} allele, neither does it rule out this hypothesis completely. Several explanations for these

Table 1
Tests of different $\underline{P}^{mm}$ alleles to promote gene action at the a_1^{m-1} locus.

Family	Source of alleles	Pericarp and aleurone phenotype of backcross ears: Mosaic P. spotted	Mosaic P. no spots	Colorless P. spotted	Colorless P. no spots
2547	Peru - from S. C. Harlan	3	3	1	0
2548	ditto	0	3	0	3
2549	Rainbow Flint - local strain	0	3*	0	3
2450	ditto	0	5	0	2
2558	P.I. 213797 - North Dakota	0	4*	0	2
2559	ditto	0	2	0	3*
2561	P.I. 214200 - Manitoba	0	2*	0	3
2562	ditto	0	1	0	5
2564	Assiniboine Flint - Manitoba	0	2	0	3
2565	ditto	0	1	0	4
2587	Medium mosaic - R.A. Brink	0	3	0	3

*a single kernel heavily spotted on one ear.

results are possible:
(1) An Spm-like element could be present in some plants of inbred A171 which I use as a recurrent parent throughout my genetic stocks. This is quite likely since I reported in 1964 that another breeding line carried an Spm-En like element. The four isolated spotted kernels, then, could more probably be contaminants. Family 2547 which seems to show independence between mosaic and spotted kernels would be explained.
(2) Spm-En occur in many states. (a) Mosaic pericarp might contain a state which does not regulate a_1^{m-1} ordinarily, but which may change into a regulating state as in family 2547. It might be expected that such a change would also be correlated with a change in pericarp phenotype. However, no difference in pericarp phenotype could be detected in ears with and without spots. (b) Inbred A171 could contain a non-activating state of Spm which changes to an activating state occasionally.
(3) All spotted kernels could have resulted from Spm contamination either this year or in a previous year.

One last comment - Some states of mosaic pericarp are difficult to distinguish from variegated pericarp. Family 2547 is one of these and it is possible that this family is really P^{vv}. As far as I know, no one has ever determined if variegated regulates a_1^{m-1} gene action. Or perhaps Family 2547 contains neither P^{vv} nor P^{mm} but another unstable allele which is controlled by an Spm-like element while the controlling element for mosaic pericarp remains unknown.

R. I. Brawn

2. A test for Spm in Diffuse pericarp.

Greenblatt has reported (M.G.C.N.L. 39:120. 1965) that the Diffuse pericarp gene Idf does not substitute for either Spm or Ac. I wish to present data which suggest that Idf may substitute for Spm.

A different tester stock was used in my studies than was used by Greenblatt. His test required the detection of dark purple spots on a dilute purple background if Idf caused instability in C_2/c_2^{mt} heterozygotes. This may be possible if Idf inhibits only the background pigment, for Greenblatt has shown that Idf does inhibit aleurone pigmentation somewhat. However, I find that C_2/c_2^{mt} Spm kernels are uniformly purple and so perhaps his test was not adequate to detect instability of c_2^{mt}.

My test involved the same a_1^{m-1} P^{ww} no Spm stock and crossing scheme described in Note No. 1. The Diffuse stock was also a fourth generation backcross to inbred A171 (P^{ww}) and so the Diffuse ears were A_1/A_1 and heterozygous P^{rr}/P^{ww} and Idf/idf. It was expected that ¼ the ears from the backcross of the F_1's to the a_1^{m-1} P^{ww} tester stock would be Diffuse, ¼ red and ½ colorless pericarp, and on each of

these ears ½ the kernels would be $\underline{a}_1^{m-1}$ and liable to spotting. The results obtained with three families are shown in Table 2.

This test is far from definitive. The seven Diffuse ears which show strong $\underline{a}_1^{m-1}$ spotting and the nine colorless ears which are spotted could constitute the ½ of the backcross populations expected to carry Idf. On the other hand, if Idf does substitute for Spm, the one Diffuse ear with no spots and the four red ears with $\underline{a}_1^{m-1}$ spots would not be expected. The several explanations advanced in Note No. 1 are also applicable here to explain these exceptional ears. In the case of Diffuse pericarp, however, it seems more probable that Idf is substituting for Spm than in the case of $\underline{P}^{mm}$ described previously.

Table 2
A test of the Diffuse pericarp gene (Idf) to promote gene action at the $\underline{a}_1^{m-1}$ locus.

Family number	Pericarp and aleurone phenotypes of backcross ears: Diffuse P. spotted	Diffuse P. no spots	Red P. spotted	Red P. no spots	Colorless P. spotted	Colorless P. no spots
2635	3	1	0	1	1	6*
2636	2	0	2	2	2	3
2637	2	0	2	2	6	1
Total	7	1	4	5	9	10 = 36

*all ears show a few kernels with a few spots.

R. I. Brawn

3. Isoalleles of $\underline{P}^{WR}$.

The cob color of the Iowa inbred B14 is noticeably darker red than most other red-eared inbreds. This difference is most likely due to modifiers of the $\underline{P}^{WR}$ allele and not to an isoallele of $\underline{P}^{WR}$.

Inbred B14 with dark red cob color and inbred W-9 with a much lighter red cob color were crossed and carried to F_2. It was not possible to detect separate classes of red; the F_2 ranged continuously from dark to light red.

The $\underline{P}^{WR}$ alleles from both B14 and W9 have been introduced into the white-cobbed inbred A171 ($\underline{P}^{WW}$) by backcrossing. By the fourth backcross no difference in cob color could be detected between the two A171 sublines with different $\underline{P}^{WR}$ alleles.

Other P^{WR} alleles have also been introduced into inbred A171 (P^{WW}) by backcrossing and they all seem to produce the same red cob color following a number of backcrosses suggesting that modified genes and not differences at the P^{wr} locus account for the different shades of red cob color.

R. I. Brawn

MAIZE RESEARCH STATION
Yousafwala (Montgomery), West Pakistan

1. Sorghoid maize.

A research project has recently been initiated at the Maize Breeding Station Yousafwala (Montgomery) to develop varieties resistant to the Asiatic Maize Borer (Chilo zonellus Swinhoe). In the quest for genetic resistance against this devastating pest of maize, a large number of open pollinated varieties were obtained from different maize growing countries of the world and planted in the borer nursery during the year 1964. Part of the seed was also grown in another field under artificial pest control where the germplasm was maintained by composite pollination.

One of the varieties received from Italy under the name Zeppetello had plants with rather condensed tassels and small sized ears with hard flinty grains. These plants were composite pollinated as usual. No detailed observations regarding the plant or ear characters were recorded. In the following year, however, this variety was grown under close observation from the seed obtained through composite pollination in the previous years. Planting was done in the 3rd week of August, 1965. Germination and growth of the plants was normal. Observations regarding different plant characters were recorded and are summarized below:

Plant:
Plants short, average height 123.6 cm, early maturing (40.6 days to mid silking); average number of leaves, 10.2; leaf size, medium to small (average length and breadth, 40.6 and 4.8 cm respectively).

Tassels:
Peduncle medium in length, extending 10-15 cms above the flag; central rachis short; branching profuse and condensed. Apparently the tassel resembles a sorghum head; female flowers frequently present in the tassel but seldom set grain.

Ears:
(i) External character: Ears short 5 to 8 cm long, 4-6 cm in diameter, conical in shape, borne on short 3-5 cm long shank; average diameter of the shank 1.26 cm; ear enclosed in 8-10 husks that extend 6-10 cm beyond the tip of the ear; ears in general appearance resemble small compact heads of sorghum with similar type of branching. Some of the branches end in a spike of male flowers.

(ii) Internal characters: Ears in most cases devoid of cobs (pith), instead there is central rachis with primary, secondary and tertiary branches; branches short and stout, female spikelets borne on primary, secondary, and tertiary branches; rachilla of the female spikelets short; glumes hard and indurated, usually bearing two grains like double seeded sorghums; lemma and palea thin and papery.

Grain:
Grain small, roundish in shape, 7.1 mm in width and 7.4 mm in length, hard flinty type, deep yellow in colour.

Most of the tassels shed normal pollen. The pollen when used on silks of other maize varieties proved to be quite effective. No grain formation was obtained in self pollinated ears. Partial to normal setting was observed in the ears pollinated by composite pollen from the plants within the variety. Ears of self pollinated plants that fail to form grain present a clearer picture of the internal structure of the ear. The unusual feature of the ear is a slender sorghum-like rachis with primary, secondary, and tertiary branches bearing female spikelets.

Studies to ascertain the genetic basis of sorghoid characters in this type of maize are being undertaken both at the maize research station, Yousafwala (Montgomery) as well as in the department of Plant Breeding and Genetics in the West Pakistan Agricultural University at Lyallpur.

A. Ghafoor Bhatti

NOTE: Photographs of plants and ears of sorghoid maize were included with this report, but could not be reproduced here. They will be preserved in the News Letter files and are available for inspection.

MARQUETTE UNIVERSITY
Biology Department
Milwaukee, Wis.

1. The null-expression of the wx gene in a monoploid sporophyte test.

It is well known that wx wx wx constitution in the endosperm and the wx constitution of the pollen grain result in a

starch change from the Wx condition so that the starch grains of the waxy type stain red with iodine instead of purple. It is equally well known that starch grains in sporophyte tissue of the constitution wx wx are found to stain purple.

One possible testable explanation for such behavior of the waxy condition is that the expression of the wx gene is dependent upon dosage of the gene in the tissue involved. While this insight seems incorrect, (pollen of tetraploid wx wx wx wx plants stain red) it was felt important enough nevertheless, to test for expression of the wx gene in roots of monoploid plants.

In order to obtain the required monoploid wx plants the following mating was made: W23 A A, c c, r r, wx wx, X W23 A A, C C, R^{nj} R^{nj}, Wx Wx. By utilizing the R-navajo allele embryos that do not exhibit the dominant conditioned R^{nj} pigment, but whose aleurones do have pigment, can be easily selected in dried seed form. Such colorless scutellum, colored aleurone kernels are then the presumptive monoploids. The genetic constitution of the monoploid would then be A c r wx.

In order to confirm the genome constitution and test for starch type, these presumptive monoploids were germinated and seedling roots obtained. Each root cap was tested with iodine while the meristematic region was used for chromosome counts.

Three different seedling roots were found to have counts of ten chromosomes in late mitotic prophase cells. Iodine tests of starch grains from these three roots all showed a uniform dark purple staining indistinguishable from Wx Wx controls.

As with diploid pollen, monoploid roots do not alter the expression of the wx gene known in usual ploidy constitutions.

Irwin M. Greenblatt

NOTE: I would like to express sincere thanks to the staff of the Department of Agronomy, University of Illinois, for making field space and help available to the projects under study in this laboratory.

The address of this laboratory effective September 1, 1966 will be Biology Department, Northwestern University, Evanston, Illinois.

UNIVERSITY OF MARYLAND
College Park, Maryland

1. Interchromosomal effects of deficiencies in chromosome 1 on association.

Homozygotes for $\underline{zb_4}$ $\underline{P^{ww}}$ $\underline{As}$ $\underline{br_1}$ in chromosome 1 were crossed with X-rayed pollen carrying $\underline{Zb_4}$ $\underline{P^{wr}}$ $\underline{As}$ $\underline{Br_1}$. Forty-four plants hemizygous for one or more of the three recessive genes were amenable to analysis at diakinesis or metaphase I. Fifteen of the deficient plants, including three monosomics showing loss of all dominant morphological markers, were variably asynaptic. Syncytes, curved spindles, and fragmentation--characteristic of asynaptic homozygotes--occurred in the deficient plants exhibiting failure of association. It seems likely that As was deleted along with linked dominant markers in the X_1 asynaptic plants. The single dose of As, contributed by the female parent, was insufficient to control normal first division association, thereby simulating the homozygous recessive.

R. L. Baker
(Dept. of Horticulture)

D. T. Morgan, Jr.
(Dept. of Botany)

UNIVERSITY OF MASSACHUSETTS
Department of Environmental Sciences
Waltham Field Station
Waltham, Massachusetts

and

HARVARD UNIVERSITY
Cambridge, Massachusetts

1. Genetic correspondence of Tripsacum chromosomes to their homeologs from corn.

Further progress has been made during the year in identifying Tripsacum chromosomes both genetically and cytologically. When a Tripsacum chromosome in a 2n+1 stock substitutes in physiological function for a dominant gene in corn by covering its recessive marker allele present in the maize chromosome complement, we can locate a Tripsacum gene on a particular Tripsacum chromosome. Thus we can map the Tripsacum chromosomes, not by their own recessive genes, but by the ability of their dominant genes to prevent the expression

of recessive marker genes, either singly or in linked series, from corn. The data so far obtained, presented below, show that one Tripsacum chromosome carries dominant genes preventing the expression of three recessives on the short arm of chromosome 2 while another Tripsacum chromosome corresponds to the other arm. A similar situation exists with respect to chromosome 4. A single Tripsacum chromosome carries dominant genes which mask four recessive genes on chromosome 7 and another Tripsacum chromosome masks five recessive genes on the short arm of chromosome 9.

Corn chromosome	Dominant from Tripsacum
1	Bm_2 *
2S	Ws Lg_1 Gl_2 (does not cover v_4)
2L	V_4 * (does not cover lg_1 gl_2)
3	A_1 *
4S	Su_1 (does not cover gl_3)
4L	Gl_3 (does not cover su_1)
7	V_5 Ra_1 Gl_1 I_j
8	J_1 *
9	Yg C Sh_1 Bz Wx

*Allo-trisomic stocks which were lost but are being redeveloped. The data for a more complete genetic map of Tripsacum will be forthcoming as crosses and backcrosses to multiple marker gene stocks of corn are made.

W. C. Galinat
P. C. Mangelsdorf

2. A planting in Florida of perennial relatives of maize.

Arrangements have been made during the year to establish and maintain at the Montgomery Foundation of the Fairchild Tropical Garden, Miami, Florida, a collection of the perennial relatives of maize. Representative specimens of the collections of Tripsacum from Mexico and Guatemala made by Dr. Raju Chaganti and Mr. Garrison Wilkes (mentioned in last year's News Letter) were delivered to Florida and are now well established in a planting protected from frost by a sprinkler system. The planting includes all of the known species of Tripsacum, some interspecific hybrids in Tripsacum, perennial teosinte, three species of Manisuris, and Elyonurus tripacoides. In February all species except T. australe were in flower. The National Science Foundation has made a grant to the Fairchild Garden to

maintain the collection for a period of five years. Maize geneticists wishing to use the collection or to add to it should get in touch with Dr. John Popenoe, Director, Fairchild Tropical Garden, Miami, Florida.

W. C. Galinat
P. C. Mangelsdorf

3. Simple dominance of a day-neutral-like condition in an F_2 generation of a corn-teosinte hybrid.

The distribution of anthesis dates in the F_2 of a cross between Gaspé Flint and Amecameca teosinte is bimodal with a large peak in the middle of July and a small peak in the middle of August. An organization of the data on the basis of these two months separates the two peaks and reveals an almost perfect 3:1 ratio, as follows:

Anthesis Date	Frequency	Anthesis Date	Frequency
July 6	2	August 2	1
8	4	4	1
10	9	6	2
12	17	8	3
14	5	10	5
16	6	12	2
18	3	14	2
20	2	16	0
22	2	18	1
24	3	20	1
26	3	22	1
28	2	24	1
30	3	26	0
TOTALS	61		20

The strong effect of Gaspé Flint germplasm in producing a day-neutral-like condition for flowering of just the main stalk, even in the presence of id id has been found by Brawn (MNL, 1963). Likewise in our segregation from Gaspé Flint x teosinte, many of the plants which flowered early in July on the main stem, continued to grow tall tillers which flowered about a month later, in August. The anthesis dates reported are only for the main stems.

Much of the material from this segregation has promise for the early synthesis of a 'day-neutral' type of teosinte of possible agronomic, genetic and evolutionary importance.

W. C. Galinat

4. Tassel-in-the-seed from Gaspé Flint?

The earliest flowering plants in the F_2 and F_3 of the cross Gaspé Flint by Amecameca teosinte have between five and seven leaves, the embryonic leaf number of corn as reported by Sass and others. The main stalk of these plants is in full bloom at the 'thinning stage' when only a foot high and about three weeks old. Then, as also reported by Brawn for his id id Gaspé Flint plants, the basal tillers begin to elongate and ultimately reach a height of about ten feet when they flower during short days six weeks later on. As suggested by Brawn for his id material, the primordial tassel was probably already induced if not differentiated in the seed at planting time, having been laid down during the short days of the previous Fall. This 'Gaspé trait' is dominant, simply inherited and may have economic value in the breeding of teosinte for the North. Such a Gaspé teosinte would produce two crops of seed, a Spring crop and a Fall crop. It is possible that the 'Spring crop' seed formed during long days would not have 'tassel-in-the-seed'. During the long days of Summer, it would tiller profusely or, if perennial, develop rhizomes.

Longitudinal sections will be cut from the embryos from the Gaspé Flint-teosinte lines to study the growing point.

W. C. Galinat

5. The corn grass and teopod loci involve phase change.

The switching from one phase of growth to the next is usually rather abrupt in typical corn as it is with other plants. That is the organs of the phytomer (internode, leaf, axillary bud and prophyll) attain a distinct form which is characteristic for the juvenile, mature vegetative, pistillate-floral and staminate-floral modes of their expression. The time of phase change is usually programmed for a certain point in development although in certain genotypes the external environment may trigger the switching as, for example, in short day corn.

In contrast to normal, phase change in corn grass, and to a lesser extent in teopod, is a gradual process. The narrow bloomy leaves of the juvenile phase integrate into the broad pubescent leaves of the adult vegetative phase followed by a gradual transition into the two forms of floral development. Although variability is common, the tendency is for an intergradation between solitary vegetative branches at the base of the plant to paired spikelets near the apex of the plant. The various intergrading forms as evidence of homologies between vegetative and floral phytomers have been described elsewhere (Galinat, 1959, Bot. Mus. Leafl., Harvard U.).

A recognition that the corn grass and teopod loci are involved in phase change may lead to a better understanding of the genetic control of differentiation in corn.

W. C. Galinat

6. Somatic mosaicism in corn grass.

Several features of corn grass (Cg gene) seemed at first to be evidence that phase change in this mutant at least was primarily 'physiological' and, therefore, to cast doubt on the suggestion of Brink that phase change involved a somatic paramutational process. Not only is the phase change process gradual in corn grass, as if following some physiological gradient, but the length of shank on which an ear is borne is usually related to how 'vegetative' the ear becomes. Ears with short shanks are usually more vegetative as if they were precociously thrust into ear formation before the vegetative phase had run its course.

However, somewhat to our surprise, the first experiment designed to detect the possibility of mutational phase change in corn grass seemed to reveal it. The first and second ears of a line of corn grass apparently homozygous for the Cg gene were pollinated by a normal inbred, A 158. The hybrid progenies from the two ears were grown the following year and there were differences reflecting the differences in the two parental Cg ears from one plant. The classifications were made on a basis of five types of terminal inflorescences. Type one was completely proliferated with no functional spikelets. Type three had a single spike male region subtended by leaves and one or more sub-tassel ears. Type five was normal, at least in regard to the tassel.

Although the data are still limited, the differences in tassel types between the progenies of vegetative and normal ears from a single plant are consistent (Table 1) and are significant (P = $<.01$).

Table 1
Frequency distributions for hybrid progeny from two types of corn grass ears borne on a single plant.

	Tassel Types					
Parental Type	1	2	3	4	5	Totals
Vegetative ear	6	16	16	9	7	54
Normal ear	0	4	15	17	22	58

W. C. Galinat

UNIVERSITY OF MINNESOTA
St. Paul, Minn.

1. Chromosome pairing studies.*

The following series of interchanges are being used in these studies: T1-5, T2-6, T4-6, and a few T1-6 and T5-9. Almost without exception homologous ends are closely associated at pachyteme in all intercrosses between stocks of interchanges that involve the same chromosomes. Intercrosses in all possible combinations between the members of each series have been made to test the applicability to corn of the intercross method as applied in barley (Kasha and Burnham, Canad. Jour. Genetics and Cytology 7(4):620-632).

John Stout
Joseph Neubauer
Ronald L. Phillips
Gary Stringam
C. R. Burnham

*supported by N.S.F.
Grant G B 1586.

2. Additional notes on the T2-6 interchanges.

T2-6 (027-4) has the break in 6 in the nucleolus organizer. In T2-6e the break in 6 is in the short arm between the centromere and the organizer. The break in 2 is also in the short arm.

Cultures of the interchange listed as T2-6 (014-11) show a chain of 6 chromosomes associated with the nucleolus. We have been unable to isolate a stock with an association of only 4 chromosomes.

Ronald L. Phillips
John T. Stout

3. Notes on the T1-5 interchanges.

In the following stocks, one chromosome is probably incorrect: 1-5 (8972), 1-5 (8347), 1-5 (018-5), 1-5 (024-5, 1-5 (4331), 1-5 (6178), and 1-5 (48-34-2). The breaks in chromosome 5 in 1-5a and 1-5 (6899), based on genetic data, are in the long arm rather than the short arm.

John Stout

4. Notes on a few of the 4-6 interchanges.

Based on cytological examination in homozygous lines, the following have the break in 6 in the short arm rather than

in the long arm: 4-6 (8591), 4-6 (025-12), and 4-6 (011-16). The following have the break in 6 in the long arm as listed: 4-6 (8428), 4-6 (8927), and 4-6b.

Ronald L. Phillips
R. Bammi

5. Non-homologous pairing in double trisomics in maize.

Double trisomics of many different combinations have been observed to show very close pairing of non-homologous univalents in pachytene. In every case the ends have been paired and one or more foldbacks is present. In no case has there been pairing of the centromeres. The configurations indicate that pairing is initiated at both ends and proceeds toward the middle of the chromosomes.

The non-homologous pairing continues into diakinesis and metaphase. The frequency of paired non-homologous univalents has been determined at diakinesis. Table 1 gives the combined frequency of the different possible configurations at diakinesis for the different double trisomics thus far observed.

Table 1
Frequency of figures at diakinesis of double trisomics.

	8II + 2III	9II + 1III + 1I	10II + 2I	11II	Total
Number	867	444	140	63	1514
Per cent	57.3	29.3	9.2	4.2	100

Kenneth Michel

6. Early hybrid with good pachytene spreading.

This double cross hybrid Minn. A.E.S. 101, which has been carried on by sib crossing for the past 5 or 6 years, has given well-spread pachytenes (reported last year in the News Letter). The four inbred parents, grown last summer, do not have superior spreading ability. All have several knobs. The N.D. 203 line has a large terminal knob on the short arm of 9. The other three lines have a medium or small terminal knob on 9.

John T. Stout
Joseph Neubauer

7. A new character, tinged, in chromosome 10.

A new seedling character from early generation selfs in the corn breeding program has been tested with a partial series of interchange lines. The seedlings are pale green in the tips of the leaves. This past summer the plants were pale green to maturity. It is closely linked with T5-10 (5290), but independent of T5-7e; hence it is probably located in chromosome 10. A test for allelism with g_1 is needed.

C. R. Burnham

8. Propionic acid cotton blue stain.

The addition of a drop or so of Watkins cotton blue stain before adding the cover slip to a preparation of spore quartets well-stained in propionocarmine was found to greatly improve the definition of cell walls and the nucleolar material was easily distinguishable. Also the spores remain as quartets within the original spore-mother-cell wall much better. The cotton blue stain used was from an old bottle in the lab made up many years ago, and was highly viscous.

A new solution, made up from the formula given in Gray is:

25cc distilled deionized water

25cc glycerin

25 gm. phenol

25cc lactic acid

This was not viscous and did not give the results obtained with the old stock. 100cc of glycerin were added to the formula and, after mixing, the solution was boiled very slowly until a fourth of the mixture was boiled away. After cooling, 1 part of stain was mixed with 2 parts of propionic acid. This solution still is not equal to the old stock in its ability to stain the cell walls but it does hold the spore quartets together. Some destaining is possible if steam heat is used. If the quartets reject destaining, less propionic cotton blue must be used. On the other hand if destaining is too drastic, not enough stain has been used.

Joseph Neubauer

9. An improvement in the aceto-carmine smear technique.

Corn anthers for pachytene, diakinesis or metaphase I analysis are removed from the acetic alcohol killer and

placed for a few minutes in 20% acetic acid before the regular staining procedure. The measurable diameter of the cells increases by 70 to 80%. Considerable improvement was noted in the spreading of pachytene chromosomes in sporocytes that were relatively poor spreaders. Prolonged exposure to the acetic acid results in loss of affinity for the stain. Similar but less pronounced effects were noted in barley. Pre-treatment with higher percentages of acetic acid was better in some cases.

Joseph Neubauer

10. Improved propiono carmine stain.

A number of years ago a worker in the radiation genetics lab noted that a batch of propiono carmine unintentionally refluxed for a much longer time seemed to give better staining. When this came to our attention recently, we prepared it as follows:

0.5 gm per 100cc. of 45% propionic acid

reflux for 6 to 8 hrs.

cool and filter

This stain gives much better results for corn than any that we have prepared by other methods.

Dilution with 45% acid may be necessary if the cytoplasm is stained too heavily, as in the tomato.

John T. Stout

11. Variable transformer for use with microscope lamp.

For a microscope lamp using a spotlight 100W, 120V, G16 ½ bulb, or for one that uses a 100W 120V T 8 ½ bulb, CC13 filament, we have used a Powerstat variable autotransformer:

Type 2PF10 input 120V, 60 cycle

output 0-130V, 1 amp.

It is manufactured by the Superior Electric Co., Bristol, Conn. A 1 ½ or higher ampere unit would probably be better.

C. R. Burnham

UNIVERSITY OF MISSOURI
Columbia, Missouri

1. Pollination with liquid suspensions.

Paraffin oil and the aqueous pollen germination medium of Cook and Walden (News Letter 39: 170; Can. J. Bot. 43: 779) were used to suspend pollen before pollination; both media allowed successful fertilization. Paraffin oil was best of the two in seed set; pollen stored in oil as long as overnight (in the refrigerator) was also successful. Either medium can include Tween detergent. An emulsion of paraffin oil-Tween-aqueous medium can also be used. One cc of pollen (estimated to contain well over 2×10^6 grains) per 1 to 6 cc of medium was used. Sequential dilution of suspensions with the aqueous medium or aqueous-Tween decreased seed sets. A manuscript is in preparation.

"Enrichment" should be possible with these media, parallel to the use of selective agents and conditions in microorganisms.

E. H. Coe, Jr.
(in collaboration with D. B. Walden and F. S. Cook, U. of Western Ontario)

2. Endosperm losses following exposure to an intermittent DC electrical field.

Three plants of ++/a sh_2 were used in a test of effects of exposure to an intermittent DC field, in collaboration with D. L. Waidelich (Electrical Engineering Dept., U. of Mo.). The three plants were uniform in developmental stage at the time of treatment (meiosis to post-meiosis). Plant No. 1 was untreated. Plant No. 3 was exposed in the tassel region to 30,000 volts DC across a 5 cm insulated gap (6,000 volts/cm) for 40 minutes, intermittently and irregularly (1 to 10 min. on, briefly off, 1 to 10 min. on, etc.). Plant No. 11 was exposed to the same 40-minute pattern as No. 3 and then continued for a total of 3.5 hours, on a regular pattern (1 minute on, briefly off, 1 minute on, etc.). No adverse effects other than localized searing (connected with corona discharge) were apparent; pollen of treated plants appeared to have slightly decreased fertility.

Pollinations on a sh_2 were made daily. Fractional losses of A Sh_2 were scored; pooled data for all pollination dates are presented in Table 1. Judging by Poisson limits, fractionals were definitely more frequent for treated males. Further experiments of better design will be needed to determine whether the effect is real.

Table 1
Fractional losses of A Sh_2 following electric-field treatments of ++/a sh_2 males.

Male	Fractional category 1/2	1/4	1/8	1/16	limit	Total	No. A Sh kernels
1 (Control)		1	4	3	8	16	991
3 (40 min)		3	3	8	5	19	686
11 (3.5 hr)	5	7	3	15	23	53	1359
Total	5	11	10	26	36	88	3036

E. H. Coe, Jr.

UNIVERSITY OF MISSOURI
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and

UNITED STATES DEPARTMENT OF AGRICULTURE

1. Preferential pairing in trisomic plants containing an irradiated chromosome.

Pollen from plants with normal chromosomes 3 containing the A_1 allele was given 1000 r and used to fertilize standard trisome 3 plants which were homozygous for a_1. The gene segregation from the resulting trisome 3 plants (A/a/a) when used as the pollen parent is given in Table 1. Corresponding control data are given in Table 2.

The control data in Table 2 indicate that the theoretical ratio of 1 A : 2 a is held to very closely. There is only one progeny out of twenty-five in which the percentage of A gametes is significantly higher than 33.3%, but this may be expected at the .05% level. The interaction chi square of 32.7 with 24 degrees of freedom is not significant. The data are homogeneous.

In the case of the trisomes with an irradiated chromosome, it is an entirely different situation. Six of the 26 plants had transmission frequencies of A gametes significantly lower than 33.3% and six others had rates which were significantly higher than 33.3%. The former was

Table 1

	No. A gam.	Total gamet.	% A	X^2 (1:2)		No. A gam.	Total gamet.	% A	X^2 (1:2)
1	523	1280	40.85	31.3**	14	218	650	33.54	0.0
2	207	532	38.91	7.6**	15	462	1379	33.50	0.0
3	462	1201	38.47	14.4**	16	703	2110	33.32	0.0
4	572	1493	38.31	16.5**	17	248	746	33.24	0.0
5	178	465	38.28	5.1*	18	278	852	32.51	0.2
6	343	931	36.84	5.3*	19	233	749	31.10	1.7
7	256	699	36.62	3.4	20	133	431	30.85	1.3
8	262	728	35.99	2.2	21	161	566	28.44	4.7*
9	375	1047	35.82	2.8	22	230	824	27.91	10.6**
10	311	1435	35.61	3.4	23	142	766	18.54	75.1**
11	689	1961	35.14	2.9	24	97	566	17.14	67.2**
12	280	817	34.27	0.4	25	83	522	15.90	76.4**
13	140	410	34.15	0.1	26	30	1101	2.97	464.2**

Table 2
Control

	No. A gam.	Total gam.	% A	X^2		No. A gam.	Total gam.	% A	X^2
1	173	468	36.96	3.7	14	279	831	33.57	0.0
2	171	467	36.62	2.2	15	135	405	33.33	0.0
3	344	943	36.48	4.3*	16	137	416	32.93	0.0
4	176	484	36.36	2.1	17	220	673	32.69	0.1
5	527	1485	35.49	3.1	18	531	1641	32.36	0.7
6	556	1581	35.17	2.4	19	196	607	32.29	0.3
7	967	2765	34.97	3.3	20	202	631	32.01	0.5
8	265	765	34.64	0.6	21	390	1229	31.73	1.5
9	463	1338	34.60	1.0	22	286	932	30.69	3.0
10	481	1401	34.33	0.6	23	167	546	30.59	1.9
11	296	865	34.22	0.3	24	162	540	30.00	2.7
12	311	911	34.14	0.3	25	180	602	29.90	3.3
13	288	851	33.84	0.1	Total	7903	23377	33.81	2.4

expected, but the latter was not, since there was no indication of "negative preferential pairing" when In 3a chromosomes were used in an earlier experiment. The term, "negative preferential pairing," may not be a good one. It is possible that the pairing is still preferential and that the tacit assumption that "the greater the structural homology between two chromosomes is, the greater is their pairing affinity at meiosis" needs to be examined critically. It has been observed that synapsis in hybrids is often more regular than in the parental inbreds. One explanation is that this is merely an expression of the greater physiological efficiency of the hybrid. However, it is possible if the following hypothesis has any merit that certain structural differences may enhance the pairing affinity of chromosomes.

Let us consider the spatial orientation of two homologous chromosomes prior to synapsis, i.e., at the leptotene stage of meiosis. To perhaps oversimplify the matter, they will either be in reverse or non-reverse position in regard to order of their pairing code units. See figures 1 and 2.

A B C D E F G H I

I H G F E D C B A

Fig. 1
Reverse Orientation

A B C D E F G H I

A B C D E F G H I

Fig. 2
Non-reverse Orientation

It may be easily imagined that the initiation of synapsis in the reverse orientation might be very difficult; one of the chromosomes or part of one would have to be rotated 180°. If, however, a small inversion has been induced as is shown in Fig. 3 and Fig. 4, then pairing could take place with less difficulty when the chromosomes are in the reverse orientation. As the chromosomes slither past each in their random movements, the directionally homologous segments could make the initial contact and facilitate synapsis along the rest of the chromosome. A small inversion would not seriously hamper synapsis when the chromosomes were in the non-reverse orientation.

A B D C E F G H I

I H G F E D C B A

Fig. 3

A B D C E F G H I

I H G F E D C B A

Fig. 4

No cases of "negative preferential pairing" were found when In 3a chromosomes were irradiated. This is probably because an inversion is already present and the chromosomes do not need help in pairing in the reverse orientation.

Any additional inversions would not help much and would hamper pairing in the non-reverse orientation.

Some derivatives of the irradiated In 3a chromosomes from the original experiment have been reintroduced into trisomic 3 plants and the amount of preferential pairing has been redetermined. This was done by taking plants grown from colored (A) kernels from the testcross, a/a X A/a/a, and crossing them to the standard trisome 3 stock (a/a/a) and crossing the resulting trisomes as the pollen parent to the a/a tester. The results are given in Table 3.

In the first case in Table 3, the A locus has probably crossed over onto a normal chromosome. The chi square tests for a fit to a 1 A : 2 a ratio. It is significantly higher than 33.3% A. Perhaps a chromosome aberration which now causes negative preferential pairing has been retained.

Table 3

Original % A	Derived Trisome case #	No. of A gametes	Total gametes	% A	(X^2)!
17.15	1	334	905	36.90	5.1*
15.65	2	58	369	15.72	0.0
18.14	3	63	463	13.60	8.7**
	4	81	743	10.90	25.6**
	5	46	447	10.29	18.5**
	6	115	1072	10.73	39.3**
	7	140	906	15.45	4.4*
	8	269	2277	11.81	61.3**
	9	360	2402	15.00	16.2**
11.54	10	161	1057	15.23	14.2**
	11	121	830	14.58	7.2**
	12	142	693	20.49	0.8
12.56	13	107	886	12.08	0.2
10.23	14	121	830	14.58	17.0**
	15	42	375	11.20	0.5
	16	192	814	23.58	1.2
	17	151	2000	7.55	15.8**
	18	147	674	21.81	0.0
	19	93	748	12.43	3.7
	20	42	347	12.10	1.6

! See text for explanation of chi square tests

In the cases 2 - 11, 13 - 15, 17, 19, and 20 the chi square tests for a correspondence between the original transmission rate of A and the derived one. In cases 2, 13, 15, 19, and 20 the rate has been unaffected. The other cases 3 - 11, 14, and 17 show a shift in the amount of preferential pairing, possibly due to the loss of positively or negatively acting aberrations by crossing over.

In cases 12, 16, and 18, the amount of preferential pairing has reverted to that expected from In 3a alone (22% A), again probably by crossing over.

Unfortunately the data are too limited to make any sweeping conclusions. It is apparent that the level of pairing affinity is heritable and that it would be possible to map the location of these "synaptic mutations."

Another method of detecting preferential pairing has been devised and tested. Pollen from wx/wx plants was given 1000 r and used to fertilize trisome 9 plants which were homozygous for Wx. The pollen from the resulting trisomic plants (Wx/Wx/wx) is stained with iodine and is scored for Wx and wx. The results of this experiment are given in Table 4.

Table 4

Control				Irradiated				
	No. wx gametes	Total gametes	% wx		No. wx gametes	Total gametes	% wx	X^2 (wx=23.22)
1	182	708	25.71	6	146	681	21.44	1.2
2	182	731	24.90	7	128	656	19.51	4.9*
3	158	684	23.10	8	114	635	17.95	9.6**
4	144	654	22.02	9	119	676	17.60	12.0*
5	129	646	19.97	10	109	621	17.55	11.1**
Total	795	3423	23.22	11	106	652	16.26	17.4**
inter. X^2 = 8.23 (not sig.)				12	88	605	14.54	31.3**

As may be seen in Table 4 the average frequency of wx pollen in the five control trisomes was 23.22%. The interaction chi square was only 8.23 with 4 degrees of freedom, so the data are homogeneous. Testing the frequency of wx pollen in the trisome 9 plants which received an irradiated wx chromosome against the value of 23.22% it was found that six out of seven plants gave an indication of preferential pairing.

The advantages of this method may be readily apparent. It is possible to examine a hundred thousand pollen grains or more if necessary and thus it is possible to detect very small differences in the level of preferential pairing. Also it is feasible to look for rare spontaneous changes in chromosome structure affecting chromosome pairing without having to plant an acre of tester plants. One possibility, which now can be tested easily, results from non-homologous pairing of a univalent (the pairing with itself) in a trisome. Crossing over in this non-homologously paired region would lead to the formation of an inversion.

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1. Japanese local races of maize resistant to the virus disease, corn stunt.

Four virus diseases are known to occur in maize under natural conditions. Stunt disease transmitted by the smaller brown planthopper, Delphaiodes striatella Fallén, is the most harmful one in Japanese maize production. A great deal of damage by virus disease is done to maize cultivation in the southern district of Japan, especially in Kyūshū.

Over a period of 2 years many varieties were tested for resistance to stunt disease at the Miyakonozyō Sub-station of the Miyazaki Agricultural Experiment Station, Miyazaki Prefecture. Seventy materials (48 Japanese local races, 17 varieties introduced from foreign countries, and 5 recommended hybrids) were tested in 1963. The results showed that all but 2 Japanese races, Kamigane-1 and Suyame-inno-1, had high susceptibility to this disease. Frequency (%) of diseased plants and index of susceptibility* was over 50% and 1.70 respectively. However, Kamigane-1 showed only 13.5% and 0.42, and Suyama-inno-1 showed 24.4% and 0.65 respectively.

In 1964, two hundred eighteen races (151 Japanese local races and 67 races collected from foreign countries, of which 17 were from Thailand), were tested. It is said that most of the Thailand races originated from the progenies of Guatemala Golden Yellow Flint Hybrid. All but some Thailand and the 2 Japanese races mentioned above had low resistance to the disease, showing similar values regarding susceptibility as the test in 1963. The values in some Thailand races varied from 9.4% to 45.2% and 0.3 to 1.4. The values of Kamigane-1 were 12.5% and 0.3, and those of Suyama-inno-1 were 6.3% and 0.1 respectively.

From the results of the 2-year test it was concluded that both Kamigane-1 and Suyama-inno-1 were resistant to the corn stunt disease and useful materials for maize breeding in Japan. These two races were collected from the environs of Mt. Fuji by our institute in 1954 (cf. Maize Genetics Cooperation News Letter 32, 1958).

*Index of susceptibility: Four numerical values (v), 0, 2, 4, and 6, are given to diseased plants corresponding to degrees from light to heavy damage, and the number of plants (n) belonging to each grade are counted. Index of susceptibility is obtained from $\frac{\sum n\,v}{\text{Total number of plants}}$.

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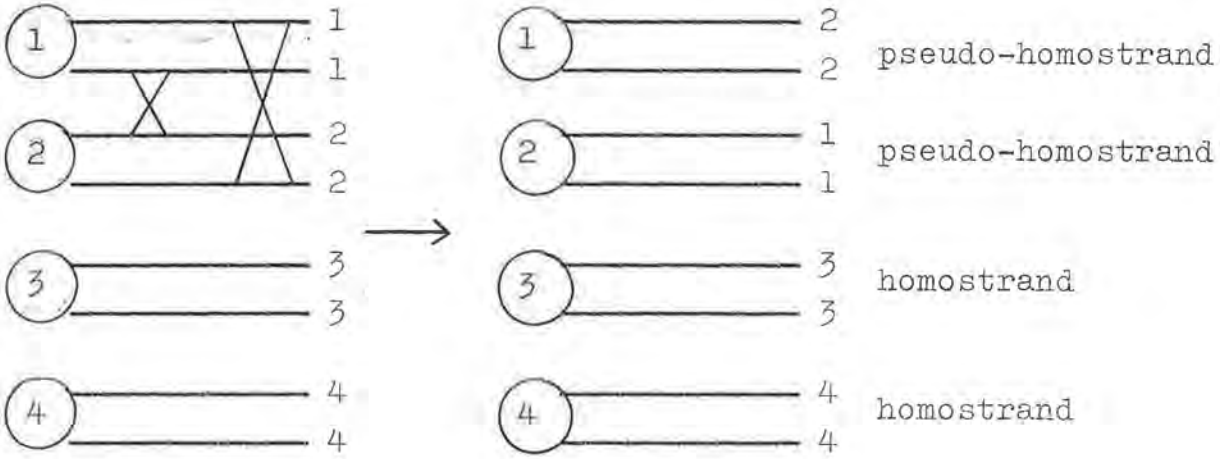
1. Considerations in the use of double reduction in autotetraploids for mapping.

The coefficient of double reduction, α, has been used in estimating crossing-over between a gene and centromere in autotetraploid maize (Catcheside, Heredity 10:205-218, 1956). In this case, α was stated to be dependent upon the coefficients: (1) the amount of crossing-over (c) between centromere and the locus; (2) the frequency (q) of quadrivalent formation; (3) the frequency (p) of adjacent (or parallel) disjunction of quadrivalents; and (4) the frequency (d) with which adjacent disjunction of the quadrivalent results in nondisjunction of the genes in paired chromosome arms. These parameters are related by the formula $\alpha = cqpd$, since the half chance of having the necessary disjunctional arrangement at division II of meiosis is offset by the double chance of the necessary crossover in each cell. Crossing-over between gene and centromere can then be determined by solving the above formula for c, $c = \frac{\alpha}{qpd}$. This formula implies a direct relationship between recombination and double reduction. Unfortunately, this is not entirely true. For example, the occurrence of a four-strand double crossover involving two of four chromosomes yields strands which are designated here as pseudo-homostrands.



If the centromeres were marked, the pseudo-homostrands would be scored as recombinant strands. However, the crossovers have placed the telomere portion of sister chromatids (i.e. 2-2 and 1-1) on the same centromere. This, of course, precludes double reduction, since at second division they must separate to different gametes. Multiple crossovers, other than four-strand doubles, can also give rise to pseudo-strands. Since recombinants can occur which actually prevent double reduction, crossing-over between a gene and centromere determined from double-reduction values will be underestimated.

A second point should be made. When recombination is estimated from autotetraploid data, it is not directly comparable with diploid estimates (Sved, Heredity 19:585-596, 1964). For instance, the upper limit of recombination in autotetraploids is 75% while it is only 50% in diploids. Therefore, if methods were available with autotetraploids for determining recombination distances between centromeres and genes, these values would need appropriate corrections to be comparable with diploid values.

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1. Neocentromeres as metaphase I chromosome markers.

Analysis of the meiotic behavior of specific chromosomes has been mostly confined to pachynema, though attempts have been occasionally made to extend the analysis to early diakinesis (for example, Miller 1960: MNL 34).

Obviously, the reason is lack of suitable landmarks which can differentiate between chromosomes. Centromeres, heterochromatic knobs, and the chromomere pattern, which so well characterize pachytene chromosomes are of little value as chromosome markers in metaphase I and subsequent stages.

The significant observation that neocentric activity is induced at knob sites in the presence of abnormal chromosome 10 (Rhoades, 1952) appears to provide a clue to overcome this difficulty. If all knobs, irrespective of their position in specific chromosomes are capable of inducing detectable neocentric activity, it may be possible to know the number of knobs present in the genome by counting the number of neocentromeres. On this assumption, if specific chromosomes are suitably marked with varying number of knobs, it should be possible to identify particular chromosomes at metaphase I as well as metaphase II.

Before trying to use neocentromeres as meiotic metaphase markers in the manner now suggested, it is necessary to test the assumption made above that all knob sites show detectable neocentric activity in the presence of abnormal chromosome 10. At least two important aspects of this problem can be recognized. (1) Under some conditions, there may be competition between knob sites, particularly if knobs of different sizes and/or physiological states are present in the same chromosome or chromosomal arm. (2) It is important to know the extent of variability in neocentric activity at any knob position due to intrinsic and external factors. Sites showing constancy in behavior should be useful as markers.

Even assuming constancy in neocentric expression and absence of any competition, not more than 3-4 chromosomes out of the ten present in the maize genome can be identified at metaphase I or II, since the same number of knobs cannot be employed to distinguish more than one chromosome of the complement. Further, if more than two knobs and hence neocentromeres are used to mark a chromosome, difficulty may be encountered due to overlapping or crowding of the chromosomal fibers at the neocentromeres.

S. K. Sinha

2. <u>A note on the possible use of neocentric activity as an additional trait for characterizing knob sites and maize races.</u>

The heterochromatic knobs, whenever present in maize races, are valuable aids for the characterization and identification of maize races. Usually observations are taken on the position and the size of the knob. Maize cytologists have tried to evaluate the activity of knob-forming positions

by grading the knobs according to their size. However, it has been felt that 'such an evaluation is not entirely satisfactory due to the personal element that is involved' (Longley and Kato, 1965: Chromosome Morphology of Certain Races of Maize in Latin America). Besides the subjective difficulty in grading, an important defect may arise in case there is no correlation between size and activity. In fact, it may be visualized that two knobs of exactly the same size may possess different physiological activity and similarly, the total activity of knobs in one race may be entirely different from that of a second race, having the same number of knobs in exactly the same positions in corresponding chromosomes. From these considerations it appears necessary to measure some form of physiological activity, which may be independent of size and can be easily estimated with a fair degree of precision. Thus an additional trait would be provided for characterizing these chromosome markers and consequently the maize races.

The neocentric activity, elicited by the abnormal chromosome 10, and possibly other abnormal chromosomes like Ab. 2 and Ab. 9, reported recently by Longley and Kato (1965) may be considered as one kind of physiological activity at a knob site. For the purely taxonomic purpose of delineating maize races, it would not matter whether and to what extent this activity is a property of the site itself or the result of interaction of the site with the rest of the chromosomal material besides the inducer, i.e. the extra heterochromatic piece in the abnormal chromosome.

Since the method of estimation is important, one must look for the stage of meiosis where this estimation can be undertaken with ease and accuracy. Metaphase II appears to be the right stage for such analysis, since precocious activity at the neocentromeres results in sufficient stretching of the chromatids, so as to permit easy measurement without the risk of the personal element. The total length of the stretched chromatid segments can be taken as a measure of the degree of neocentric activity. For estimating the total knob activity within the meiocyte, the following procedure may be adopted. The maize race to be studied may be crossed with a standard homozygous line, carrying abnormal 10, but few knobs. From the total activity of this hybrid material, half of the activity in the standard line may be deducted, and next this difference may be multiplied by two. The logic of this procedure is fairly simple. By suitably marking the chromosomes with varying number of knobs, it should be possible to identify specific chromosomes as discussed earlier and thus the activity at particular knob sites can possibly be estimated.

S. K. Sinha

3. The relation of heterozygosity to environmental variation with reference to some seedling traits in maize.

It is now generally believed that developmental homeostasis with respect to many characters is associated with heterozygosity, particularly in the cross-fertilized species. To state in simple terms, the phenotypic (=environmental) variance of inbreds is greater than that of hybrids. Recent investigations in several organisms indicate that this may not hold good for all characters. With respect to certain traits it has been observed that the variance in hybrids is greater than the variance in inbreds. Attempts have been made to explain this anomaly in terms of the nature of variation, i.e. whether it is developmental or due to adaptive response. As suggested by Falconer (1960), developmental variation, which may be an expression of the degree of buffering or canalization of development, would be expected to be maximum in inbreds and minimum in hybrids. On the contrary, variation due to adaptive response, which may be associated with the greater fitness of hybrids, should be high in hybrids but low in inbreds. If this argument is generally valid, a clue is provided for knowing the nature of the environmental variation in different characters by observing the difference in the degree of variation between inbreds and hybrids.

Starting with this premise, we have undertaken a study of the difference in environmental variation in inbreds and hybrids with respect to seedling as well as adult plant traits. The preliminary data on a few seedling traits lead us to the following tentative inferences: (1) In the case of a few characters like mesocotyl length, coleoptile length and the number of seminal roots, the environmental variance is greater in the inbreds than in the hybrid. According to the view expressed by Falconer (1960) and others, the variation in inbreds with respect to these characters may be of a developmental nature, probably 'arising from accidents of development'. Heterozygosity would lead to developmental homeostasis of these characters. (2) In another group of characters, the variance in the hybrid is strikingly more than that in the inbred parents. The greater variance in the hybrid can presumably be ascribed to the 'adaptive responses' of characters such as radicle length, the average length of seminal roots, and the average number of vascular strands in seminal roots. (3) Perhaps, there is a third category of characters, e.g. the number of vascular strands of the radicle, in which not much difference can be observed between inbreds and the hybrid.

We are further extending this study to (1) other seedling traits, (2) certain aspects of chromosomal behavior like synapsis, chiasma frequency, division synchrony, (3) variation in nucleolus and (4) pollen grain variation.

It is hoped that such a study will yield more information on the nature of variation in these traits and also help us to select additional traits for a thorough characterization of inbred lines. Further, this study has an important bearing on the problem of choice of material (inbreds or hybrids) for experimental studies, especially for evaluating the effect of different factors on growth and development including the meiotic events. This point will be elaborated elsewhere.

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1. Photoresponse of albescent maize.

Wrapping the bases of stems of young, field-grown al/al plants with aluminum foil proved useful in prolonging the period during which green tissue was produced. Plants with the typical albescent top reverted to the production of green foliage after a 15-cm length of aluminum foil was secured to shade the bases of such plants. Following treatment, the greening response was evident within 48 hours in the tissue just emerging from the leaf rolls. Most responsive to the treatment were those albescent plants which had produced the most green tissue in the lower leaves. This technique should be of use whenever al/al pollen is required; treated early, albescent plants would probably produce enough green foliage to sustain a moderate seed set.

Green tissue produced on al/al plants appeared in thin-layer chromatographic separations to have carotenoid and chlorophyll complements similar to those of +/al foliage. Illuminated al/al seedlings also contained a component in the white tissue with an absorption peak near 340 mμ, tentatively identified as phytofluene. Dark-grown al/al and +/al seedlings contained similar amounts of protochlorophyll. Dark or brightly illuminated albescent seedlings failed to develop a content of carotenoids equivalent to that of heterozygotes. On the other hand, in dim light, somewhere below 0.06 m watt/cm^2, total pigment content of al/al seedlings approached that of heterozygotes. Red or blue light under higher intensity illumination appeared to be most effective in preventing pigment accumulation whereas a green cellophane filter allowed moderate pigment formation. In seedlings as in field-grown

plants, shading of the apical meristem enhanced development of photosynthetic tissue.

These observations suggest that albescent plants can produce functional photosynthetic tissue by means of a light-requiring pathway. Inhibition of greening by red or blue light would seem to correspond to photodestruction of protochlorophyll in the absence of sufficient carotenoids. Transverse green bands found at times on field-grown albescent plants are apparently produced when the apical meristem is below the soil surface with the emerged foliage acting as a light filter. There is a striking parallel between albescent responses noted here and the light requirement for carotenoid formation in Neurospora reported by Zalokar (Arch. Biochem. and Biophys. 56: 318-325. 1955).

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1. Genetics of resistance to Maize Dwarf Mosaic Virus.

The inbred Pa. 11 has exhibited a high degree of resistance, but not immunity, to the Ohio Type Strain of M.D.M.V. in both greenhouse and field trials. Repeated inoculations of Pa. 54 (susceptible) x Pa. 11 have failed to produce symptoms. The virus has not been recovered from this single cross after repeated inoculations.

Of 988 seedlings of the single cross selfed, following two inoculations, 659 were symptomless; the remaining 229 were infected. Of the infected, 24 showed symptoms as broad bands (tolerant) but these were classified as susceptible. No distinction in symptom severity of the other 205 susceptibles was observed. The X^2 probability for a 3:1 segregation is 0.25.

Pa. 32 shows resistance to M.D.M.V. in the seedling stage and in the field until anthesis. Pa. 444 [(Pa. 54 x Pa. 11) (Pa. 32 x Pa. 33 susc.)] was selfed and the S_1 seedlings were inoculated to determine if the genetics of resistance of Pa. 32 was similar in expression to that of Pa. 11.

816 seedlings were classified for reaction to M.D.M.V. as follows: 362 symptomless, 170 mildly infected, 224 moderately infected, 30 severe and 30 showed symptoms as broad bands. No simple segregation ratios could be fitted to the data.

However the data indicate that the inheritance of resistance is not so complicated that the back-cross method of transferring resistance could not be used.

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1. Partial restorer and full restorer genes in a common genetic background.

Four partial restorer inbreds, each having a partial restoring gene allelic to $\underline{Rf_1}$ but of less restoring strength, have been crossed to SK2-T, of genotype $\underline{rf_1}\,\underline{rf_1}\,\underline{Rf_2}\,\underline{Rf_2}$. SK2 has a full complement of modifying genes for $\underline{Rf_1}$. Each cross has now been backcrossed (as female) to SK2 6 or 7 times, selecting fertile plants in each generation. As a control, SK2-T $\underline{Rf_1}\,\underline{rf_1}$, segregating for the full restorer gene from WG3, has been carried along also, with the same selection.

In each winter generation (Florida) all backcrosses segregate approximately 1 sterile to 1 "fertile". The "fertiles" given by the partial restorer sources usually are class 4, with class 5 being of normal full fertility. The fertiles with the WG3 source are, as expected, nearly all class 5.

In each summer generation the backcross with the WG3 gene continues to segregate 1 sterile: 1 fully fertile. However, the four backcrosses with partial restorer source typically have 80 - 95 per cent completely sterile plants, with the fertiles being class 3 or less (a few, weakly fertile anthers are exserted). Obviously the environment prevents most of the partial restorer genotypes from expressing themselves.

As backcrossing continues the different sources of partial restoration resemble each other more and more, in restoration strength, but it appears that the gene from one source (L) is more powerful than those from the other 3 sources, although it clearly is less powerful than the gene from WG3.

Segregations obtained in Florida, 1964-5, in BC_4, are shown in the following table:

Restorer Source	Sterile	Partially Fertile	Fully Fertile	S:"F"
	(No.)	(No.)	(No.)	(No.)
B	19	18	0	19:18
G	25	17	0	25:17
L	17	14	6	17:20
M	23	16	1	23:17
WG3	11	3	18	11:21
Total				95:94

Chi square tests show no significant differences from the ratio 1 sterile: 1 "fertile" for the individual restorer sources, nor for the pooled data, and the interaction chi square is not significant. Nevertheless the WG3 gene clearly has more restoration power than any of the other four genes.

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1. DNA from maize with B-chromosomes.

Heterochromatin, that is, chromosome material showing heteropycnosis, has been reported to differ from euchromatin in coiling cycle, time of DNA replication and turnover of DNA as well as in content of identifiable oligogenes. The suggestion has been made (e.g. Herskowitz, Genetics, p. 371) that heterochromatin might differ from euchromatin in the base composition of its DNA. An investigation of this possibility was made by comparing base ratios of DNA prepared from two lines of Black Mexican Sweet Corn isogenic with the exception that one contained a variable number of largely heterochromatic B-chromosomes in addition to the 20 A-chromosomes.

DNA was extracted from the endosperm of kernels in the milk stage of development. The yield of DNA from the B-chromosome

line was 60% higher than that of the normal line although cytological determinations showed the average number of B-chromosomes present to be only 2 per haploid set of A-chromosomes. Using the pachytene lengths given by Rhoades and assuming that a B-chromosome is about the length of the 10th chromosome, one can see that a chromosome of this size would contribute only about 7% of the total length of a haploid set of chromosomes. If all the extra DNA in the B-chromosome line comes from the B-chromosomes and if they contributed an amount of DNA per unit length equivalent to that of the A-chromosomes, more than 8 B-chromosomes per haploid set would be required to make up the extra 60% of DNA. It must be concluded then, that the B-chromosomes contain on the average, 4 times as much DNA per unit length as the A-chromosomes.

The yield of DNA for the normal Black Mexican line was very similar to that obtained for a white dent commercial inbred line.

Base ratios of the DNAs were determined by paper chromatography and by the bromination reaction. A summary of the results is give in table 1.

Table 1
Purine and pyrimidine constituents of DNA-Na of three lines of maize.

Source	Per cent Bases	
	adenine + thymine	guanine + cytosine + 5-methyl cytosine
K64r Commercial white dent inbred	56	44
Black Mexican Sweet Corn without B-chromosomes	45	55
Black Mexican Sweet Corn with B-chromosomes	30	70

It can be seen from table 1 that the B-chromosome line contains 15% more C-G base pairs than does the normal Black Mexican line. If the extra 60% of DNA presumably contributed by the B-chromosomes is composed largely or entirely of C-G bases, the alteration in base ratios expected is very close

to that actually observed. It would seem then, that DNA from the heterochromatic B-chromosomes of maize is made up largely or entirely of C-G base pairs.

An unexpected result was the difference in base ratios between the normal Black Mexican line and a white dent commercial inbred which is typical of all the maize for which base ratios have been determined in this laboratory. It has been assumed generally that normal individuals of the same species show about the same base ratios in their DNA. Our results indicate that this is not necessarily true for maize. Further studies are being undertaken to investigate this aspect of the problem.

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2. Transmission of the P locus and Modulators in reciprocal crosses.

Reciprocal crosses were made between 31 plants heterozygous for light variegated (P^{rr} Mp + transposed-Mp) and a white commercial inbred line (P^{ww}, no Mp) in order to study the transmission of the P locus and Modulators through male and female gametes. The light variegateds used were all from families which had shown close linkage between the P locus and the transposed-Modulator in previous generations.

Three comparisons were made for each pair of reciprocal crosses:

1. number of colorless ears to colored ears to determine if the transmission of the P locus itself was normal

2. number of medium variegated ears (P^{rr}Mp) to light variegated ears (P^{rr}Mp + tr-Mp) to compare the transmission of the transposed-Modulator through male and female

3. number of red ears (P^{rr}) to variegated ears to compare the transmission of the Mp at the P locus through male and female.

The results of the reciprocal crosses were compared for each of the three comparisons by means of X^2-tests for 2 x 2 contingency tables. In cases where either the expected values or the totals were too small to use the X^2-test, the probability was computed directly.

The results are summarized in table 1.

Table 1

Comparison	No. of reciprocal crosses giving: Significantly different transmission through ♂ & ♀	Not sig. different
colored to colorless	4	28
medium to light var.	1	30
red to variegated	5	26

In all cases a cross showing a different ♂ and ♀ transmission for one characteristic did not show a significant deviation for the other two comparisons.

The abnormality of transmission of the $\underline{P}^{rr}$ gene is puzzling. In each of the 4 significant cases, the color gene was transmitted at a much lower frequency when the variegated was the male parent. It may be that there is a male gametophytic lethal closely linked to the $\underline{P}^{rr}$ gene in these families.

The most striking difference that appeared in reciprocal crosses was the difference in the proportion of red ears. Five of the 31 plants tested (16%) gave significantly ($P < .05$) more reds when the variegated plant was used as male parent. The 26 crosses which did not give significantly different proportions of red ears individually showed a highly significant difference in the same direction when lumped together. This seems to indicate that the Modulator at the P locus is more likely to undergo transposition and/or crossing over in the tissues giving rise to the male gametes than in the corresponding female tissues. The transposed-Mp present in the original light variegated plants did not show a corresponding increase in transposability. In only one of the 31 plants was there a significant difference between the proportion of medium and light variegated ears in the reciprocal crosses. In this one case, the tr-Mp was closely linked to P when passed through the female gametes but independent of P when the variegated plant was used as male parent. It is interesting to note that in this single case the transposition of Mp occurred in the male tissue thus behaving in the same way as the Mp's at the P locus.

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1. Reaction of monogenic resistance of Lady Finger popcofn to the natural infection of Helminthosporium turcicum observed in Kulu Valley, India.

Northern leaf blight disease of maize caused by Helminthosporium turcicum Pass. (Trichometasphaeria turcica Luttrel) is the most severe leaf disease in the hilly tracts of Punjab and Himachal Pradesh and according to Mitra it was first observed in India (Bihar) in 1907.

In a bid to control the damage caused by Helminthosporium turcicum to the maize crop in the Sub-Himalayan tracts of Punjab a backcross program to incorporate the high degree of resistance of Lady Finger popcorn in the adapted maize stocks was started in 1964. The seeds of Lady Finger popcorn were obtained from Dr. Hooker of University of Illinois. The material was for the first time planted in Kulu Valley, India in the monsoon season of 1965. The observations made indicated high susceptibility of Lady Finger popcorn to the natural infection of Helminthosporium turcicum in this tract. The lesions on the leaves were dark brown and quite large with an average size of 5" x ½". The lesion type is clearly distinct from the one described by Dr. Hooker for this genotype. The differential response of the variety in this region indicates the presence of a race complex of the pathogen which carries the necessary genetic complement for virulence on this particular stock. The differential reaction of Lady Finger popcorn to the Helminthosporium turcicum isolates used by Dr. Hooker and the natural infection at Kulu Valley, India could be important from the point of genetic and physiological studies and the variety may serve as a useful differential for classifying the virulence of the pathogen complexes found in different geographical regions.

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1. New endosperm mutant tentatively designated opaque-4.

An opaque phenotype endosperm mutant has been isolated from an "exotic" composite. Negative allele tests have been obtained with du, h, bt_1, bt_4, o_1, o_2 and ae. Also, it does not show the floury phenotype dosage effect. Analyses show that it is normal in amylose level and in lysine content.

Paul L. Crane

2. Induced mutation rates produced by treatments with four alkylating agents to the proembryo of Zea mays L.

The study reported here involves the use of seedling marker genes Lg_1 and Gl_2 at positions 11 and 30 in the short arm of chromosome 2 and Yg_2 at position 7 in the short arm of chromosome 9 as a system of testing and comparing the mutagenicity of ethyl methanesulfonate (EMS), diethyl sulfate (DES), ethylenimine (EI), and diepoxybutane (DEB) treatments applied to the proembroyos of maize.

Homozygous lg_1 gl_2, Yg_2 C Sh_1 Bz Wx female stocks were crossed with homozygous Lg_1 Gl_2, yg_2 c sh_1 bz wx male stocks. The proembryos 24 and 48 hours after pollination were treated with 20 ml solutions of one of the four alkylating agents. The treatment concentrations for each of the agents were as follows: EMS-0.2, 0.1, and 0.01653M; DES-.045M; EI-0.2, 0.1, and 0.05M; and DEB-0.01, 0.005, and 0.0025M. As a control, deionized glass-distilled water was used. All solutions were freshly prepared in deionized glass-distilled H_2O at pH 6.4 with phosphate buffer. The proembryos were prepared by carefully making a longitudinal incision in the ear shoot, plying back the husks from the ear sufficiently to allow one to wrap absorbent cotton around the ear. The ear shoots were soaked with the treatment solutions and covered with a bag. The cotton swab was allowed to remain for 2 hours and then it was removed. The ear was thoroughly washed with deionized glass-distilled H_2O, the husks were closed back around the ear and held by rubber bands and the ear was covered with a bag.

The mature ears were scored for genetic losses of partial and whole endosperm and seedling markers and are shown in table 1. This communication reports only the results of the pooled genetic losses of seedling markers. The mutant phenotypes were scored in seedling material from the first through the sixth leaf stage. Many seedling mutation events were also scored as very minute streaks of recessive tissure in addition to those partial events which were 1/2, 1/4, 1/8, 1/16th part of the seedling leaf.

Table 1
Pooled seedling mutation rates following treatment of maize zygotes and proembryos 24 and 48 hours after pollination by EMS, DES, EI and DEB.

Treatment No.	Chemical	conc. (M)	Age of zygote or proembryo at treatment (hrs.)	No. of seedlings	Total mutation rate %	Limits[a] .05 level
1	EMS	0.2	24	380	28*	23.27-32.37
2	EMS	0.2	48	72	28*	18.06-39.62
3	EMS	0.1	24	1100	25*	22.23-27.37
4	EMS	0.1	48	756	13*	10.47-15.56
5	EMS	0.01653	24	1685	5*	3.57- 5.62
6	EMS	0.01653	48	561	7*	6.07- 8.98
7	DEB	0.01	24	220	10*	6.40-14.75
8	DEB	0.01	48	77	6	2.14-14.56
9	DEB	0.005	24	246	7*	4.40-11.00
10	DEB	0.005	48	191	5	2.54- 9.41
11	DEB	0.0025	24	450	5*	3.46- 7.84
12	DEB	0.0025	48	167	5	2.49-10.00
13	D-H_2O	--	24	2738	3	2.13- 3.38
14	D-H_2O	--	48	697	4	2.46- 5.42
15	DES	0.045	24	691	10*	7.48-11.99
16	DES	0.045	48	275	9*	5.69-12.70
17	EI	0.2	24	588	6*	4.19- 8.19
18	EI	0.2	48	136	10	5.74-16.68
19	EI	0.1	24	861	2	1.52- 3.71
20	EI	0.1	48	217	5	2.56- 8.88
21	EI	0.05	24	315	0	0- 1.17
22	EI	0.05	48	50	0	0- 7.38

[a] Calculated according to Stevens, 1942.

* Mutation rate exceeds respective aged control at .05 level of significance.

Ethyl methanesulfonate produced the greatest seedling mutation yield (28%). The 0.2M EMS solutions applied to both 24 and 48 hour old proembryos yielded 7 to 9 times their respective D-H_2O controls and about 3 times the treatments which gave the highest rate of loss of genetic markers in each of the DES, EI, and DEB chemical treatments. The latter treatments were 2 to 3 times greater in mutation yield than their respective controls. All of the EMS and DES treatments were significantly better than control when applied to both 24 and 48 hour old proembryos. However, the DEB solutions at all concentrations used were only significantly greater than control when applied to the 24 hour old proembryos. The 0.2M solution was the only EI treatment which was significantly greater than control. With the exception of the 0.1M EMS treatments, there were no significant differences in mutation yield between the 24 and 48 hour old proembryos treated with the alkylating agents at each of the treatment concentrations. For each of the chemical treatments where there was a concentration gradient, in general there was an increase in the rate of loss of genetic markers with an increase in concentration.

It was particularly noted that EMS produced a high proportion of 1/2 to 1/16th part leaf sectors showing the genetic loss for $\underline{Yg}_2$ and $\underline{Gl}_2$ in addition to the small streaks. The larger sectors produced in proembryo treatments encourage the use of this type of treatment for screening for true gene mutations since the chance of survival against diploidal elimination of mutant sectors seems greater. The induction of several single locus whole seedling mutations by EMS for either $\underline{Yg}_2$ or $\underline{Gl}_2$ and 3 multiple locus whole seedling mutants for both $\underline{Yg}_2$ and $\underline{Gl}_2$ strengthens the suggestion that perhaps EMS is producing "true" gene mutations at the substructural level of the chromosome.

D. V. Glover

3. Further tests for the location of small plant (spl) on chromosome 6.

The location of a small plant (spl) character has been shown to be on chromosome 6 near the Y locus (MGCNL 39:152, 1965). Further evidence that it is on chromosome 6 comes from the following testcross data in the presence of a series of waxy and chromosome-nine translocations.

Small plant (spl) mutant stocks were crossed to stocks homozygous for the waxy marked chromosome-nine translocations. The F_1 plants were backcrossed to a small plant waxy line. The starchy and waxy seeds from each translocation cross were planted out separately and the plants were classified for small plant (spl) segregations. A chi-square test for independence, utilizing fourfold contingency tables with one degree of freedom, was used to determine if the populations from the two classes of seeds

were different. A significant value was found only for the cross involving T6-9$^{4505-4}$ at the one per cent level. The data from the progenies involving T6-9$^{4505-4}$ (6L.13 and 9 ctr.) were as follows: starchy seeds gave 55 normals and 49 spl; waxy seeds gave 77 normal and 23 spl giving a x^2 = 11.94 and a P-value of less than .01. Within the waxy class there was a significant deviation from the expected (50%) ratio of small plants (X^2 = 29.16, $P < .01$).

D. V. Glover

4. The effects of dimethylsulfoxide (DMSO) upon germination in Zea mays L.

Dimethylsulfoxide (DMSO) is known as a universal solvent for protein and carbohydrate materials. Biological materials are very permeable to this solvent and reports have indicated that DMSO is an effective carrier for some systemic herbicides. These characteristics of DMSO suggested the possibility of using it as a carrier for alkylating mutagen agents in treating mature seed of Zea mays.

In chemical mutagen experiments using mature seed it is desirable to obtain rapid absorption and uptake of mutagen solutions and subsequent interaction with active groups. Some alkylating agents possess a short half-life and therefore soon lose their potency as a mutagen if not incorporated rapidly by the seed.

A small experiment was initiated to determine the effects of DMSO on seed germination prior to using it as a carrier in mutagen experiments. Mature corn seeds of the single cross W23/L317 were soaked for 4, 12 and 24 hours in 0, 5, 10, 15, 20, 25, 50, 75 and 100 per cent by volume concentrations of DMSO in phosphate-buffer solutions. Fifty ml treatment solutions were used. The pH ranges varied from 6.1 for buffer solutions without DMSO to 11.4 for 100% DMSO solutions. Each treatment consisted of 20 seeds. After treatment the seeds were washed with deionized water for 3 minutes and germinated for 7 days on folded blotter germination paper.

The results of the treatments are shown in table 1. Treatments at concentrations greater than 50% V/V for 4 hours or more were completely lethal to the mature seed. The data suggested an increasing lethal effect with increased treatment time; however some of this effect may have been confounded with oxygen effects on germination. The control showed decreased germination with increased treatment time, suggesting the effect of insufficient oxygen. The results of mutagen treatments of mature seed using DMSO as a carrier are being analyzed.

D. V. Glover

Table 1
The effect of several dosage rates of dimethylsulfoxide (DMSO) upon the germination of Zea mays L. (W23/L317)

Treatment No.	DMSO treatment Conc. % V/V	DMSO treatment Time hrs.	pH of treatment solution	Per cent germination
1	100	4	11.4	0
2	100	12	11.4	0
3	100	24	11.4	0
4	75	4	9.0	0
5	75	12	9.0	0
6	75	24	9.0	0
7	50	4	8.25	100
8	50	12	8.25	30
9	50	24	8.25	0
10	25	4	6.9	100
11	25	12	6.9	90
12	25	24	6.9	70
13	20	4	6.75	90
14	20	12	6.75	95
15	20	24	6.75	80
16	15	4	6.6	80
17	15	12	6.6	90
18	15	24	6.6	60
19	10	4	6.4	100
20	10	12	6.4	85
21	10	24	6.4	70
22	5	4	6.4	100
23	5	12	6.4	90
24	5	24	6.4	80
25	0	4	6.1	100
26	0	12	6.1	95
27	0	24	6.1	90

5. Mutation in maize following the application of chemical mutagens to the pollen and proembryo.

The mutagenic effect of several alkylating agents upon the expression of several maize endosperm loci following their application to the mature seed was reported in the 1965 News Letter. Molar concentrations of the chemicals were also applied to cotton wrapped maize tassels 3 to 5 days prior to anthesis in order to determine their effect upon the developing male gametophyte.

The treatment was accomplished by saturating the cotton wrapped tassel with an aqueous chemical solution for a period of 3 hours. The treated tassels were not rinsed with water or covered, but were allowed to dry. Successive daily pollinations were made to the recessive seed parent and the resulting progenies were scored for whole or partial mutant endosperm events. The chemicals, molarities, and per cent mutation are presented in the following table.

Chemical mutagen	Conc. M.	No. of progeny	Mutation rate %
Ethyl Methanesulfonate (EMS)	.0125	281	1.06
	.025	428	1.17
	.05	1190	2.94*
	.1	343	4.08*
	.2	263	7.60*
Diepoxybutane (DEB)	.0012	952	1.05
	.0025	225	.89
	.005	785	2.93*
	.01	458	3.28*
Diethylsulfate (DES)	.045[a]	616	1.79
Ethyleneimine (EI)	.0125	1236	1.38
	.025	1105	.45
	.05	457	.44
	.1	1363	.51
Control	0	3057	.75

*Mutation rate exceeds the control at the .05 level of significance following correction for small numbers.

[a]Saturated solution at 20°C.

The non-treated parts of the tassel were removed immediately after treatment and the successive pollinations were made starting at the time of first pollen shed which was three days following treatment. Comparisons were made of the mutation rate of the treatments with the control for the same pollination date. There was a significant increase in mutation rate for the pollination dates which occurred five and six days after the initial treatment. The data indicate that the less mature pollen grains were most sensitive to the alkylating agents.

The chemicals were also applied to cotton wrapped ears at 24, 48, and 72 hours after pollination in an effort to induce massive endosperm and embryo chimeras. The endosperm mutation rates, chemicals, age of proembryo, and molarity of treatment solution are presented in the following table. All treatments were of 2 hours duration.

Age of proembryo	Chemical mutagen	Conc. M.	No. of progeny	Mutation rate %
24 hrs.	DEB	.0025	825	5.30*
	EMS	.1	737	1.90
	DES	.045[a]	498	.80
	EI	.1	1127	.62
	Control	0	805	.62
48 hrs.	DEB	.0025	1259	2.30*
	EI	.1	2294	.44
	EMS	.1	2458	.37
	DES	.045	939	0
	Control	0	1397	.14
72 hrs.	DES	.045	2199	.41
	DEB	.0025	2039	.39
	EI	.1	1873	.27
	EMS	.1	2183	.18
	Control	0	1432	.07

*Mutation rate exceeds the control at the .05 level of significance following correction for small numbers.

[a]Saturated solution at 20°C.

The DEB treatment was the only significantly effective treatment in the 24 and 48 hour age groups. None of the chemical applications were effective in the 72 hour age group. Nearly all of the mutants that were produced were partial mutants.

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Department of Botany and Plant Pathology

1. The difference in Wx frequency between male and female gametes from wx^{Coe}/wx^{90}.

In 1963 a conventional genetic analysis of the heterozygote Bz +90 V/bz C+ v, ae ae showed an interesting difference in Wx frequency in the male and female gametes. When the heterozygotes were used as males onto the bz wx^{Coe} v, ae tester, 76 apparent Wx, ae recombinants were found in 133,358 kernels. This is a frequency of 57×10^{-5}. The weighted average of Wx frequency in the pollen of the heterozygotes was 75×10^{-5}. When heterozygous plants of the same genotype were used as female parents, 19 apparent Wx, ae recombinants were found in 94,158 kernels or a frequency of 20×10^{-5}. The probability that the observed distribution would be found if Wx gametes were equally likely for both male and female populations is .0001 (from expansion of the binomial distribution and summation).

The verification of the presumed recombinants was hindered by poor germination. Test pollinations (by bz wx^{Coe} v, ae) were obtained on only 36 plants. Of these 31 came from Wx, ae recombinants, 2 from Wx Ae contaminants, and 3 from wx ae gametes. These latter could arise by heterofertilization events or misclassification.

The same type of test was repeated in 1965. Plants of the constitution Bz +90 V/bz C+ v, ae ae were used as male and female parents in crosses with the tester stock bz wx^{Coe} v, ae. When the heterozygotes were used as males, 18 presumed Wx ae were found in 35,497 kernels. This is a frequency of 51×10^{-5}. The weighted average of Wx in the pollen of the plants used as male parents is 72×10^{-5}. When the heterozygotes were used as female parents, 17 presumed Wx ae were found in 85,679 kernels or a frequency of 20×10^{-5}.

The agreement between the results of 1963 and 1965 indicates that the difference in Wx frequency between male and female gametes for wx^{Coe}/wx^{90} heterozygotes is real and reproducible.

In tests with Bz Wx V/bz wx v plants that are as closely related as possible to the Bz wx^{90} V/bz wx^{Coe} v heterozygote, no differences were found for the bz wx interval (♂♂ 20.0% and ♀♀ 19.1%) or the wx v interval (♂♂ 5.6% and ♀♀ 5.4%).

Oliver Nelson

2. Reconstitution of the R^{st} allele.

Near-colorless aleurone mutants from $R^{r}R^{st}$ are associated with crossing over between outside markers and possess all

or part of the paramutagenic action characteristic of the $\underline{R}^{st}$ parental allele. These facts suggest that the stippled phenotype may depend on two or more components that are separable by crossing over. Tests have been made for $\underline{R}^{st}$ reconstitution in various heterozygous combinations of mutants derived from $\underline{R}^{st}$, and an apparently successful test involved the following alleles:

$\underline{R}^{scl}113$: Self color mutant from $\underline{R}^{lst}$; nonparamutagenic.

$\underline{R}^{scl}132$: Self color mutant from $\underline{R}^{lst}$; as paramutagenic as $\underline{R}^{st}$.

$\underline{r}^{g}(I)^{2}$: Near-colorless aleurone, green plant mutant isolated from $\underline{R}^{r}\underline{R}^{st}$; unstable seed color giving mutations to self color; stable plant color. Mutants of this type are not associated with recombination when isolated from $\underline{R}^{r}\underline{R}^{st}$ plants, also occur in $\underline{R}^{st}\underline{R}^{st}$ plants, and are as paramutagenic as $\underline{R}^{st}$.

$\underline{r}^{g}(I)^{3}$: Near-colorless aleurone, green plant mutant isolated from $\underline{R}^{r}\underline{R}^{st}$; stable seed color; unstable plant color giving mutations to red plant. Mutants of this type are associated with recombination when isolated from $\underline{R}^{r}\underline{R}^{st}$ plants, and are as paramutagenic as $\underline{R}^{st}$.

The two near-colorless mutants were made heterozygous with each of the two self color mutants, and plants of the four heterozygous combinations were pollinated with $\underline{r}^{g}$, $\underline{wx}$ pollen. Stippled kernels were selected from these ears and grown out for verification. The results are shown in Table 1. Tests to definitely exclude the possibility of the stippled kernels having resulted from pollen contamination are not yet complete, but evidence to date makes this very unlikely.

One of the three $\underline{R}^{st}$ mutants isolated from $\underline{R}^{scl}113/\underline{r}^{g}(I)^{3}$ was atypical in phenotype, the colored spots being smaller than those characteristic of the standard $\underline{R}^{st}$ allele. The two $\underline{R}^{sc}$ alleles were not tested for back mutations to $\underline{R}^{st}$ in homozygous plants, but McWhirter (MGNL 35:142) tested 98 $\underline{R}^{sc}$ mutants for back mutations to $\underline{R}^{st}$ and none were recovered in over one million gametes.

Positive verification of the reconstitution of $\underline{R}^{st}$ in certain of the heterozygous combinations would indicate that: (1) the stippled phenotype is dependent on two or more genetic components, (2) the components of $\underline{R}^{st}$ can be separated and reassembled by crossing over, (3) the component(s) of $\underline{R}^{st}$ carried by the near-colorless crossover mutant was complementary to the one(s) carried by the $\underline{R}^{sc}$ mutants, (4) the component(s) of $\underline{R}^{st}$ carried by the

near-colorless noncrossover mutant, if any, was not complementary to the one(s) carried by the $\underline{R}^{sc}$ mutants, (5) mutations of $\underline{R}^{st}$ to $\underline{R}^{sc}$ and to near-colorless alleles not associated with crossing over involve alterations of a common $\underline{R}^{st}$ component, (6) paramutagenic and nonparamutagenic $\underline{R}^{sc}$ mutants carry the same unaltered components of $\underline{R}^{st}$, and (7) secondary changes may occur in the separation and reassembling of $\underline{R}^{st}$ components as evidenced by the altered phenotype of one of the reconstitued $\underline{R}^{st}$ alleles.

Table 1
Occurrence of reconstituted $\underline{R}^{st}$ in four heterozygous combinations of $\underline{R}^{sc}$ and near-colorless aleurone, green plant mutants, and in two near-colorless, green homozygotes.

Combination of alleles	Total No. of kernels scored	No. of stippled kernels			
		Selected	Verified as $\underline{R}^{st}$	Non-mutant	Not verified
$\underline{R}^{scl}113/\underline{r}^{g}(I)^{3}$	24,459	5	3	1	1
$\underline{R}^{scl}132/\underline{r}^{g}(I)^{3}$	14,877	1	1	0	0
$\underline{R}^{scl}113/\underline{r}^{g}(I)^{2}$	28,033	0	-	-	-
$\underline{R}^{scl}132/\underline{r}^{g}(I)^{2}$	19,952	0	-	-	-
$\underline{r}^{g}(I)^{3}/\underline{r}^{g}(I)^{3}$	22,260	0	-	-	-
$\underline{r}^{g}(I)^{2}/\underline{r}^{g}(I)^{2}$	32,155	0	-	-	-

R. B. Ashman

3. Seed color mutations from $\underline{R}^{r}\underline{R}^{sc}$ heterozygotes.

Three general classes of mutations to or toward colorless aleurone in $\underline{R}^{r}\underline{R}^{st}$ plants have been identified: near-colorless aleurone, green plant; near-colorless aleurone, red plant; and colorless aleurone, red plant. The near-colorless, green mutants do not form a homogeneous group, varying in seed and plant color stability and in their association with recombination between outside markers. Tests have shown that near-colorless mutants possess either all or part of the paramutagenic action of $\underline{R}^{st}$, and that colorless mutants are nonparamutagenic. The apparent association between the near-colorless phenotype and paramutagenic action was examined further in the following test.

Self colored mutants (R^{sc}) from R^{st} are known to vary from non-paramutagenic to as fully paramutagenic as R^{st}. Two paramutagenic R^{sc} mutants, $R^{sc}(1-1)$ and $R^{sc}(1-5)$, and two non-paramutagenic R^{sc} mutants, $R^{sc}(1-2)$ and $R^{sc}(1-9)$, were made heterozygous with standard R^{r}. The four heterozygous combinations were pollinated with r^{g}, wx pollen, and the colorless and near-colorless seed color mutants were selected and grown out for verification. The results are shown in Table 2; data from the earlier test of $R^{r}R^{st}$ are included for comparison.

Table 2
Occurrence of near-colorless and colorless aleurone mutants in $R^{r}R^{st}$ plants and in $R^{r}R^{sc}$ plants of four heterozygous combinations involving the paramutagenic R^{sc} mutants 1-1 and 1-5 and the non-paramutagenic R^{sc} mutants 1-2 and 1-9.

Heterozygous combination	Total No. of kernels scored*	Number of Mutants: Near-colorless, green	Near-colorless, red	Colorless red
$R^{r}R^{st}$	92,820	13	15	14
$R^{sc}(1-1)/R^{r}$	27,621	0	6	4
$R^{sc}(1-5)/R^{r}$	28,568	0	4	8
Pooled	56,189	0	10	12
$R^{sc}(1-2)/R^{r}$	27,669	0	0	7
$R^{sc}(1-9)/R^{r}$	18,469	0	0	7
Pooled	46,138	0	0	14

*Adjusted for proportion of selected kernels verified.

The heterozygous combinations involving the two paramutagenic R^{sc} mutants carried proximal and distal outside markers, and all 10 of the near-colorless, red mutants, and 11 of the 12 colorless, red mutants were recombinant for these markers. The combination of outside markers was the same as that obtained in the isolation of these kinds of mutants from $R^{r}R^{st}$ plants, i.e. the proximal marker from the R^{r} chromosome and the distal marker from the R^{st} chromosome. Heterozygous combinations involving the two non-paramutagenic R^{sc} mutants were marked proximally only, and all 14 mutants received the marker from the R^{r} chromosome.

Absence of the near-colorless, green class of mutants in the heterozygotes involving either paramutagenic or non-paramutagenic R^{sc} mutants indicates that mutation of R^{st} to R^{sc} alters an R component essential for the near-colorless, green phenotype, or alters the pairing relationships of the R components in such a way that a crossover necessary for the isolation of such mutants cannot occur.

The recovery of near-colorless mutants from the heterozygous combinations involving the paramutagenic R^{sc} mutants, but not from the heterozygous combinations involving the non-paramutagenic R^{sc} mutants is additional evidence for a close association between the near-colorless phenotype and paramutagenic action.

R. B. Ashman

4. A new dominant mutant.

A dominant mutant, clumped tassel (Ct), has been recovered from inbred M14. This mutant gives a compact, shortened tassel, some dwarfing of the plant and modified ear morphology. The homozygous Ct Ct is not easily recovered. Classification is fair in most backgrounds. Preliminary linkage tests indicate Ct is located on chromosome 8.

L. F. Bauman

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1. Further studies on disjunction at anaphase I of the chromosomes of a trivalent configuration.

In 21 chromosome maize plants carrying a normal chromosome 2, a 2^T chromosome and a T^2 chromosome a genetic test of frequency of nondisjunction at anaphase I of the 2^T and T^2 chromosomes is readily available. From plants carrying recessive ws lg and gl only on the T^2 chromosome the test gives frequency of nondisjunction following crossing over; from plants in which only the 2^T chromosome carries dominant alleles the test gives frequency of nondisjunction regardless of chiasma formation. Results of the former type of test have been published (Genetics 49:69-80, 1964). Data have recently been accumulated from the latter type of test with the expectation that differences might be attributable to the pattern of distribution of univalents. From a total of 922 plants it now appears that the frequency of nondisjunction from the second type of test is very much higher (average 38%) than that found in the first (19%). Even if all the univalents were distributed nondisjunctively at first anaphase,

this would leave an average frequency of 31% nondisjunction from trivalents. It is suspected that genetic background may strongly influence frequency of non-disjunction.

In some stocks a substantial deficiency of 21 chromosome progeny (and excess of 20 chromosome progeny) from non-disjunctive distribution were found (total average = 12%). These deficienceies and excesses were not correlated with mortality (although mortality was high and a potential source of error).

It is thought that they may have resulted from a tendency at metaphase I for trivalents destined to have nondisjunctive distribution to orient so that only the 2^T chromosome is directed toward the basal position. Such a tendency would not have been detected in the previous study and would have resulted in a slight underestimation of frequency of nondisjunction and of crossover frequency.

Further tests are underway. B chromosomes, which are similar in length to the T^2 chromosome, are being added to the stocks for study of their possible effects on disjunction.

M. P. Maguire

2. Recombination studies in maize with segmental substitution from Tripsacum.

Although a segment derived from a Tripsacum chromosome has been found to carry dominant alleles for markers on the short arm of chromosome 2 of maize (ws, lg, gl), crossing over between this segment and the corresponding region of chromosome 2 rarely occurs. Previous results have suggested that in maize stocks which carry this segment as a heterozygous substitution such crossover inhibition is accompanied by an enhanced frequency of crossing over in adjacent regions. Disomic stocks heterozygous for the substitution were constructed to test the frequency of crossing over in the gl B, B sk, and sk v regions. Unfortunately, only pollen from plants heterozygous for sk was available from the tester stock at the appropriate time, and severe spring weather reduced the testcross progenies to a total of 622 plants. Results suggest that the b locus is very near the proximal end of the Tripsacum segment, that the recombination frequency between B and sk is high (13 per cent in these studies) and between sk and v very high (50 per cent). The tests are being repeated with pollen from sk sk plants and hope of low spring mortality,

Similar tests in 21 chromosome plants carrying a normal chromosome 2, a 2^T chromosome and a T^2 chromosome also suffered from high mortality and the use of an Sk sk tester, but similarly suggested high recombination frequency in the sk v region. These tests are also being

repeated. They provided convincing evidence, however, that the T^2 chromosome carries an Sk allele in its Tripsacum region.

M. P. Maguire

3. The relationship of crossing over to chromosome synapsis in a short paracentric inversion.

Frequencies of (any) reverse pairing at pachytene and bridge and/or fragment formation at anaphase I have been compared in three plants heterozygous for inversion 1 Lh (Longley 5083). This inversion is listed by Longley as having breakpoints at .70 and .87 in the long arm of chromosome 1, and is thought to contain well less than 50 crossover units. Pooled data (homogeneous at the 5 per cent level in chi square tests) are as follows:

Plant	Frequency of reverse pairing at pachytene		Combined anaphase I bridge and fragment, and fragment only frequency	
	No.	%	No.	%
1	182/505	36.0	466/1303	35.8
2	149/495	30.1	303/1023	29.6
3	190/544	34.9	426/1244	34.2

Since 2 strand double crossovers within the inversion are rare, the anaphase I data are considered a measurement of crossover frequency within the inversion. Such a close correspondence of frequency of homologous pairing at pachytene and crossover frequency in a region of considerably less than 50 map units is interpreted as further evidence that either crossing over is a precondition for homologous pachytene synapsis or invariably follows pairing of the tested region.

M. P. Maguire

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1. Genetics of tillering.

During 1965 crosses not obtained before because of different dates of maturation were made. Parts of two sets of crosses between the 17 translocations and the 7 tillering stocks were planted out; of these no group showed any segregation of tillering related to waxy versus non-waxy seeds. Studies are continuing during 1966.

Norton H. Nickerson

2. Studies involving the gene rootless.

As reported in the 1965 MGCNL, two states of the gene rootless (rt/rt) apparently occur. The first of these, designated "really rootless," forms only 6-8 adventitious roots in its lifetime, grows 2/3 to 7/8 the size of heterozygous sibs, and develops both a tassel and an ear of normal proportions. The second state, designated "regular rootless," forms no true brace roots, grows about 7/8 the size of heterozygous sibs, does develop quantities of fibrous roots just above nodes at those nodes which are below ground in the early stage of plant development. Selfs of both these types were obtained in 1964 and planted out in 1965; results are summarized below.

a. Hilling of partially-mature plants did not alter root development in any way over that in unhilled sibs. The conclusion is that if light has any effect on root development the effect is manifested early in plant ontogeny.

b. A scale of root development from 0 (6-8 adventitious roots totally) to 5 (the normal mass of 60+ adventitious roots at 7 or more nodes) has been developed. A score of 3 means no roots above ground; 4 means some are visible above ground. Typical results for plants of both rootless genotypes are given below. Controls are treated with distilled water, given daily in the same amount (1 ml) as the solutions of the growth substances TIBA (tri-iodo benzoic acid) and IAA (indole acetic acid).

	Numbers of plants in each category					
Scale of root development	0	1	2	3	4	5
Family 65-43 (really rootless)						
H_2O (control)	5	33	13	2		
daily TIBA(250µg)			8	18	7	
daily IAA (500µg)	3	9	6			

cont'd.

Scale of root development:	Numbers of plants in each category 0	1	2	3	4	5
Family 65-45 (really rootless)						
H_2O (control)	15	13	1			
daily TIBA (250µg)	3	1	8	12	2	
daily IAA (500µg)	13					
Family 65-47 (regular rootless)						
H_2O (control)	8	11	31	18	2	
daily TIBA (250µg)	4	5	9	5		
daily IAA (500µg)	2	3	4			

These data show that TIBA, which makes normal plants rootless, enhances root development in really rootless plants and has less effect on regular rootless ones. IAA tends to enhance the expression of rootlessness. The conclusion reached is that the gene rootless forms no roots because of an excess production of IAA, rather than because of a dearth of this substance in the nodal meristems of the lower stalk of the plant as was thought heretofore. On this assumption the above data are explainable in terms of known effects of TIBA on IAA concentration and root initiation in normal systems.

c. Differing dates of tassel anthesis in the two types of rootless plants also occur, as summarized in the following table:

Days in August, 1965	10	11	12	13	14	15	16	17	18	19	20
65-43 (really rootless) (H_2O)	15		17	8		2	4		1		
65-47 (regular rootless) (H_2O)						1	13	11	28	3	1

IAA and TIBA, based on limited data, show no clear effects on altering the dates of anthesis of either state of the rootless gene. NAA treatment of 500 µg per day completely

prevents anthesis in the tassels of both types of rootless plants. Studies are continuing.

Norton H. Nickerson

3. Studies involving the gene Knotted.

Development of double-knotted plants Kn/Kn was checked by selfing three suspected plants and at the same time crossing each one to standard L317/W23 female, in 1964. During 1965 these three checks, of 64, 65, and 61 plants, all developed knots. They were not as well expressed in the first group, possibly indicating the presence of modifying genes. Thus the assumption is that double-knotted plants were indeed obtained. These stocks were treated with various growth substances. TIBA had little effect on masking or altering the expression of Kn in either Kn/+ or Kn/Kn plants. NAA does suppress or retard elongation and development of knots; since there is also a slowing down of tissue maturation with this chemical, the "suppression" of Kn may simply mean non-development of knots which ordinarily appear relatively late in ontogeny. IBA and IAA had no demonstrable effects, but the number of plants employed were small. When NAA was administered in a 5% solution of DMSO (Dimethyl sulfoxide), penetration of the NAA was apparently enhanced, as Kn manifestations practically disappeared. A side effect of DMSO at this level was death of areas within the leaves, however. Controls treated with 5% DMSO only showed a slight dwarfing as well as death in leaf areas. The numbers of plants involved in the studies, distributed among 20 seed stocks, are given in the following table:

Substance:	H_2O (control)	TIBA	NAA	IBA	IAA	DMSO	DMSO-NAA
No. of plants treated daily (includes both Kn/+ and Kn/Kn)	227	145	92	23	32	20	46

Anatomical study of knots from Kn/Kn and from Kn/+ leaves shows no vascular proliferation. All cells are essentially the same size as their counterparts in other places of the same leaf. Compared to cells from +/+ sibs, these cells are smaller with thinner walls, but there are many more of them. In knotted plants treated with NAA, there is a progressive lack of development of the transfusion tissue around

the vascular bundles. In general, cells from Kn/+ and Kn/Kn plants are less differentiated and more numerous than in normal leaves, as they have been so far examined. A knot seems to be a section of leaf in which there is maintained the same relative cell patterns as in the rest of a leaf, but there is conspicuously less differentiation of certain cell types. Studies are continuing.

Norton H. Nickerson

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Department of Botany

1. Studies at the su_1 locus.

As part of an undergraduate research project, four genotypes of maize pollen were tested for their starch content. The starch was extracted with perchloric acid, hydrolysed and then measured using the Somgi method of determining reducing sugars.

Three samples of each genotype were used for the starch determination while a fourth was used to measure the dry weight of the pollen.

Results

Genotype	Fresh wt.	% H_2O	Dry Weight	% Starch (dry wt.)
+ +	0.100 gm.	36%	0.064	14.8
su_1 +	0.100 gm.	27%	0.073	11.0
+ su_2	0.100 gm.	23%	0.077	10.9
su_1 su_2	0.100 gm.	32%	0.068	5.3

It is interesting to note that these results resemble those obtained by R. G. Creech for 28 day old endosperm (Genetics 52: 1175):

Genotype	% Starch (dry weight)
normal	73.4
su_1 su_1 su_1	35.4
su_2 su_2 su_2	64.6
su_1 su_1 su_1 su_2 su_2 su_2	18.9

The analysis of hexoses, pentoses, disaccharides and H_2O soluble polysaccharides is continuing for the pollen genotypes listed above.

John Vandermeer

2. Preliminary biochemical investigation of the yg_2 locus.

As part of an undergraduate research project, a study of the leaf pigments of yg_2/yg_2, $yg_2/+$, and $+/+$ plants was initiated. Using spectrophotometric techniques, the amount composition of chlorophyll A, chlorophyll B, xanthophylls and carotene were determined after extraction from fresh tissue.

At maturity (pollen shedding) the following observations were made:

(1) yg_2/yg_2 possessed less chlorophyll B and carotene than $+/+$, on a dry weight basis;

(2) $+/+$ possessed more xanthophyll than yg_2/yg_2;

(3) the chlorophyll A content was the same in both genotypes;

(4) $yg_2/+$ presented the spectra of the $+/+$ genotype.

Chlorophyll A and B were estimated for 55 day old yg_2/yg_2 and $+/+$ plants (11 leaves) grown under controlled supplemental lighting, November-December 1965. The top three leaves demonstrated the differences noted above, whereas the middle four leaves from the two genotypes were not different.

Comparison of tetraploid vs. diploid stocks ($yg_2/yg_2/yg_2/yg_2$ vs. yg_2/yg_2) did not yield any differences in the relative amounts of pigment per unit dry weight or the distribution of the pigment.

M. C. Weir

3. Smear technique for obtaining large numbers of metaphases in corn root tips.

The method for root tip smears of Wolff and Luippold (Stain Technology 31: 201-205, 1956) was modified for corn as follows:

(1) Orient seeds with embryos up on moistened filter paper in Petri dishes. Incubate 36-40 hours under intense, constant light at 30°C. (The radicle should be 3-5 mm in length).

(2) Transfer the seed to a new dish, same conditions, except that a 0.2% colchicine solution has been added to the filter paper. (A drop of tween-80 added to the solution seems to yield more cells in metaphase). Incubate for 8 hours.

(3) Fix immediately in Carnoy's. Transfer to fresh Carnoy's and incubate for 24 hours at 60°C.

(4) Pour off Carnoy's. Rinse thoroughly with distilled water.

(5) Hydrolyze in 1NHCl at 60°C for 20 minutes. Rinse thoroughly with distilled water.
(6) Place in leuco-basic fuchsin for 30-45 minutes.
(7) Wash in distilled water for 1 hour.
(8) If the root tip has not previously been cut off, excise and place in a 5% cellulose - 5% pectinase solution at pH 4.2 for 2 hours.
(9) Cut the deeply stained tip onto a clean slide and macerate in a drop of propionic carmine. A flattened end of an ivory stick is suitable for maceration and spreading.
(10) Heat gently.
(11) Add cover slip and flatten with the rounded end of a glass or steel rod. Invert onto bibulous paper and apply pressure with thumbs.
(12) Seal. Temporary smears may be stored several months in a freezer. Temporary smears may be made permanent by conventional techniques.

R. M. Brown

4. The mitotic karyotype of maize.

Using the smear technique outlined in the previous note, a number of metaphases suitable for photographing have been studied. Two notable features have emerged as the karyotype has been prepared.
(a) A definite gradation in chromosome size from large, nearly metacentric chromosomes to small, submetacentric chromosomes; and consistent arm ratios of the three classes of chromosomes - large, metacentric; medium, submetacentric and short, submetacentric.
(b) A secondary constriction with satellites is easily seen in most preparations in two chromosomes.

At present we are interpreting the karyotype on the basis of the measurements (Rhoades, Jour. of Heredity 41: 58-67, 1950) presented for pachytene chromosomes. Arm ratios quite similar to those reported for pachytene chromosomes are found in the mitotic metaphases. We are attempting to further qualify the mitotic karyotype with the use of trisomics.

R. M. Brown
D. B. Walden

5. Influence of calcium concentration on pollen germination.

A suitable medium for the germination of "Seneca 60" ($\underline{su}_1/\underline{su}_1$) hybrid corn pollen was reported in MCNL 39: 169. This medium, consisting of 0.35 M sucrose, 100 ppm H_3BO_3 and 300 ppm $CaCl_2.2H_2O$, may be used simply as aqueous drops or with either 0.7% Difco Special Agar-Noble or 1% to 2% methyl cellulose. Calcium ion has been shown to be required for corn

pollen germination and a series of experiments were designed to investigate changing calcium concentrations on per cent germination.

Fresh "Seneca 60" pollen produced in the greenhouse was placed on drops of media in well-slides and allowed to germinate for 30 minutes. Densities were kept well above those which might reduce germination because of a "population effect" (see following note).

Sucrose and boron concentrations were held constant at the levels given above. The data were recorded and stored on film.

The addition of 1% methyl cellulose to the aqueous medium had no influence on per cent germination. Agar was not tried. Only a trace of germination was recorded at 50 ppm calcium and below. Optimum concentrations were in the range 300-400 ppm. Above 400 ppm the per cent germination slowly fell off but was still above 50% of optimum at 900 ppm. 100 ppm and 200 ppm gave 20-40% and 60-80% of optimum values, respectively.

F. S. Cook
D. B. Walden

6. The "population effect" in corn pollen germination.

Brewbaker and Kwack (1963) described a pollen population effect whenever pollen grains are germinated in vitro. Small populations germinate poorly or not at all under conditions which support good germination of larger populations. Although no population effect with corn pollen could be shown on the medium to which Noble agar had been added (MNL 39: 170), the effect was evident with small populations on the aqueous medium containing only 0.35 M sucrose, 100 ppm H_3BO_3 and 200 ppm $CaCl_2,2H_2O$, i.e. sub-optimal concentrations of calcium.

Greenhouse-produced "Seneca 60" corn pollen was placed in wells of slides containing 20 µl of the medium and allowed to germinate for 30 minutes. Numbers of grains and per cent germination were counted under a dissecting microscope. Germination in all experiments was compared with that of "high" populations.

Below 10 grains per 20 µl there was only a trace of germination. At 100-150 grains per 20 µl, per cent germination approached a maximum. These results are similar to those reported by Brewbaker and Kwack for the pollen of Saintpaulia, Haworthia and others.

F. S. Cook
D. B. Walden

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1. A collection of pericarp factors.

A collection of factors conditioning pericarp pigmentation has recently been sent to the National Seed Storage Laboratory in Fort Collins, Colorado. These factors have been incorporated into a common genetic background by successive backcrossing to the colorless pericarp white cob inbred strain 4 County 63. The collection represents a comprehensive selection of diverse origin and phenotype. One group of factors form a continuous series from dark red through orange and tan to colorless; others group according to degree of variegation or mosaicism. The factors are individually described in the accompanying tables. Seed is now available, from Fort Collins, for genetical studies. The 4 Co 63 inbred strain is of a maturity suited to Southern Wisconsin.

R. A. Brink
Derek Styles

Collection of factors conditioning pericarp pigment incorporated, by successive backcrossing, into the colorless pericarp white cob inbred line 4 County 63.

A. Uniform Pericarp Pigment--This collection represents a continuous series from dark red through orange and tan to colorless or near colorless. Although not tested individually for allelism, they are probably all alleles of the P locus. They are ordered according to intensity of pericarp pigment: "11" representing the darkest red in the collection, and "1" representing colorless.

Field No.	Pericarp intensity	Cob color	Available information on origin and source	No. backcross generation to 4 Co 63	Notes
65-CFS-33	11	Red	Red husked pointed popcorn	5	
65-CFS-272	11	Red	Variegated stock from Caingang Ivai aberto A; from H. C. Cutler, Nov., 1949; originally from F. C. Brieger, Sao Paulo, who raised it from Indian seed from Parana, Southern Brazil.	5	
65-CFS-303	10-11	Red	Peru, 1948; from Paul Mangelsdorf, Harvard Univ., April, 1949.	6	
65-CFS-548	10-11	Red	M13 PP	4	Possibly linked with $\underline{zb}_4$ $\underline{ts}_2$
65-CFS-36	10	Red	90 PP	5	
65-CFS-61	10	Red	Argentina red popcorn; from W. L. Brown, Pioneer Hybrid Corn Co., Jan., 1952.	6	
65-CFS-237	10	Red	Variegated stock from Ames, P. I. 168001	5	May be unstable

65-CFS-266	10	Red	Georgia, U.S.A.	5	
65-CFS-305	10	Red	M13 PP	6	
65-CFS-43	9-10	Red	R. W. Richardson, Minn., March, 1951.	5	
65-CFS-71	9-10	Red	Brazil-18; ex Caingang stocks from F. C. Brieger, Sao Paulo, Brazil, April, 1951.	4	
65-CFS-79	9-10	Red	Brazil-23; ex Caingang stocks from F. C. Brieger, April, 1951.	4	
65-CFS-238	9-10	Red	Madison variegated stocks, of U.S.A. origin	3	"Unstable"-- occasional lighter or darker striping on uniform background.
65-CFS-315	9-10	Red	Mosaic from Dr. Matlock, Arizona	6	May be unstable.
65-CFS-316	9-10	Red	Dakota Squaw Mosaic from A. M. Strommen, Agric. Exp. Sta., Spooner, Wis.	6	
65-CFS-53	9	Red	Brazil-1A; ex Caingang stocks from F. C. Brieger, April, 1951.	5	
65-CFS-186	9	Red	Dakota Squaw Mosaic from A. M. Strommen	3	Linked T1-2b
65-CFS-302	9	White	Mosaic from Paul Weatherwax, April, 1945 as 1872.119 Originally from Huancayo, Peru, 1945.	5	
65-CFS-332	9	White	Bloody Butcher; from T. C. Warwick & Son, Blenheim, Ontario, Canada.	6	

Field No.	Pericarp intensity	Cob Color	Available information on origin and source	No. backcross generation to 4 Co 63	Notes
65-CFS-75	8-9	Red	Brazil-21; ex Caingang stocks from F. C. Brieger, April, 1951.	4	
65-CFS-293	8-9	Pale	Mosaic from A. Johnson, Waukesha, Wisconsin.	6	Pale crown
65-CFS-327	8-9	Red	R. Andrew, Agronomy Dept., Univ. of Wisconsin.	6	
65-CFS-342	7-9	White	Northwestern Dent variety from Olds' Seed Co. Madison, Wisconsin, April, 1950.	6	Pale crown
65-CFS-57	8	Red	Brazil-17; ex Caingang stocks from F. C. Brieger, April, 1951.	4	
65-CFS-181	8	Pale	Pisac, Peru; from H. C. Cutler, Missouri Bot. Garden, St. Louis, November, 1949.	3	Pale crown, linked T1-2b.
65-CFS-320	8	Red	"Hardware Orange" mutant from colorless; of unknown parentage; from N. P. Neal.	5	
65-CFS-334	8	White	Bloody Butcher from O. & M. Seed Co., Green Spring, Ohio, March, 1948.	6	Pale crown
65-CFS-324	7-8	White	W1376; from N. P. Neal, Agronomy Dept., Univ. of Wisconsin.	6	
65-CFS-140	7	Red	Quebec 36; from R. Brawn, Macdonald College, P.Q., Canada, April, 1951.	4	Possibly linked zb_4 ts_2

65-CFS-321	7	Red	A mutant in W153, a Wisconsin inbred strain.	6	
65-CFS-330	7	White	Ames, Iowa, P.I. 183765, Jan. 1951.	5	
65-CFS-350	7	White	Quebec 63M; from R. Brawn.	6	
65-CFS-40	6-7	Red	W153, from J. Maloney, Agronomy Dept., Univ. of Wisconsin, November 1951.	5	Darkly pigmented silk scar.
65-CFS-325	6-7	White	Mosaic from Will's Rainbow Flint, North Dakota.	6	Pale crown
65-CFS-364	6-7	White	Tesuque, N. Mexico; from E.S. Anderson, Missouri Bot. Garden, January, 1948.	6	Pericarp slightly "grained".
65-CFS-317	6	Red	Charasani, Bolivia; from H. C. Cutler, March, 1949.	6	
65-CFS-336	5-6	White	Bloody Butcher; from O. & M. Seed Co., Green Spring, Ohio, March, 1948.	6	
65-CFS-263	5	White	Uncertain origin.	7	
65-CFS-369	5	White	Mexico, Sin 2; from E. J. Wellhausen, Rockefeller Foundation, Mexico City, November, 1950.	5	
65-CFS-47	4-5	Red	J. H. Lonnquist, Univ. of Nebraska, Lincoln, December, 1950.	5	
65-CFS-345	4-5	Red	Bloody Butcher; from T. C. Warwick & Son, Blenheim, Ontario, April, 1948.	6	
65-CFS-285	3-5	Pale	Ecuador 1184b; from Paul Mangelsdorf, April, 1949.	5	Darkly pigmented silk scar.

Field No.	Pericarp intensity	Cob Color	Available information on origin and source	No. backcross generation to 4 Co 63	Notes
65-CFS-576	3	Red	Unknown	4	Linked T1-2b, and possibly $\frac{zb_4}{ts_2}$
65-CFS-381	2-3	Red	Unknown	5	Linked T1-2b
65-CFS-319	2	Red	Mosaic from Paul Weatherwax, April, 1949, as: 1872-119 Huancayo, Peru, 1945.	5	
65-CFS-365	2	Red	Krug 1-1-1; from W. L. Brown, Pioneer Hi-Bred Corn Co., Dec. 1950.	6	
65-CFS-308	1-2	Red	Cologaita, Potosi, Bolivia; from H. C. Cutler, March, 1949.	5	
65-CFS-309	1-2	Red	Bloody Butcher; from O. & M. Seed Co., Green Spring, Ohio, March, 1948.	6	
65-CFS-376	1	White	Probably the 4 Co 63 inbred strain itself.	5	Linked T1-2b (1S.43; 2S.36)

B. Variegated--Arbitrarily grouped into "Dark", "Medium", "Light", and "Very Light", according to the degree of variegation. Such groupings are not meant to indicate known modulator dosage.

Field No.	Degree of pericarp variegation	Available information on origin and source	No. backcross generation	Notes
65-CFS-138	Dark	Jose-17; from E. S. Anderson, Missouri Bot. Garden, September, 1948.	4	Possibly linked $\underline{zb}_4$ $\underline{ts}_2$
65-CFS-155	Dark	Magdelena-23, Colombia; from Paul Mangelsdorf, April, 1949.	4	Possibly linked $\underline{zb}_4$ $\underline{ts}_2$
65-CFS-195	Dark	Magdelena-23, Colombia; from Paul Mangelsdorf, April, 1949.	5	Linked T1-2b
65-CFS-250	Dark	Quetzaltenango above Zunil, Guatemala; from E. S. Anderson.	5	
65-CFS-252	Dark	Zapalate, Chico; from E. G. Anderson, April, 1949.	5	
65-CFS-278	Dark	Brazil 6a; ex Caingang stocks from F. C. Brieger, April, 1951.	5	
65-CFS-96	Medium	Open-pollinated variety; from J. D. Brown.	4	Linked T1-2b
65-CFS-110	Medium	Zapalate, Chico; from E. G. Anderson, April, 1949.	4	Possibly linked $\underline{zb}_4$ $\underline{ts}_2$
65-CFS-245	Medium	Cotogaita, Potosi, Bolivia; from H. C. Cutler, March, 1949.	6	
65-CFS-246	Medium	Reyes, Bolivia; from H. C. Cutler, March, 1949.	6	
65-CFS-249	Medium	Peru 1233; from Paul Mangelsdorf, April, 1949.	5	

Field No.	Degree of pericarp variega-tion	Available information on origin and source	No. backcross generation	Notes
65-CFS-253	Medium	Peru 1209; from Paul Mangelsdorf, April, 1949.	5	
65-CFS-255	Medium	Chacan, Peru 2276; from Paul Weatherwax, April, 1949.	5	
65-CFS-259	Medium	Linkenmeyer Bros., Ricerville, Iowa; from E. S. Anderson, June 1948.	5	
65-CFS-262	Medium	Uncertain origin	6	
65-CFS-264	Medium	Crow Creek, U.S.A.	6	
65-CFS-265	Medium	Minnesota stripe from R. R. St. John, Purdue University, March, 1948.	6	
65-CFS-273	Medium	Georgia, U.S.A.	5	
65-CFS-275	Medium	Brazil-2a; ex Caingang stocks from F. C. Brieger, April, 1951.	3	
65-CFS-276	Medium	Brazil 5a; ex Caingang stocks from F. C. Brieger, April, 1951.	5	
65-CFS-279	Medium	Brazil 7a; ex Caingang stocks from F. C. Brieger, April, 1951.	5	
65-CFS-280	Medium	Brazil 8b; ex Caingang stocks from F.C. Brieger, April, 1951.	5	
65-CFS-282	Medium	Brazil 13; ex Caingang stocks from F.C. Brieger, April, 1951.	5	
65-CFS-283	Medium	Brazil 19; ex Caingang stocks from F. C. Brieger, April, 1951.	3	
65-CFS-284	Medium	Mexico 1a ex Chis 101; from E. J. Wellhausen, Mexico City, November, 1950.	5	

65-CFS-286	Medium	Caingang Ivai aberto A; from H. C. Cutler, Nov., 1949. Originally from F. C. Brieger, Sao Paulo, who raised it from Indian seed from Parana, S. Brazil.	5	
65-CFS-497	Medium	D. F. Jones, Conn. Agric. Expt. Sta., New Haven, Conn.	5	Possibly linked $\underline{ts}_2$
65-CFS-501	Medium	Unknown origin.	5	Possibly linked $\underline{ts}_2$
65-CFS-506	Medium	J. D. Brown	6	Possibly linked $\underline{zb}_4$
65-CFS-116	Light	Peru 1233; from Paul Mangelsdorf; April, 1949.	4	Possibly linked $\underline{zb}_4$ and $\underline{ts}_2$.
65-CFS-256	Light	J. D. Brown, April 15, 1948.	5	
65-CFS-261	Light	Cornell; from R. Andrew, Agronomy Dept., Univ. of Wisconsin.	6	
65-CFS-281	Light	Brazil-12; ex Caingang stocks from F. C. Brieger, April, 1951.	4	
65-CFS-226	Very light	Ames, Iowa; P.I. 168001, Jan., 1951.	5	

C. Mosaic

Field No.	Available information on origin and source	No. backcross generations	Notes
65-CFS-286	Calea, Near Cuzco, Peru-2169; from Paul Weatherwax, April, 1949.	5	
65-CFS-287	From Dr. Matlock, Arizona.	5	
65-CFS-288	Bolivia: from W. S. Brown, October, 1948.	4	
65-CFS-289	Possible origin: San Juan, Pueblo, New Mexico; from Paul Weatherwax, April, 1949.	5	
65-CFS-290	Chas. Fountaine (U.S.A.).	5	
65-CFS-291	R. R. St. John, Purdue University, March, 1948.	5	
65-CFS-292	Purchased from Madison store by R. Brawn.	5	
65-CFS-294	Will's Rainbow Flint (North Dakota).	5	
65-CFS-297	Tesuque, New Mexico; from E. S. Anderson.	5	
65-CFS-301	R. W. Richardson, Minnesota, March, 1951.	5	
65-CFS-556	Chas. Fountaine (U.S.A.)	6	Linked T1-2b

D. Miscellaneous

Field No.	Description	Available information on origin and source.	No. backcross generations	Notes
65-CFS-25 65-CFS-29 65-CFS-124	$\underline{P}^{\underline{ov}}$ $\underline{ov}$ - Orange variegated. Varying degrees of light & dark variegations on an orange-red background. For further information refer to F. Valentine, 1957, Ph.D. Thesis, U. of Wisconsin Library, Madison.	Ricerville, Iowa F. Valentine F. Valentine	5 5 4	Possibly linked $\underline{zb}_4$ $\underline{ts}_2$
65-CFS-167	Orange background, grained with red.	Province of Boyaca, Colombia, collected by Schultes; from Paul Mangelsdorf, April, 1949.	5	Possibly linked $\underline{zb}_4$
65-CFS-355	Orange background, grained with red.	Province of Boyaca, Colombia, collected by Schultes; from Paul Mangelsdorf, April, 1949.	7	
65-CFS-360	Diffuse, variable expression.	Peru, 1948; from Paul Mangelsdorf, April 1949.	5	$\underline{P}$ allele is red pericarp, red cob.

Field No.	Description	Available information on origin and source.	No. backcross generations	Notes
65-CFS-80	Purple-red pericarp	"$\underline{r}^{ch}$: Burbank"		Stock carries Pl. Has not been verified as an R allele.
65-CFS-69	Purple flushed pericarp and purple cob core. Probably a Pl allele.	Venezuela-1. var. Refugio: from Obregon, 1951.	4	

2. Enhancement of R expression in plants hemizygous for the R locus.

In the 1965 News Letter it was stated that paramutable R alleles are metastable, i.e., they have a capacity to vary heritably in plants not carrying an overtly paramutagenic allele. One evidence of such metastability is that alleles conditioning a mottled phenotype in single dose are enhanced in level of action toward self-color when maintained through successive generations heterozygous with a recessive r allele. R^g_2 (a plant color mutant from standard R^r, comparable with standard R^r in paragenetic properties) has been maintained for three successive generations in stocks heterozygous with r^r, and also in otherwise comparable stocks hemizygous for the R locus. Enhancement has occurred in parallel fashion in both cases. The mating scheme consisted of an initial pollination of $R^g_2R^g_2$ on silks of r^r r-x_1 plants (r-x_1 = deficiency in chromosome 10 spanning the R locus), followed by recurrent pollinations of $R^g_2r^r$ and R^g_2 r-x_1 sibs on r^r r-x_1 females. Mean single dose aleurone scores for the parental $R^g_2R^g_2$ stocks ranged from 5.38 to 5.72 (seven class scale; 1 = colorless, 7 = self-colored). Mean scores from two $R^g_2r^r$ lines after three generations of heterozygosity were 6.46 and 6.50. Mean scores from two comparable R^g_2 r-x_1 lines after three generations of hemizygosity were 6.61 and 6.24. It appears, therefore, that enhancement may occur autonomously; i.e., it is not of necessity directed by the partner allele as with paramutation of R to R' in R R^{st} heterozygotes.

Derek Styles

3. Complete reversion of R'.

$R^g_2R^{st}$ plants from $R^g_2R^g_2$ X $R^{st}R^{st}$ matings were used as pollen parents in crosses with r^r r-x_1 plants (r-x_1 = deficiency in chromosome 10 spanning the R locus). R^g_2 r^r and R^g_2 r-x_1 sibs were then used to establish R' lines which were further subdivided at each generation by recurrently mating with r^r r-x_1 females and separating again into R'r^r and R' r-x_1 sublines. A number of R' lineages were obtained in this manner, some of which were successively heterozygous (R'r^r), and some successively hemizygous (R' r-x_1). As there was no consistent difference in reversion pattern between heterozygous and hemizygous lineages, the separation into R'r^r and R' r-x_1 classes at each generation served only as a basis for establishing new sublines. Change in R' aleurone expression was followed by testcrossing representatives of each lineage at each generation on W23 r^gr^g females.

Testcross scores of homozygous $R^g_2R^g_2$ plants average ca. 5.50 (seven class scale; 1 = colorless, 7 = self-colored). The R' class on testcross ears from the three $R^g_2R^{st}$ plants used to start this experiment scored 2.10, 2.04, and 2.72.

Reversion toward the original R expression occurred at each generation and was consistent on the average, but the amount of reversion from one generation to the next within any one subline was irregular and unpredictable. The overall mean scores from all sublines in the first, second and third generations were 3.86, 4.28, and 4.72 respectively. Forty-eight sublines had been established by the third generation, and although expressions within sublines were relatively uniform, their mean scores ranged from 2.22 to 6.39. Thus in some lineages there had been essentially no reversion in three generations. In other lineages, however, reversion had progressed to the point that the reverted R'had a mean score higher than that of the parental R^gR^g stock. Further "reversion of R'" in this case could be described equally well as "enhancement of R". The phenomenon of R' reversion further reflects the innate metastability of paramutable alleles.

Derek Styles

4. Paramutation of standard R^r in $a_1a_1;R^rR^{st}$ and $c_1c_1;R^rR^{st}$ plants.

Evidence so far indicates that paramutation of standard R^r in R^rR^{st} heterozygotes occurs in somatic tissues, and that there is no direct correlation between the pigmenting action of paramutagenic alleles and their paramutagenicity. A small scale test has been conducted to determine whether the actions of other genes concerned with pigment production have any effect on the process of paramutation. Matings were of two types:

Mating	Class of interest	No. of Plants	Aleurone color score when testcrossed on W22 ACr^gr^g ♀♀
$A_1a_1;R^{st}r^r$	$A/-;R^rR^{st}$	7	3.21
X $A_1a_1R^rr^g$	$a\ a;R^rR^{st}$	6	3.00
$C_1c_1;R^{st}r^r$	$C/-;R^rR^{st}$	15	3.20
X $C_1c_1;R^rr^g$	$c\ c;R^rR^{st}$	8	3.28

There is no indication that overt function of the A_1 or the C_1 gene is a requirement for paramutation of standard R^r in R^rR^{st} heterozygotes.

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Addendum:

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1. Further studies on spontaneous chromosomal variation in Coix aquatica.

Spontaneous chromosomal variation was observed in certain populations of Coix aquatica from India (Maize News Letter, 1965). With a view to isolate different cytologically abnormal types the several collections of Coix aquatica from India were grown intermixed and open pollinated. Seed was collected from a male sterile plant in the field and, in the progeny raised, six plants cytologically checked were all found to be heterozygous for a translocation. Thereafter plants were grown in isolation for two generations and in the population raised from seed of the second generation 18 plants were cytologically checked which showed the following categories:

Cytological Class	Remarks	No. of Plants
2n = 10	Meiosis normal	6
2n = 15	Triploid	1
2n = 20	Tetraploid	2
2n = 13	Aneuploid with bridge and fragment at anaphase I	1
2n = 17	Aneuploid with association of three and four chromosomes at diakinesis and metaphase I	1
2n = 10	Bridge and fragment at anaphase I	2
2n = 10	Ring or chain of four chromosomes at diakinesis and metaphase I	3
2n = 10	Ring or chain of four chromosomes at diakinesis and metaphase I; bridge and fragment at anaphase I	1
2n = 10	Desynaptic; eight to ten univalents at diakinesis and metaphase I	1
	Total	18

The progeny consists of plants with 10, 13, 15, 17 and 20 chromosomes as somatic number. Plants with the aneuploid numbers (13 and 17) and the desynaptic plant were completely sterile and did not set seed. The rest were partly fertile. The plants with 2n =10 (desynaptic) and 2n = 13 were dwarf, and had a bushy habit with narrow, short, thick, and dark green leaves. The plant with 2n = 13 had aborted ovaries also. Plants with 2n = 15, 2n = 17 and 2n = 20 all showed the gigas characters usually associated with polyploidy. From the occurrence of chromosome numbers varying from 10 to 20 in this progeny it appears that in Coix aquatica gametes with n, 2n and intermediate numbers as well function successfully in fertilization.

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M. Krishna Rao

2. Meiosis in a spontaneous tetraploid of Coix aquatica.

Two spontaneous tetraploids with 2n = 20 were located in the progeny of a population of Coix aquatica known to contain plants heterozygous for translocations (See this News Letter: Further studies on spontaneous chromosomal variation in Coix aquatica). Chromosome pairing, in one of the two plants, was studied at metaphase I in 40 nuclei. In addition to bivalents, trivalents and quadrivalents, associations of five, six and eight chromosomes were present. Univalents were also observed. The mean frequency of chromosome pairing was 0.05_{VIII}, 0.2_{VI}, 0.075_{V}, 1.6_{IV}, 0.4_{III}, 4.9_{II}, 0.625_{I}. In only one case two associations of six chromosomes (one ring and one chain of six) were observed in a cell; otherwise, as at diakinesis, only one association of five or more chromosomes occurred per cell. Probably during open pollination of plants heterozygous for translocations fusion of two gametes with 2n chromosome number gave rise to this tetraploid.

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3. Further studies on apomixis in Coix aquatica.

In last year's News Letter (1965) Venkateswarlu and Chaganti reported apomixis in Coix from attempted crosses between maize and Coix. The following additional observations have been made in this regard. In Coix aquatica it has been observed that at the upper nodes on a culm both male and female flowers are produced while at the lower nodes, down from the fifth or sixth node, only female flowers are produced which are suspected to set apomictic seed. Embryo sacs were studied in squash preparations according to the method of Bradley (1948) from these flowers. Preliminary observations revealed occurrence of two to three embryo sacs per ovule all of which were four nucleate. This is suggestive of the occurrence of apomixis in Coix aquatica.

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4. Chromosome pairing in autopolyploid Coix lachryma-jobi.

Autopolyploidy was induced in Coix lachryma-jobi by the following method. Seedlings at a four to five leaf stage were root treated with 0.4% aqueous solution of colchicine for 24 hours. After the treatment roots were washed in tap water over several hours and the seedlings were transplanted and kept in the greenhouse till they recovered from the shock of the treatment. Of the 25 seedlings treated only four survived. Though all chromosome counts in squashes of pollen mother cells showed only the diploid number, the high percentage of seed sterility and the occurrence of some big sized seeds led us to suspect induction of sectorial polyploidy. From the seeds of these plants 13 plants were raised and one was more vigorous from the early seedling stage and showed gigas characters. A chromosome count of n = 20 in dividing pollen mother cells confirmed its tetraploid nature.

Chromosome pairing was studied in 40 nuclei at metaphase I. On the whole 212 quadrivalents, three trivalents, 369 bivalents and five univalents were observed. The mean frequency of chromosome pairing was observed to be 5.3_{IV}, 0.075_{III}, 7.5_{II} (ring type), 1.725_{II} (rod type), 0.125_{I}. Thus, on an average more than 50 per cent of the chromosomes of the complement regularly pair as quadrivalents. The number of quadrivalents varied from two to 10 per cell.

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1. Regulation of catechol oxidase.

Investigations on the genetic control of catechol oxidase in maize have revealed the existence of three distinct classes. Type A is designated as the constitutive form since in inbred lines of this class the enzyme is regularly synthesized in the plumule of the young seedling. Type B is classified as the inducible form. No active enzyme occurs in the seedlings; however, active enzyme is produced if the seeds are treated with maleic hydrazide prior to germination. Type C is the non-inducible form. The enzyme is not detected in either

control or maleic hydrazide treated seedlings. This pattern is reversed in the aerial roots. Type A plants which contain active enzyme in the untreated seedlings do not show the enzyme in the aerial roots while type B which does not contain active enzyme in the untreated seedlings forms enzyme in the aerial root. Type C is deficient in both seedlings and aerial roots. These preliminary studies suggest that plants of type C contain an inactive mutant form of the catechol oxidase gene while types A and B contain active genes. However, the A and B lines have different allelic forms which show differential activity in various tissues of the plant.

Toru Endo
Drew Schwartz

III. LINKAGE MAPS OF MAIZE CHROMOSOMES

The following is a linkage map which I propose to use in a forthcoming publication of color pictures of the usable mutants of maize that have been located to chromosome. It includes unpublished information volunteered by a number of corn workers. Most of it is very good but in some cases placement is based on small amounts of data. The reasoning behind this proposal is that publication of any map is subject to error and since this is a good one it is better published, with certain errors understood, than held for an indefinite verification of all facts. This is essentially the same proposal as was made to the Allerton Maize Genetics Conference March 12, 1966, where permission was requested for use with proper qualifications of the data collected by Dr. Earl Patterson in completing this map. I wish to take full responsibility for preparing and presenting the map in this form but to acknowledge that credit for locating the markers goes to many participants in the Maize Coop and that full credit for collecting and arranging the information goes to Dr. Earl Patterson. If you have serious objections or suggestions for changes please write to me before June 1, 1966. The characters in parentheses are those with some doubt as to position. If you have information leading to removal of this doubt or raising a question about established locations, please advise me so the change can be made. Your comments will greatly appreciated.

M. G. Neuffer

Chromosome #1

0	sr_1	Striate
1	vp_5	Vivipary
14	ag	Resistance to grasshoppers
15	ga_6	Gametophyte factor
19	zb_4	Zebra striped
23	ms_{17}	Male sterile
24	ts_2	Tassel seed
26	P	Pericarp & cob color
28	zl	Zygotic lethal
56	as	Asynaptic
58	pa	Pollen abortion
(o)		
64	hm	Susceptible to Helminthosporium carbonum
81	br_1	Brachytic
85	Vg	Vestigial glumes
86	f_1	Fine stripe
102	bz_2	Bronze seed & plant
104	an_1	Anther ear
(108)	ad_1	Adherent
119	Ts_3	Tassel seed
127	Kn	Knotted leaf
(128)	lw_1	Lemon white
135	gs_1	Green stripe
(154)	vp_8	Vivipary
158	Ts_6	Tassel seed
161	bm_2	Brown midrib

Chromosome #2

0	ws_3	White sheath
4	al	Albescent
11	lg_1	Liguleless
(rp)		Susceptible to Puccinia sorghi
30	gl_2	Glossy leaf
(34)	d_5	Dwarf
49	B	Booster of plant color
54	gs_2	Green stripe
56	sk	Silkless
68	fl_1	Floury endosperm
74	ts_1	Tassel seed
o		
83	v_4	Virescent seedling
(107)	w_3	White seedling
(127)	Ht	Resistant to H. turcicum
141	Ch	Chocolate pericarp

NOTE:

( ) = location uncertain

Chromosome #3

0	cr_1	Crinkly leaf
18	d_1	Dwarf plant
25		
26	ra_2	Ramosa ear
31	Cg	Corn grass
(38)	cl_1	
40	rt	Rootless
(45)	Rf_1	Fertility restoration
46	Lg_3	Liguleless
48	Rg	Ragged leaf
50	gl_6	Glossy leaf
55	ts_4	Tassel seed
72	ba_1	Barren stalk
(75)	w_{7748}	White seedling
83	lg_2	Liguleless
(86)	na_1	Nana
111	a_1	Anthocyanin
111.25	sh_2	Shrunken endosperm
122	et	Etched endosperm, virescent seedling
128	ga_7	Gametophyte factor

Chromosome #4

0	de_1	Defective endosperm
35	Ga_1	Gametophyte factor
(55)	st	Sticky chromosomes
56	Ts_5	Tassel seed
(60)	la	Lazy
66	sp_1	Small pollen
71	su_1	Sugary endosperm
73	lo	Lethal ovule
74	de_{16}	Defective endosperm
84	zb_4	Zebra striped
86	gl_4	Glossy seedling
107	Tu_1	Tunicate
112	j_2	Japonica
118	gl_3	Glossy seedling
	(c_2)	Aleurone color
(138)	Idf	Diffuse

Chromosome #5

(0)	am	Ameiotic
14	gl_{17}	Glossy seedling
15	a_2	Anthocyanin
18	vp_2	Vivipary
19	ps	Pink scutellum
21	bm_1	Brown midrib
22	bt_1	Brittle endosperm
25	v_3	Virescent seedling
27	bv_1	Brevis
(35)	ga_2	Gametophyte factor
(37)	ae	Amylose extender
46	pr	Red aleurone
	(gl_8)	Glossy seedling
	(lw_2)	Lemon white
55	ys_1	Yellow stripe
87	v_2	Virescent seedling

Chromosome #6

(0)	rgd	Ragged leaf
4	po_1	Polymitotic
17	Y_1	Yellow endosperm
17	pb_1	Piebald
(19)	ms-si	Male sterile-silky
37	pg_{11}	Pale green seedling
(43)	Dt_2	Dotted
48	Pl	Purple plant color
49	Bh	Blotched aleurone
(57)	su_2	Sugary endosperm
58	sm	Salmon silk
(59)	Pt	Polytypic
68	py	Pigmy

Chromosome #7

0	Hs	Hairy sheath
16	o_2	Opaque endosperm
18	y_8	Lemon yellow endosperm
20	in	Intensifier
24	v_5	Virescent seedling
(25)	vp_9	Vivipary
32	ra_1	Ramosa ear
36	gl_1	Glossy leaf
46	Tp_1	Teopod
50	sl	Slashed leaf
52	ij	Iojap
71	Bn	Brown aleurone
109	bd	Branched silkless
(112)	Pn	Papyrescent glume

Chromosome #8

0	v_{16}	Virescent seedling
14	ms_8	Male sterile
28	j_1	Japonica
	mn	Miniature seed

Chromosome #9

0	Dt_1	Dotted aleurone
7	yg_2	Yellow green plant
26	c	Aleurone color
29	sh_1	Shrunken endosperm
31	bz_1	Bronze aleurone and plant
44	bp	Brown pericarp
59	wx	Waxy endosperm
	ga_8	Gametophyte factor
62	d_3	Dwarf
(64)	pg_{12}	Pale green seedling
(65)	ar	Argentia
66	v_1	Virescent seedling
67	ms_2	Male sterile
69	gl_{15}	Glossy seedling
79	bk_2	Brittle stalk
104	Wc	White cap
134	Bf_1	Blue fluorescent
138	bm_4	Brown midrib

Chromosome #10

0	Rp	Rust resistant (*Puccinia sorghi*)
(12)	oy	Oil yellow
16	Og	Old Gold stripe
24	nl	Narrow leaf
28	li	Lineate stripe
33	du_1	Dull endosperm
35	zn	Zebra necrosis
38	l_8	Luteus
43	g_1	Golden
	(Tp_2)	Teopod
57	R^r	Aleurone and plant color
58		Red leaf stripe
(63)	M^{st}	Modifier of stippled
73	w_2	White seedling
(92)	sr_2	Striate
(99)	l_2	Luteus
	K_{10}	Abnormal chromosome appendage

IV. REPORT ON MAIZE COOPERATIVE

During the summer of 1965, about 125 reciprocal translocations were increased at Urbana and by Dr. D. S. Robertson at Iowa State University. An approximately equal number is planned for increase at the two locations this year. This will complete the task of growing the entire collection of reciprocal translocations and transferring them to the Maize Cooperative for continued preservation. A portion of the collection has been grown at Urbana each year, beginning in 1960. It is hoped that during this year the work of cataloguing and filing the whole series can be completed in order that the entire collection will be available for requests. It is also planned that samples of all of them will be sent to the National Seed Storage Laboratory at Fort Collins, Colorado for long-term storage. Those translocations which can be supplied at present are listed in the accompanying catalogue of stocks.

A substantial increase was made of dwarfs, chlorophyll traits, and endosperm traits. Because of the increasing interest in physiological and biochemical studies of these categories of traits, some effort is being made to derive stocks of individual traits free of other markers.

Work is continuing on chromosome location and allele testing of unplaced genes in the collection. The bulk of these represent seedling chlorophyll traits. A considerable amount of time is also being spent in determining and confirming pedigrees, and in upgrading the vigor of stocks. At the present time, there are more than 75,000 individually-pedigreed samples in our collection.

We have available for distribution a number of copies of the gene symbol index to Volumes 12 through 35 of the Maize News Letter. This summary, which was prepared by Dr. E. H. Coe, Jr., Field Crops Department, University of Missouri, Columbia, Missouri, was issued July 1, 1962 as an Appendix to Volume 36 of the Maize Genetics Cooperation News Letter.

Tables 1 and 2 summarize the distribution of genetic stocks by the Maize Cooperative from the time of initiation of project work at the University of Illinois in 1953 through calendar year 1965. During this period, genetic stocks were supplied to more than 300 different people in 38 States of the United States and in 34 foreign countries. Of total samples supplied, 12% were distributed within Illinois, 65% were sent to other States of the United States, and 23% were sent to foreign countries. Of stocks supplied to foreign countries, about 20% were sent to India.

Dr. R. J. Lambert will assume full responsibility for the maize genetic stock program in the immediate future, as soon as the switch can be effected. Future requests for seed

samples and correspondence relative to the stock program should be addressed to Dr. Lambert, S-116 Turner Hall, University of Illinois, Urbana, Illinois 61801.

The accompanying catalogue of stocks represents a complete listing of currently available stocks. The interchange positions of reciprocal translocations are listed in the following publication: Longley, A. E. Breakage Points for Four Corn Translocation Series and Other Corn Chromosome Aberrations. U. S. Dept. of Agr., Agr. Res. Serv. ARS 34-16, 40 pp., 1961.

Table 1
Distribution of Seed Samples from Maize Cooperative Genetic Stock Collection, University of Illinois (May 1, 1953 - December 31, 1965)

Summary of Stock Distribution and Sources of Requests by Areas and Years

	Number of Samples Supplied			Number of Requests		
Year	U.S.	Foreign	Total	U.S.	Foreign	Total
1953	74	4	78	8	1	9
1954	207	42	249	21	2	23
1955	694	73	767	34	6	40
1956	760	223	983	42	6	48
1957	459	215	674	52	9	61
1958	574	80	654	71	5	76
1959	926	516	1,442	72	23	95
1960	917	114	1,031	79	5	84
1961	737	334	1,071	59	15	74
1962	1,413	519	1,932	83	17	100
1963	997	419	1,416	81	11	92
1964	1,390	224	1,614	86	9	95
1965	1,317	396	1,713	88	22	110
Totals:	10,465	3,159	13,624	776	131	907

Table 2
Distribution of Seed Samples from Maize Cooperative Genetic Stock Collection, University of Illinois (May 1, 1953 - December 31, 1965)

Summary of Stock Distribution and Sources of Requests by Individual States and Foreign Countries

	No. of samples supplied	No. of requests		No. of samples supplied	No. of requests
A. United States					
Alabama	137	6	Nebraska	123	15
Alaska	-	-	Nevada	-	-
Arizona	6	1	New Hampshire	45	1
Arkansas	93	2	New Jersey	84	5
California	350	34	New Mexico	-	-
Colorado	33	4	New York	555	45
Connecticut	187	7	North Carolina	138	14
Delaware	-	-	North Dakota	27	3
Florida	155	12	Ohio	157	13
Georgia	199	16	Oklahoma	36	3
Hawaii	142	6	Oregon	28	3
Idaho	7	1	Pennsylvania	265	14
Illinois	1,625	161	Rhode Island	-	-
Indiana	1,505	117	South Carolina	-	-
Iowa	629	51	South Dakota	103	6
Kansas	90	9	Tennessee	271	16
Kentucky	40	3	Texas	241	21
Louisiana	-	-	Utah	-	-
Maine	-	-	Vermont	-	-
Maryland	423	16	Virginia	161	10
Massachusetts	358	32	Washington	-	-
Michigan	96	10	West Virginia	22	2
Minnesota	633	27	Wisconsin	408	42
Mississippi	24	3	Wyoming	-	-
Missouri	994	41			
Montana	24	4	Total, U.S.	10,465	776

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(Continued on next page)

(Table 2 continued from page 173)

	No. of samples supplied	No. of requests		No. of samples supplied	No. of requests
B. Foreign Countries					
Argentina	92	2	Netherlands	27	3
Australia	146	8	Nigeria	12	1
Brazil	217	9	Peru	60	2
Canada	454	23	Philippines	173	2
Colombia	5	1	Poland	3	1
Egypt	72	1	Portugal	11	2
England	17	2	Puerto Rico	8	1
France	82	6	Rep. of S. Africa	81	2
Germany	14	2			
Greece	8	1	Scotland	3	1
Hong Kong	20	2	Spain	6	1
Hungary	3	1	Sweden	11	1
India	644	26	Taiwan	13	1
Indonesia	10	1	Thailand	4	1
Israel	132	5	U.S.S.R.	230	1
Italy	126	10	Venezuela	279	3
Japan	47	1	Total,		
Mexico	93	3	Foreign	3,159	131
Morocco	39	2			
			GRAND TOTAL	13,624	907

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

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

sr_1

sr_1 P^{WR} an_1 bm_2

sr_1 P^{WR} bm_2

sr_1 P^{WR} an_1 gs_1 bm_2

Chromosome 1 (Continued)

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 (includes locus of an_1)

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

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

Chromosome 2 (Continued)

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

Chromosome 3

A$_1$ ga$_7$; A$_2$ C R

A$_1$ sh$_2$; A$_2$ C R

A^d-3l; A$_2$ C R

A^d-3l; A$_2$ C R Dt$_1$

A^d-3l 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$_1$

Chromosome 3 (Continued)

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

cr$_1$

d$_1$

d$_1$ gl$_6$

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$_6$

gl$_6$ lg$_2$ a$_1$ et; A$_2$ C R Dt$_1$

gl$_6$ Lg$_3$

gl$_6$ v$_{17}$

gl$_7$

lg$_2$ a$_1$ et; A$_2$ C R Dt$_1$

lg$_2$ a$_1$ et; A$_2$ C R dt$_1$

lg$_2$ a$_1$ sh$_2$ et; A$_2$ C R Dt$_1$

lg$_2$ a$_1^{st}$ et; A$_2$ C R Dt$_1$

lg$_2$ a$_1^{st}$ sh$_2$; A$_2$ C R Dt$_1$

lg$_2$ pm

Lg$_3$

Lg$_3$ Rg

na$_1$

Chromosome 3 (Continued)

pg_2

pm

ra_2

ra_2 gl_6 lg_2

ra_2 lg_2 pm

ra_2 Rg

Rg

rt

ts_4 na_1

vp_1

Primary trisomic 3

Chromosome 4

bm_3

bt_2

bt_2 gl_4

$de_{(1 \text{ or } 16?)}$

Ga_1 Su_1

Ga_1^s Su_1

gl_3

la su_1 gl_3

lo

lw_4; lw_3

o_1

st

su_1 bm_3

su_1 gl_3

su_1 gl_4

Chromosome 4 (Continued)

su_1 o_1

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

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

Chromosome 5 (Continued)

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

sh^{fl} = "sh_4"

"sh_3" = allele of $\underline{bt_1}$

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

si_1

wi

y_1 l_{10}

Chromosome 6 (Continued)

y_1 $ms_{(1?)}$

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

Y_1 pl; seg w_1

y_1 Pl; seg w_1

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

va_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

Wx pg_{12}; y_1 pg_{11}

Chromosome 9 (Continued)

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

r^r; A_1 A_2 C

r abnormal 10; A_1 A_2 C

R^g sr_2; A_1 A_2 C

Chromosome 10 (Continued)

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

"oil yellow" (seedling and plant)

Primary trisomic 10

Unplaced genes

ct

el

fl_2

gl_{12}

gl_{14}

gl_{16}

h

l_3

l_4

mn

ms_5

ms_6

ms_7

Unplaced genes (Continued)

ms_9

ms_{10}

ms_{11}

ms_{12}

ms_{13}

ms_{14}

Mt

rd

Rs_1

rs_2

"sh_5"

v_{13}

va_2

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^g Pr B pl lg_1 y_1

A_1 A_2 C R Pr

A_1 A_2 C R Pr wx

Multiple gene stocks (Continued)

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 su_1

A_1 A_2 C R pr su_1 y wx

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 su_1

A_1 A_2 c R Pr y_1 wx

A_1 A_2 c R Pr y_1 sh_1 wx

A_1 A_2 C r Pr su_1

A_1 A_2 C r Pr su_1 y_1 g_1

A_1 A_2 C r Pr y_1 wx

A_1 A_2 C r Pr y_1 sh_1 wx

bm_2 lg_1 a_1 su_1 pr y_1 gl_1 j_1 wx g_1

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

Reciprocal translocations (Continued)

*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

RECIPROCAL TRANSLOCATIONS

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

Reciprocal Translocations (Continued)

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

Symbol	Translocation	Symbol	Translocation
8001	1-9	8441	2-6
8004	4-8	8443	3-4
8006	3-7	8447	3-9
8023	3-8	8452	1-6
8027	2-4,	8457	5-9
8032	3-9	8460	1-9
8041	1-5	8465	3-9
8045	2-7	8483	2-3
8048	1-3	8491	1-10
8069	4-5	8525	8-9
8103	4-7	8528	3-5
8104	3-5	8536	6-9
8108	4-5	8541	4-10
8143	6-7	8558	7-9
8219	2-10	8562	3-9
8219	5-6	8563	1-4
8249	1-4	8580	7-8
8302	1-9	8590	5-6
8321	2-5	8591	5-9
8322	2-7	8591	4-6
8339	4-6	8602	1-4
8345	5-10	8607	4-8
8347	1-5	8609	1-6
8349	3-10	8622	4-5
8350	3-8	8628	1-2
8351	3-5	8634	3-4
8367	3-8	8636	4-9
8368	1-4	8637	1-3
8374	4-7	8640	1-8
8375	1-10	8645	6-10
8376	2-8	8649	4-9
8380	4-6	8651	6-10
8383	7-9	8658	1-6
8386	5-9	8659	7-9
8388	1-5	8662	2-3
8389	1-9	8663	1-4
8395	4-5	8665	5-6
8397	3-4	8666	3-8
8405	1-3	8667	3-8
8407	2-4	8670	3-8
8412	3-10	8671	5-7
8415	1-6	8672	3-6
8420	5-8	8679	5-7
8428	4-6	8683	1-8
8439	6-9	8696	5-6

Symbol	Translocation
8746	5-8
8764	4-6
8768	6-9
8770	1-10
8782	1-5
8786	2-6
8796	5-8
8806	5-8
8818	5-6
8854	5-9
8864	2-10
8886	1-9
8890	1-9
8895	5-9
8904	6-10
8906	6-9
8919	1-8
8927	4-6
8951	8-9
8963	3-6
8972	1-5
8987	4-8
8995	1-3
8997	5-8
9002	2-6
9020	8-10
9028	4-10
48-34-2	1-5
48-40-8	4-7
001-3	1-10
001-5	8-10
001-13	1-8
001-15	3-7
001-15	2-6
002-12	4-5
002-16	2-5
002-17	5-8
002-19	1-4
003-5	2-8
003-16	4-6
004-3	7-8
004-7	3-7
004-7	4-9
004-11	1-2
004-13	2-4
004-17	5-6
005-7	1-8
005-14	2-3
006-7	4-5
006-10	2-8
006-11	5-10
006-17	3-4
007-17	5-8
007-19	1-10
008-17	1-8
008-18	5-9
009-19	2-5
010-4	2-4
010-10	2-3
010-12	1-7
011-7	2-4
011-11	6-7
011-16	4-6
011-20	2-8
012-16	3-4
013-3	5-7
013-8	6-7
013-9	1-3
013-11	5-8
013-17	2-8
014-5	5-8
014-12	2-3
014-17	7-8
015-3	2-5
015-9	1-10
015-10	5-9
016-15	7-8
016-17	3-6
017-3	1-2
017-18	2-4
018-3	2-4
018-4	4-5
018-5	1-5
018-18	1-2
019-1	2-5
019-3	7-10
020-5	3-9
020-7	5-9
020-19	1-8

Symbol	Translocation	Symbol	Translocation
021-1	7-8	024-14	1-3
021-3	4-5	024-16	4-10
021-5	4-10	025-4	2-5
022-4	2-7	025-12	4-6
022-11	5-9	026-2	1-8
022-15	7-10	027-4	2-6
022-20	5-10	027-6	6-7
023-2	2-3	027-9	7-9
023-5	2-3	027-10	4-5
023-13	5-7		
023-15	2-5		
024-1	6-8		
024-5	1-5		
024-7	1-9		
024-11	3-8		

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