E.G. anderson

### MAIZE GENETICS COOPERATION

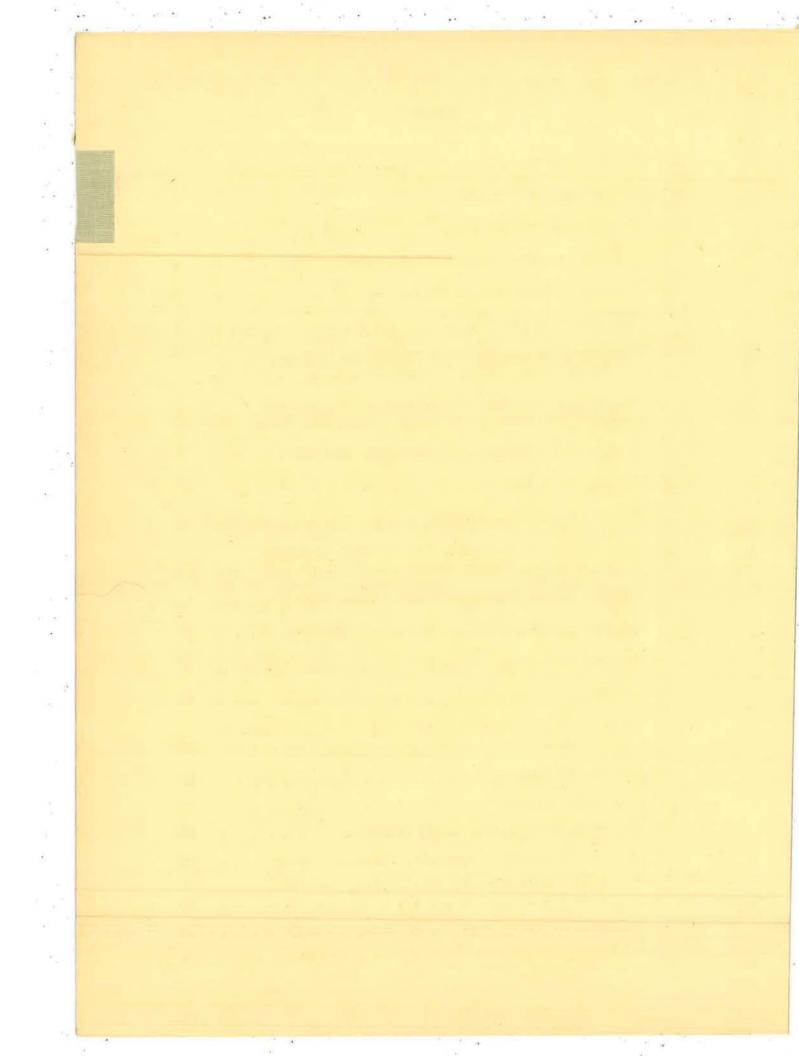
NEWS LETTER

33

April 1, 1959

The data presented here are not to be used in publications without the consent of the authors.

Department of Botany Indiana University Bloomington, Indiana



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#### I. ACKNOWLEDGEMENT

In response to our request for funds to help defray the publication of the 1959 Maize Genetics News Letter, contributions were received from the organizations listed below. We wish to express our appreciation for this financial aid. The Department of Botany at Indiana University pays for cutting the stencils and for all clerical and editorial work incidental to the preparation of the News Letter, but the cost of mimeographing, assembling, binding, covers, paper and postage constitutes out-of-pocket expenditures amounting to more than \$400 for each issue and it is for these costs that funds were requested. A total of \$470 was received which should be sufficient for this year's News Letter.

To the following companies we express our thanks:

Pioneer Hi-Bred Corn Co. DeKalb Agricultural Association, Inc. Ainsworth Seed Co. Bear Hybrid Corn Co., Inc. Coker's Pedigreed Seed Co. La rood and of the one Crow's Hybrid Corn Co. Greenwood Seed Co. Northrup, King and Company Pfister Associated Growers, Inc.
Clyde Black and Son Clyde Black and Son Ferris Watson Seed Co. and the grad of the party of the Funk Bros. Seed Co. United-Hagie Hybrids, Inc. Eastern States Farmer's Exchange Green Giant Co. Moews Seed Co. American Maize-Froducts Co. Earl May Seed Co.

We also wish to acknowledge belatedly the contributions made by Northrup, King and United-Hagie toward the expenses of the 1958 Maize Genetics News Letter.

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M. M. Rhoades

#### II. REPORTS FROM COOPERATORS

BLANDY EXPERIMENTAL FARM University of Virginia Charlottesville, Virginia

### 1. Homozygous "Old Gold".

In the processes of developing multiple gene stocks for our radiation program we have developed a stock which is <u>Og Og</u>. Each of these stocks exists in two or more inbred lines and hybrids of all the stocks have been made. The following stocks are available:

BL39 Pwr B A<sub>1</sub> Su A<sub>2</sub> Pr Y Pl IC Sh Bz Wx R also may be Og, b or pl

BILL PWr B Al Su Al Pr Y Pl C Sh Bz Wx R also may be Pww, b or pl

BLLL PWr B Al Al pry Pl C sh bs wx R also may be PWW, b pl or c

BL27 PWW b al sh2 Ap pr y pl C R

BL3 PWW b A1 su A2 pr y pl C sh wx r

These stocks are all homozygous for the genes listed.

Alan Caspar

### 2. Reconstruction of Dent Corn

It is well known that the best theory on the origin of dent corn is that it resulted from an accidental cross of an early northern flint type and the many-rowed Gourdseed corn, a type that grew in the southern part of this country, especially in Virginia. (See Wallace and Brown "Corn and Its Early Fathers," Michigan State Press, 1956.) Following the publication of that book I wrote to Dr. Brown to see if it would be possible to obtain any of the Virginia Gourdseed variety. Fortunately seed of this was available and we grew it for the first time in the spring of 1957. It was a vigorous single stalked variety with no tillers, as illustrated in Wallace and Brown. This was crossed with an early yellow flint corn, Canada Yellow Flint, which we secured from the Comstock-Ferre Company in Weathersfield, Connecticut. The F<sub>7</sub> hybrid was unusually

vigorous and showed considerable variation with most of the ears intermediate between a flint and a dent. A number of these were selfed last year, both with and without the benefit of radiation, and we plan to grow a small isolation plot of this for several years to see whether any progress can be made toward reconstructing a good dent type. Selfed seed is available of the Canada flint, of the Virginia Gourdseed and of the F<sub>1</sub> hybrid.

W. Ralph Singleton

### 3. Mutable Pericarp and Plant Color.

Several years ago a mutation grose from an intensely pigmented plant color much more intense than the A B Pl. Also the character appears early in the seedling stage or shortly thereafter. In addition to the intense color in the plant the pericarp is colored very dark, almost black, which must be considerably darker than cherry pericarp. Like the cherry pericarp it has been observed only in stocks which are A B Pl. The silk color of plants possessing this character are deep wine in color. The anthers usually are a sort of mottled dark and light red. One of the interesting things about this character is that we have not yet been able to get a homozygous stock of it. It keeps mutating back to the normal A B Pl color. It is almost but not quite completely recessive when crossed with other stocks. There is almost a complete correlation between the type of pericarp color and the type of silk and anther color, although classification is somewhat difficult and not completely satisfactory. Seed is available.

W. Ralph Singleton

### 4. Height Potential in Brachytic-2 and Brachytic-3 Types.

Both brachytic-2 and brachytic-3 are mutations from the inbred R4. They are about equal in height, 114 centimeters for br2 and 113 centimeters for br3 in 1958. In crosses back to the R4 they contribute about equally to the height of the plant, giving hybrids that were 227 and 230 centimeters, respectively, for br2 and br3 hybrids. However, almost without exception, when these two inbreds are crossed to unrelated stocks the brachytic-3 contributes much more height to the hybrid than does the brachytic-2. Crosses with an unrelated type, reduced 38-11, gave the following types: rd38 x R4 = 273 (av. 2 rows), rd38 x R4br2 = 241 (av. 4 rows), and rd38 x R4br3 = 304 cm (av. 3 rows). When crossed with wf9, the following heights resulted: wf9 x R4br2 = 233 cm (1 row), wf9 x R4br3 = 265 cm (av. 2 rows). These data agree with our observations in previous years. More extensive tests are planned. In addition to being somewhat shortened br2 hybrids usually show some of the enlarged stalk characteristic of brachytic-2.

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In the brachytic-2 inbreds themselves, there is considerable variation for height from about 80 cm for the shortest to 170 cm for the tall type in 1958. In crosses between the short and tall types, tall is dominant, a fairly good segregation occurs in F<sub>2</sub> and test-crosses. The height relationships of brachytic-2 types are being investigated further.

W. Ralph Singleton

# CALIFORNIA STATE POLYTECHNIC COLLEGE Pomona, California

### 1. Location of gene responsible for nutrient element uptake in corn.

Thene color and pare intense than the A F.S. also bee character

Certain inbred lines of corn have been found to require different amounts of mineral nutrients for optimum growth. Dr. J. D. Sayre of the United States Department of Agriculture sampled field-grown plants for magnesium content and found a sevenfold difference between the highest and the lowest lines. Other inbred lines have been shown to be low accumulators of calcium, potassium and phosphorus. Under low or sometimes where normal mineral nutrition is provided, inbred lines with these characteristics will show leaf deficiency symptoms first, when grown with other lines which are high accumulators.

Studies were initiated to determine, if possible, the location of the gene character responsible for this differential uptake of magnesium and other elements. A number of inbred lines were crossed to the waxy translocation series in 1956. The inbred lines used were as follows: Oh 28, Oh 33, Oh 40B, Oh 51A, Cl 187-2, L-317, Ind WF9, and Illa.

In 1957 the F<sub>1</sub> were backcrossed to the related inbred lines and to the available waxy inbred lines.

In 1958, due to limited space, only two of the lines from crosses were planted. These were Ind WF9 and Illa. Tissue samples were collected for analysis when each plant was approximately one meter in height. The WF9 line is being tested for high magnesium and low phosphorus accumulation. Illa is being tested for low calcium accumulation. Data from the tissue analysis will be used in an attempt to locate any major gene affecting the uptake of these elements.

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### CALIFORNIA INSTITUTE OF TECHNOLOGY Pasadena 4, California

### 1. Location of blue-fluorescent-2 in chromosome 10.

The gene <u>bf</u><sub>2</sub> has been placed in chromosome 10 by crossing to a series of chromosome 9 translocations involving waxy and testcrossing to <u>wx</u> fluorescent-2. The testcrosses using T9-10b showed clear-cut linkage of fluorescent-2 with <u>wx</u>. Comparison of the linkage data obtained with the previously known cytology and linkage relations of T9-10b gives some information on both the map location and the cytological position of <u>bf</u><sub>2</sub>.

The cytological positions of the breaks in T9-10b have been given by Dr. Longley as 98.11 and 108.28. These are fairly close to both centromeres. Precise determination of position is perhaps less reliable than for positions further removed from one or both centromeres. Linkage tests give the map order C-wx-T with 5.7 percent crossing-over between wx and T. In chromosome 10, the tests indicate the order T-g-R with 16.3 percent crossing-over between T and g. In the homozygous translocation, wx and g are linked with 17.3 percent crossing-over (62/359).

Two crosses involving T9-10b, wx and bf2 have been test-crossed, and the testcross seed grown for seedling classification.

F <sub>1</sub>	wx-bf2 non-crossovers	wx-bf <sub>2</sub> crossovers	Total	cross-	percent
wx T/bf <sub>2</sub>	848 928	19 31	1876	50	2.7
stand/wx T bf2	491 463	28 29	1021	57	5.6
Total	e rein f		2897	107	3.7

The crossing-over between wx and bf, is essentially the sum of the wx-T and the T-bf, percentages. Checks of pollen on cross-overs indicate that the translocation is closer to bf, than to wx.

Tests of homozygous T9-10b plants which were heterozygous for both wx and bf<sub>2</sub> testcrossed to wx bf<sub>2</sub>, gave 50 wx-bf<sub>2</sub> crossovers in a total of 1032 seedlings or 4.8 percent. Thus the two genes are in the same translocated chromosome, just as in the case of wx, g, and R. But bf<sub>2</sub> is closer to the locus of the translocation than is g, and therefore further to the left. The distances indicated are:

C	WX	4.8	bf2		F	1
С	wx			17.3	g	R

These data suggest a map position in the general neighborhood of lineate but they do not tell which side of the centromere.

E. G. Anderson Ron Burkholder

### 2. Blue fluorescence.

Blue fluorescence under ultraviolet light is due to the accumulation of anthranilic acid or very closely allied substances in the seedlings and in the anther walls and filaments. The two blue fluorescent genes which have been isolated both lead to the accumulation of anthranilic acid or related compounds but otherwise differ markedly.

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Blue fluorescent-1, when homozygous gives fluorescent seedlings which increase in fluorescence until about the third or fourth leaf, after which there is a gradual weakening or fading out. The plants increase in size very rapidly, and there is little, if any, further accumulation of anthranilic acid until anther development. The anther walls and filaments fluoresce brilliantly although the pollen itself shows no fluorescence.

Paper chromatograms of fresh material show three closely associated fluorescent spots, one of which is anthranilic acid. From extracts, all the fluorescent material appears as a single spot of anthranilic acid. When the gene is heterozygous the seedlings do not fluoresce, but the anther walls and filaments show a strong fluorescence as in the homozygote. Thus the gene can be handled as a recessive in the seedlings, as a dominant in the anthers. In most of the linkage tests and stock building, it has been convenient to use it as a dominant. Thus it is perhaps more appropriate to list the gene as a dominant for which the symbol Bf<sub>1</sub> can be used. This gene has been located in the distal portion of the long arm of chromosome 9. It shows about 45 or 46 percent recombination with wx. By linkage tests with translocations, it has been placed at or near 9L.9.

Blue fluorescent-2, when homozygous gives a brilliant fluorescent in the early seedling stage with its maximum brilliance immediately after germination. The coleoptile tip is brilliant, and the first seedling leaf has its maximum fluorescence as soon as unfolded. The succeeding leaves show decreasing fluorescence. The chromatographic picture shows most of the fluorescent substance concentrated in a single spot which is identical with one of the three spots shown by fluorescent-1. The fluorescence of anthers and filament is less pronounced. In the heterozygote, the seedlings do not fluoresce, and the anther fluorescence is somewhat weaker than in the homozygote. For most purposes, it is most conveniently handled as a seedling character. So we prefer to list this gene as a recessive, with the symbol bf.

E. G. Anderson

### UNIVERSITY OF CALIFORNIA Los Angeles 24, California

### 1. Gibberellin-like substances from maize.

Initial acetone extracts from green shoots of maize show no evidence for gibberellin-like activity when applied to the seedlings of the mutants d1, d2, d3, d5, and an1. However, fractions obtained from the chromatographic purification of these extracts have been found to be highly active in the gibberellin bioassay. There is evidence for at least two gibberellin-like substances from normal maize. These substances are highly active on the mutants d2, d3, d5, and an1; they are inactive on the mutant d1. (Gibberellic acid and many gibberellin-like substances from flowering plants are active on all 5 of the dwarf mutants). This information provides direct evidence for the presence of native gibberellins in maize. These native gibberellins are probably one of the controlling factors in the growth of the maize plant.

Preliminary cross-feeding studies have been made with extracts from each of the five mutants,  $\underline{d_1}$ ,  $\underline{d_2}$ ,  $\underline{d_3}$ ,  $\underline{d_4}$ , and  $\underline{a_1}$ . Purified extracts from the green shoots of  $\underline{d_1}$  plants have been found to be inactive on  $\underline{d_1}$  seedlings, and highly active on the other  $\underline{u}$  dwarf mutants. Apparently the mutant  $\underline{d_1}$  is making a gibberellin that it cannot use. Since the other  $\underline{u}$  mutants respond to the  $\underline{d_1}$  extract, the data support the interpretation that the gene  $\underline{d_1}$  controls a terminal step in the sequence of reactions leading to a native gibberellin necessary for normal growth in maize.

Bernard O. Phinney Charles A. West

### COLLEGE OF AGRICULTURE AND EXPERIMENT STATION Potchefstroom, Union of South Africa

### 1. A comparison of male sterile single crosses and their fertile counterparts.

Yields of 15 male sterile, single crosses from Tx61Ms and Ky 27 ms sources, and their fertile counterparts were compared in a yield trial, using 2x10 hill plots in rows 3 feet apart and the plants spaced 3 feet in the rows.

Three sterile crosses yielded significantly more than their corresponding fertile forms. In most cases, however, there were no significant differences between the fertile and sterile forms, although the yields of the fertile crosses were always lower than those of their sterile counterparts.

H. C. Kuhn F. J. Dijkhuis

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2. A new cycle of recurrent reciprocal selection with maize varieties that combine well.

Although a reciprocal recurrent selection program was started some time ago, no lines of outstanding merit could thus far be obtained from the local varieties chosen for their adaptability to the local hot and dry climatic conditions. A large number of varietal crosses was, however, compared in a yield trial, and fairly high yields in some cases showed good combining ability between certain varieties. In this respect it was noticeable that the highest yields came from dent-flint crosses. The two best combining white and yellow varieties were chosen for a new recurrent reciprocal selection cycle.

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### 3. Hybrid maize seed production.

Mr. C. Kuhn resigned from the Union of South Africa Department of Agriculture during September 1958 to take up the appointment of maize breeder to the Golden West seed company at Klerksdorp. Considering that hitherto crop breeding work has been almost entirely in the hands of the Department of Agriculture, this is a step in the direction followed in the United States where, it is understood, mainly fundamental work is carried out at the experiment stations, and private initiative furnishes hybrid seed under well-trained guidance.

In South Africa the experiment station at Pretoria, Bethlehem and Potchefstroom conduct the initial breeding work and hands over to the Maize Control Board the inbreds, recommended for large-scale production. The Board has a team of officers who (a) arrange contracts for the multiplication of inbred and single cross seed and (b) certify the hybrid seed produced by its agents who are either seedsmen or cooperative societies.

J. Sellschop

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION

New Haven 4, Connecticut

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MISION BIOLOGICA DE GALICIA

Pontevedra, Spain

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1. Breeding for sugar in the stalk: correlation between refractometrical reading and resistance to Diplodia and Gibberella Zea.

Nine different hybrids of medium maturity period were elected for

this study: four were standard double crosses and five were experimental single crosses of the breeding program to increase sugar in the stalk at and after maturity. Three of the last ones were expected to have high refractometrical reading after maturity. Each hybrid was planted in a single row. Ten days after pollination six plants of each hybrid were inoculated with Diplodia and another six plants were inoculated with Gibberella ("tooth pick" technique). This was done again 15 days later and 30 days later after the first inoculation. The total number of plants inoculated with Diplodia and Gibberella were 36 in each hybrid.

On November 28 refractometrical readings were made on each inoculated plant and on December 1 the same plant was cut from base to top to observe the degree of damage caused by the inoculation. Scores for degree of infection were from zero (no damage) to five (five or more internodes showing the infection). Plants completely killed by infection were also scored five. Fractions of internodes showing infection were scored as corresponding fractions of 1. Correlation analyses for pairs of the individual plant values (for refractometrical reading and infection scores) are shown in Table I. This high correlation between resistance to these two diseases of the stalk and roots confirm previous observations.

Table I

Fig. 1527 manufaction at myor or fastingues were and country of a finish.

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Hybrid	11.17		-		Coefficient	of	correlation	for	each	hybrid
Standard	double	cross	362	IIV.	-0-49	12				0
	11	11:	363		-0.62		DAMES TO			
TIC.	11	111	364	No.	-0.78					
H.	11	- Ho a	365	1-2	-0.66		A DESCRIPTION OF			
Exp. sin	gle cros	88	366	200	-0.57	for	r: 4 0.425	P:	0.01	
116 11	n n	177 1	367		-0.56				12.0	
n n	tti		368		-0.73		10 - 11 - 12 - 12 - 12 - 12 - 12 - 12 -	3.1	301	
101 11	111		369		-0.81					
11	n:	y	370		-0.51	2 -	min sugar	mi		

Correlation coefficient of the hybrid means for refractometrical reading and score of infection, r = 0.897, P = 0.001

Jose L. Blanco
Mariano Blanco

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION

New Haven 4, Connecticut

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### 1. Production and testing of pollen restoring inbreds.

Many of the standard Northeastern and Northcentral corn inbreds

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have been converted to T sterile cytoplasm for use as seed parents and to restored fertile lines carrying T cytoplasm to be used as pollen restorers. The conversion to fertility restoration has been accomplished by crossing the sterile version of an inbred by a good restorer, such as Ky21, I153, C21, C236, Mo940, NC77, and other sources, or restorer inbreds derived from these sources, backcrossing on the sterile line as the recurrent parent for 3 to 6 generations and selfing for 2 or more additional generations. Many of these restored fertile inbreds are homozygous for the necessary restoring genes while some are still segregating for fertile and sterile plants.

These restoring inbreds have been tested for several seasons on many standard sterile seed parents with generally satisfactory results. Homozygous selections produce all fully fertile progenies. Others segregate in varying ratios ranging from a few to many plants fully fertile with no delay in pollen shedding after the silks appear. In some cases there are partially fertile plants with varying amounts of apparently good pollen. These partially fertile plants usually delay shedding until after the silks have emerged on the same plants.

the control of the co

After the inbreds have been converted to type in appearance they are further selected and tested both for pollen restoration and combining ability by selfing selected individual plants in each progeny and putting the pollen from these selfed plants on appropriate sterile seed parent single cross testers. For example, 26 test crosses of ClO3TF(Ky21) on (WF9Tx38-11) as the sterile seed parent tester gave 19 progenies all fertile and 7 progenies segregating fertiles and steriles in the combined ratios of 131 completely fertile and 89 completely sterile plants. This is a significant departure from a 1:1 ratio expected on a one factor difference. All of the segregating progenies have an excess of fertile plants. This excess of fertiles is shown by many other test crosses and is significant (P<.01).

Eighty single plant test crosses of KrTF(Ky21) restorers were also grown this past season. They were made on two sterile seed parents, (WF9TxR2) and (WF9TxW22). Many lines were tested on both seed parents. Forty-eight of these test crosses gave all fertile progenies with no delay in pollen shedding. Thirty-two segregated, again with an excess of fertile plants deviating significantly from a 1:1 ratio. In stalk growth, yield and time of maturity most of these test crosses were practically identical in performance with the same crosses made with the original fertile lines. A few of the progenies were noticeably poorer in some respect and some seemed to be an improvement in both stalk quality and yield. These will be tested further in a replicated yield test. Many other restored sterile inbreds were tested and gave similar results.

The restored versions of Hy, however, behaved quite differently. Two series were grown, all crossed on to (WF9Tx38-11) as the sterile seed parent tester. Nineteen test crosses of HyTF(Ky21) gave no fully fertile progenies. Eight of these progenies segregated into 81 fully fertile plants and 153 either partially fertile or completely sterile

plants. There were 9 progenies with only partially fertile and sterile plants and 2 progenies with all completely sterile plants. Unlike all the other test crosses the segregating progenies gave an excess of sterile plants. Three progenies seemed to be segregating 1:1 and 5 progenies in a 1:3 ratio of fertile and sterile which indicates either one or two fertility restoring genes with complementary action with this sterile tester.

Thirty-four test crosses of HyTF(C236) gave 3 progenies all fertile, 2 segregating fertile and partially fertile or sterile in a 2:1 ratio and 32 progenies all sterile. The C236 source is a Leaming inbred out of the same strain that Hy was derived.

When the single cross of ClO3TF(Ky21) x HyTF(Ky21 or C236) was tested on the same sterile seed parent the results were 5 progenies all fertile and 10 segregating 140 fertile and 80 sterile. Two of these progenies seemed to be segregating 3:1 and the remaining in a 1:1 ratio.

In these test crosses the seed was sown by hand, one seed in a place, about 9 inches apart in rows 3 feet apart. Germination was unusually good and no plants were thinned out. A full stand had 37 plants. Very few rows had less than 25 plants. The differences in the number of fertile and sterile plants in the progenies segregating about 1:1 were plotted against the number of plants in the row. There is no tendency for the thinner stands to give an excess of either fertile or sterile plants and therefore there seems to be no differential elimination.

No satisfactory explanation for the excess of fertile plants in the segregating progenies is at hand. It apparently is not due to selective elimination as stated above. It could be due to selective fertilization favoring the restored fertile plants. It is most probably due to minor modifying genes segregating in the seed parent single crosses as well as in the pollinator inbreds.

The inbreds used as sources of pollen restoring genes were also tested in various combinations with each other on (WF9Tx38-11) as the sterile seed parent. The following combinations gave progenies with all completely fertile plants. Apparently all of these inbred sources have the necessary restoring genes in common and in the homozygous condition.

	Seed Parent x 38-11)	*	Restoring Pollinator (Ky21 x C21)
171	N:	4.11	(C21 x Ky21)
131	11		(K55 x Ky21)
The	10		(NC77 x Ky21) I153
n	116		1153 (NC77 x Ky21)

Ky21 is a Kentucky inbred out of the Johnson County White variety.

C21 is a Connecticut inbred out of Illinois Low Protein originally from
the Burr White variety formerly widely grown in southern and central Illinois.

K55 is a Kansas inbred out of Pride of Saline, a white variety widely grown in the west central plains. NC77 is a very late white inbred of a white southern prolific variety. Il53 is a short stalked early yellow inbred out of a U.S. Department of Agriculture open pollinated selection 133 of unknown origin. The slightly reddish pericarp suggests that it may have come from Northwestern Dent. There are several selections of this old inbred, all with restoring ability, such as A344, A293, W153R. NY16 out of Webber Dent is another early inbred that gives good restoration with all T sterile inbreds and single crosses with which it has been tested.

when the simple area of Cloriff(ly21 a Hylf(ly21 or C236) as tested

.D. F. Jones

### 2. Independence of cytoplasm and genes.

A sterile inbred, ClO6T, restored by Ky2l has been selfed for 8 generations. It has produced only fertile plants after it was reduced to homozygosity for the restoring genes. When this fertile inbred, carrying sterile cytoplasm, was crossed by normal ClO6 the F<sub>1</sub> generation was all fertile and the selfed F<sub>2</sub> grown last year in three separate progenies gave 37 normally fertile and 11 completely sterile plants where 36 and 12 were expected in a monofactorial segregation. For 8 generations the sterile cytoplasm has persisted in fully fertile plants. Also ClO6T restored by Ky2l was backcrossed on to ClO6T for 5 generations then selfed 2 generations to give an all fertile progeny. One of these restored fertile plants was crossed by normal ClO6 and in the F<sub>2</sub> generation selfed grown last year gave 19 fertile and 5 completely sterile plants. This is clear evidence that different cytoplasms and genes can remain together in the same organisms for many generations without altering each other.

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### 3. Producing restored sterile hybrids.

There are several ways of producing hybrid corn seed without detasseling now in commercial use. The method of producing two lots of seed of the same genotype, one on a sterile seed parent and one on a normally fertile seed parent by detasseling, and mixing these two lots of seed in various proportions is being widely used. This is a temporary measure and will be superseded by the use of restoring pollinators as soon as these are available. Various ways of using restoring pollinators are being tried.

The method that eliminates all detasseling in the production of the foundation single crosses as well as the final double cross is to use sterile inbreds as the seed parent of both single crosses. The pollinator for the seed parent single cross must be an inbred that has been tested for non-restoration. The pollinator for the pollen parent single cross must be a good restorer. The formula for this type of double cross is:

(A-TffxB-ff)(C-TffxD-TFF) where A B C D are the four inbreds, T is the Texas type of sterile cytoplasm, FF the necessary T restoring gene or genes in the homozygous condition, and ff their recessive alleles. This combination will usually give about 50 percent of the plants shedding normal amounts of pollen in the farmer's fields. This method eliminates all detasseling in the propagation of the inbreds and also in the production of both single crosses as well as the final hybrid. It also gives an automatic check on fertility restoration in the seed production fields, and may give an increased heterotic effect. Any loss of fertility restoring ability will show up in the form of sterile plants in the pollinator rows. Many of the outcrosses in the sterile inbreds and the sterile seed parent will show up in fertile tassels and can be rogued out.

Other methods can be used to give from 25 to 100 percent restoration in the final hybrid. If sterile inbreds are not available the pollinator single crosses can be made by detasseling or by hand pollination. The following different formulas can be used:

### Percent Restoration

cape . . . .

11	man	n 00	\( \alpha = 0 \text{ mm} \)	700
(A-	·III 2	C B-II	)(C-FF x D-FF)	100
	n	n.	(C-ff x D-FF)	50
	11	11	(C-Ff x D-Ff)	50
mrifi	n	111	(C-ff x D-Ff)	25
711.2	n	th	(C-TFF x D-TFF)	100
0	11	11:	(C-Tff x D-TFF)	50
	n	111	(C-TFf x D-TFf)	66
	n	H .	(C-Tff x D-TFf)	50

- For and - Fa

The percent restoration listed applies when only one restoring gene is needed. Actually the amount of pollen shed will vary widely as shown by the test crosses listed above and from the results of experimental and commercial hybrids already in production. Some of the methods listed above that give 50 percent restoration in the farmer's fields will not give full pollen production in the pollinator single cross in the seedsman's crossing fields and allowance must be made for this to have enough pollen shedding plants to give a good set of seed. All combinations must be tested thoroughly under different soil and seasonal conditions both for pollen restoration and combining ability in the region in which the hybrids are to be grown.

Most corn produces too much pollen and higher yields can be expected in the combinations with less than 100 percent pollen production. An interaction between sterile cytoplasms and restoring genes seems to be a factor for increased production and deserves further study.

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D. F. Jones

4. Further report on relationships between cytoplasmic and genic male sterility.

Jones has earlier reported (MNL. 1954, p.20; Brookhaven Sym. in Biol. No. 9, 1956) the results of crosses which clearly indicated independence between restorer genes for cytoplasmic male sterility and the conventional ms genes. Dominant restorer genes did not act as dominant Ms\_1 genes when crossed to ms\_1/ms\_1 male sterile plants. Jones also pointed out that Ms genes obviously do not act as restorer genes for sterile cytoplasm, since the former genes are present in all normal inbreds which have been converted to cytoplasmic male sterility. Further evidence on the independence of the cytoplasmic and genic male sterile systems comes from crosses which placed an ms\_1/ms\_1 genotype in plants carrying S type cytoplasm plus restorers for S cytoplasm.

Fertile plants of Al58SF4-1A (S type cytoplasm, restored by Ky21 restorer, backcrossed four generations to Al58, followed by one generation self) Y Ms\_/Y Ms\_ were pollinated by Y Ms\_/y ms\_ males in a WF9 genotype. All F\_ plants from this cross (ca. 15 plants) were fertile, indicating that the female parent was homozygous for the S-restorer, since the Y Ms\_/y ms\_ male parent was in a WF9 background and presumably carried no S restorers. The F\_ plants, then, possessed S cytoplasm and were heterozygous for the restorer genes. In addition, 50% of the F\_ plants were heterozygous Y Ms\_/y ms\_ (ignoring X-overs). The F\_ plants were selfed, and white (or light yellow) seeds from ears segregating yellow-white endosperm color were planted.

The white seeds should give rise to y ms, /y ms, plants in S cytoplasm, except for X-overs (4-5%). Since the F1 plants were heterozygous for the S restorer gene, 75% of the y ms /y ms plants should have carried the restorer gene. Actually, according to Buchert's findings on the male transmission of S restorers in A158SF families (MNL 1958, p.15) all of the y ms /y ms, plants would possess the restorer gene, since only the restorer allele is transferred through the pollen. If the S restorer gene prevented the ms, gene from expressing itself, or if ms, were not capable of acting in S cytoplasm, at least 75%, and probably 100%, of the y ms,/y ms, plants would be expected to be fertile. In fact, out of a total of 46 plants in two progenies, 43 plants were completely sterile and only 3 normal fertile (6.5% fertile). The proportion of fertile plants is about that expected as a result of X-overs between the y and ms loci plus hetero-fertilization and mistakes due to misclassification of light yellow endosperm. It is concluded, therefore, that ms /ms plants with S cytoplasm and S restorer genes are sterile; the ms, gene is not inhibited by S restorers, by S cytoplasm, or by a combination of both.

Harry T. Stinson, Jr.

5. Inheritance of a chlorophyll defect in a male fertile WF9S stock.

Previously reported (Jones, MNL 28, p.19; MNL 29, p.14) was a case

involving a change from cytoplasmic male sterile to male fertile in a WF9 line carrying S cytoplasm. The evidence indicated that the loss of sterility was not due to a mutation to either a dominant or recessive pollen restorer gene. It was suggested that the alteration which brought about normal pollen fertility occurred in the S cytoplasm. Also pointed out was the fact that associated with the fertile WF9S plants was a rather severe yellow and pale green streaking, more pronounced than in normal WF9. The first record of this chlorophyll abnormality appears in both of the first generation selfed progenies from two fertile WF9S plants which had previously been backcrossed 6 generations by normal WF9. It was present every year throughout 1 subsequent generations of selfing. The character is variable in expression, ranging from a degree of yellow streaking similar to that found in some normal WF9 stocks to a verysevere streaking and reduction in vigor. The estimations of the degree of streaking that have been made are subjective ones, and in some instances may be open to question. A green plant is one judged to be no more streaked than normal WF9, i.e. to be "normal WF9 green".

In the fifth generation selfed material grown in 1956 it was noted that all 17 plants in one family appeared to be normal WF9 green, while in a second family 9 plants were clearly streaked and 8 were green. An attempt was begun to follow the inheritance of the chlorophyll abnormality and to determine what bearing, if any, it might have on pollen fertility. Specifically the breeding behavior of green and streaked plants was compared in selfs and in reciprocal crosses with normal WF9.

To date, it has not been possible to isolate a line that breeds true for green. Two selfed progenies were grown in 1957 from the family mentioned above where all 17 plants were judged to be green. One progeny was again all green, but on selfing (1 plant) yielded 20 streaked plants and none green in 1958. The other progeny gave 4 green and 3 streaked plants, and a self of one of the four green plants gave all streaked plants (16). The behavior on selfing of two of the eight green plants in the second family grown in 1956 (which had 9 streaked and 8 green plants) was similar. These produced in 1957 progenies of green and streaked plants — 14 green, 2 streaked in one; 8 green, 8 streaked in the other. A further self of a green plant in each case gave all streaked plants in 1958.

Selfs made on the yellow streaked plants have given rise to progenies consisting exclusively of streaked plants - 11 plants in 1957, 14 plants in 1958. Based on only 2 generations and a small number of plants the difference in the breeding behavior of green and streaked plants on selfing appears to be that green plants can yield green as well as streaked plants whereas streaked plants give only streaked progeny. If this is true, then the green plants that were originally observed in the two families grown in 1956 have been lost, since by 1958 their descendants were all streaked. The essential correctness of the initial judgement made in 1956 that one family consisted of all green plants is indicated by a planting of remnant seed of this family in 1958, where again all 10 plants were about normal WF9 green. Thus in 2 years of selfing these plants have become streaked. However, the progenies descended from the green plants

on the whole probably are less severely streaked than the progenies from the streaked plants.

Pollen fertility seemed to be about normal for WF9 in all of the above families, so that it was not possible to establish any connection between the chlorophyll abnormality and the change to pollen fertility that occurred in this material. One difficulty in estimating pollen fertility is the general reduction in vigor encountered in the most severely streaked plants. Such plants tend to produce reduced amounts of pollen.

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Green and streaked plants have also been crossed reciprocally with normal WF9. Normal WF9 crossed as female parent by either green or streaked plants has given only green offspring, and the plants have remained green in a first backcross by green and streaked respectively. In contrast, the reciprocal cross green x WF9 gave green and streaked plants. Green plants from this cross were again pollinated by WF9, giving all streaked plants. These results in crosses and backcrosses by WF9 parallel those of the green plants on selfing. Streaked plants crossed and backcrossed by normal WF9 pollinators have produced only streaked plants, just as the selfs on streaked plants. Again, all plants from crosses with WF9 were judged to be normal fertile for this inbred.

These differences in reciprocal crosses with normal WF9 indicate that the chlorophyll abnormality is apparently transmitted only through the female parent. This suggests that some cytoplasmically inherited factor or factors, perhaps the chloroplasts themselves, are responsible for the alteration in chlorophyll phenotype. The role that nuclear genes play is unknown, since only a WF9 genotype has thus far been involved.

The behavior of what appear to be green plants both on selfing and backcrossing by WF9 is somewhat puzzling. The failure of green plants to breed true may mean the chlorophyll abnormality is easily modified by environment and in some plants fails to show in somatic tissue, but the altered cytoplasmic constituent is nevertheless present in some egg cells and hence is transmitted. Or plants may appear green because of the presence of only a relatively few altered plastids, or other cytoplasmic elements, which fail to manifest themselves visibly in somatic tissue, but which are present in sufficient numbers in certain egg cells to give rise to streaked offspring. It is also possible that the alteration which brings about the streaking is recurring in green plants; but if this is the case the change must be more or less restricted to germinal tissue, or be taking place so late in development that it fails to show somatically. There does appear to be a real difference in the breeding behavior of green and streaked plants in the proportion of streaked plants they give in selfs and in crosses, as maternal parents, with WF9. Evidently in streaked plants all, or nearly all, egg cells receive large enough numbers of altered cytoplasmic elements to produce recognizable streaked offspring. The variability in the degree of expression could be a consequence of the relative numbers of "good" or "bad" cytoplasmic elements in the zygote, which could be distributed randomly during somatic development.

The result reported here of apparent cytoplasmically inherited constituents which affect chlorophyll and vigor are generally similar to the examples cited by Brown and Duvick (MNL 32: 120). The relationship between the chlorophyll aberration and the change from male sterility to fertility remains obscure. The two in fact may not be causally related.

Harry T. Stinson, Jr.

### 6. Male gametophytic selection as the mechanism for non-segregation in the restoration of cytoplasmic male sterility.

In the 1958 issue of Maize News Letter a non-segregation of restorer genes for cytoplasmic male sterility was reported. A further investigation has revealed the mechanism of this phenomenon. For brevity only a summary of part of the data will be presented here.

The following symbols will be used:

S = a male-sterile plant with the "S" type cytoplasmic factor

SF(Het) = a fertile plant with S type cytoplasm and heterozygous for the restorer from Ky21.

SF(Hom) = same as above but homozygous for the restorer.

Type of cross	No. of crosses made	Residual genotype	No.of plts. per progeny	
SxSF(Het)	15	A158	10 - 48	all fertile
SF(Het) selfed	4	n:	20 - 80	n n
SF(Het)xSF(Het)	3	111	19 - 47	n n
SF(Het)x Inbred	23	111	41 - 80	1 fertile:1 sterile

Thus, in the above data, whenever an SF(Het) plant was used as a male no segregation occurred; while, when one of these was not used as a male, segregation always resulted.

Microscopic observation of the pollen from SF(Het) plants revealed that about one half of the pollen in each anther was aborted. Since there is a correlation between the percentage of pollen grains carrying the restoring gene and the percentage of apparently viable pollen, and since all the pollen grains effecting fertilization have the restorer, the inheritance pattern can be explained by assuming that only the pollen grains with the restorer live, while those with the alternate allele abort.

This hypothesis has been tested in the following manner: (1) If an SF(Het) plant were self-pollinated half the progeny would be expected to have about 90% normal pollen (normal for the Al58 inbred) and half the plants to have slightly more than half of the pollen aborted. Pollen from

plants in two such "F2" cultures was examined. In one family eight plants had about 90% normal pollen, while eleven plants had about half the pollen aborted. With the other progeny eight were about 90% and fifteen were about 45%. The progeny of a selfed SF(Hom) plant was also checked. Also as expected, all eleven checked were about 90%. (2) If these "F2" plants with about 90% normal pollen were pollinated by the inbred (A158) only fertile offspring would be expected; while, from a similar pollination of the 45% segregates, a 1:1 fertile-to-sterile ratio should ensue. A total of twelve 90% plants from the three progenies were pollinated by A158 and each gave rise to an all-fertile progeny. Thirteen plants of the 45% type from among the two "F2" cultures were crossed with the inbred in the same manner; each segregated 1:1.

This selection phenomenon is not limited to material with the A158 residual genotype. The following is a summary of all S-sterile material observed:

Source		No.of progeny	Observation on basis of:
of Restorer	Background genotype	observed	Pollen % Progeny Test
Ky21	A158	45	selection selection
881	MI4	20	Dr. III
121	P39	12	n
Tr.	WF9 x Ky21	2	and a section of the
A206	A158 x A206	1	selection "
11	(ML4xWF9)(A158xA206)	1	P. Sandaria
Q703	MILL x Q703	1	n:
W22	W22 x A158	1	no selection
S.P.R.*	S.P.R.	1000	intermediate
n.	(WF9x38-11)S.P.R.	1	intermediate

\* Southern Prolific Restorer, a closed pedigree single cross produced by McCurdy.

It can be seen that this type of selection is wide-spread, though not universal in S material and therefore is not a necessary consequence of the S cytoplasm. There are indications that this inheritance pattern can be modified by both the restorer and the residual genotype; however, until the evidence is complete it will not be presented or discussed.

Preliminary observation suggests that this same type of phenomenon can occur with T cytoplasm and that it is dependent upon the residual genotype.

Janson G. Buchert

7. The location and critical time of primary gene action as a mechanism of male gametophytic selection.

Since 50% of the pollen grains of the SF(Het) plants described in

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article 6 (above) abort, it can be concluded that there has been no way for a sufficient amount of an essential product of the restoring gene to get into their cytoplasm. This could only come about if the critical restoring action were to have occurred after the formation of crosswalls during microsporogenesis; after the time when it became impossible for an active restorer product to travel to the other microspores. The spores not containing the restorer then degenerate due to the activity of the sterility factor.

If this is indeed the mechanism determining the male gametic selection and, as a consequence, the degree of pollen abortion and inheritance pattern in this S material, then the restorer would not be selected for if, while keeping the residual genotype constant, it were put with a type of cytoplasm other than S. The segregation (as would be disclosed by a progeny test) for the S restorer has been checked in normal and in T type cytoplasm. A number of fertile plants resulting from the cross A158T6 x A158SF5 (A158SF5 was also heterozygous for a T-restorer) were examined, and in no case was any more pollen aborted than is exhibited by the A158 inbred (about 10%). Offspring from A158 x A158SF5 likewise showed no excessive abortion. Thus, with normal, as well as with T cytoplasm, no selection manifests itself.

The time and location of the action in restored T-steriles is prob-

that the V at 2 April 2 April

Janson G. Buchert

### 8. On the role of the tapetum in pollen abortion.

The idea that the mechanism of abortion of pollen grains in cytoplasmic male sterile plants may be a starvation due to the withholding of food by the tapetum can be discussed in the light of the observations discussed in article 6 above. If this idea were correct, then, in those cases where there was incomplete sterility (50% of the pollen aborted), there should have been no correlation between those pollen grains aborted (or conversely, those which survived) and a gene contained by them. This, however, was not the case. There the presence of a gene in the maturing pollen grain determined which grains would be aborted by the sterility mechanism. It can therefore be concluded that in cases of the S type male sterility, the abortion is not caused by a starvation due to the withholding of the food by the tapetum.

This is probably also true in T-steriles.

Janson G. Buchert

### 9. Time of the critical cytoplasmically-induced action causing pollen abortion.

The conclusions drawn in article 7 above help to establish the time when the critical step in the abortion mechanism occurs in S-steriles.

Because more than one step must precede it, among them, the primary restorer gene action and the restoration process, and since the critical times for these processes occur after microsporogenesis, this critical stage determining the abortion must take place during the maturation of the pollen grain - only a short time before the deterioration can be seen.

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### 10. Effect of environment on pollen restoration of T (Texas) type cytoplasmic male sterility.

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It has frequently been observed in T-sterile material where the restorers from Plu. P39, or NJ113y are being employed, that one part of a tassel may be more fertile than another. Occasionally plants will be found where the first day's shedding area will be fertile, the second day's will be less-than-half fertile, the third day's will be better-than-half fertile, and the base perfectly normal. Sometimes the tassel on the main stem is almost sterile, while the tassels of the tillers will be normally fertile or nearly so. This behavior appeared attributable to the environment. To investigate this, samples of seed from the same ear, produced by the cross (Cl3T x NJ143y) Cl3, were planted at about 5 to 7 day intervals from May 15 to July 23, 1958. From the data of three backcrosses and one F2 progeny, it had been previously established that the restoration brought about by NJ143y segregated as a single dominant gene. Furthermore, seed from the same packet had been sampled the previous year and found to segregate in a 1:1 ratio; it had been planted May 31. Therefore, on the basis of both the previous sampling and the previous behavior of this restorer, each planting in 1958 should have contained fertile and sterile plants in equal numbers. Those planted from May 15 through May 25 and from June 30 through July 23 had a significant (P<.01) excess of steriles, while the samples planted June 2 through June 16 had a significant (P=.02) excess of fertiles. Samples planted on May 28 and June 23 appeared to give a one gene ratio. There was no concious selection in removing seeds from the packet; also, some samples included various degrees of "partials" while others did not. The environmental factor(s) responsible is not known.

Janson G. Buchert

### 11. The effect of environment on pollen restoration of the S-type cytoplasmic male sterility.

When grown during the 1955 and 1956 seasons, all cultures having only plants with S cytoplasm, mainly the P39 residual genotype, and the

S-restorer from Ky21 exhibited various degrees of fertility. Never, however, were any plants completely fertile, nor were any completely sterile. The P39SF material grown in 1958, however, expressed itself differently. Conclusions are based on the following data.

Cross	Year Grown	Fertility *	Extrusion of anthers**
P39S8 x P39SF1	1955	all P	all M
P39S9 x P39SF2	1956	all P	all M
P39S9 x P39SF2	1956	all 13 P	all 13 M
P3989 x P398F2	1958	5P 32 S	all 37 M
P39810 x P398F3	1958	1P 37 S	36 M 2 F
P39SF1 x P39	1955	10 P 7 S	10 M 7 F or N
P39SF1 x P39	1955	9 P 3 S	9 M 3 F or N
P39SF2 x P39	1958	all 49 S	30 M 4 F 15 N
P39SF2 x P39	1958	all 32 S	15 M 8 F 9 N
P39SF3 x P39	1958	1 P 45 S	23 M 13 F 10 N
P39S10 x P39	1958	all S	all F or N

<sup>\*</sup> On the basis of pollen shed. P = partial fertile S = sterile

Except for seven, all of the plants in 1958 were sterile, regardless of the type of cross they had resulted from. These seven, however, were the latest plants to flower; therefore, the critical environment may have been different for these plants. Although the expression of fertility was different in 1958, the degree of extrusion was not. In the 1955 and 1956 grown cultures all of the fertile plants had many anthers extruded, while the steriles had only a few or none. If this same relationship had existed in 1958 the same ratios of fertile:sterile as was expected (on the basis of male gametophytic selection - see article 6 above) would have resulted. That is, while the progenies of the crosses of the type S \* SF were expected to have only fertile plants, almost all the plants were sterile, but, virtually all had many anthers just as the nonsegregating families in 1955 and 1956. SF x inbred families had almost all sterile plants, but they segregated in a 1:1 ratio for many: few or no anthers extruded, just as similar pedigreed families in 1955 and 1956. P39Sll was listed for comparison.

The environment responsible for this different expression in 1958 is not known. There was one major difference in the environment in

<sup>\*\*</sup> M = many (usually more than 150) anthers; F = few (usually less than 15) anthers; N = no anthers extruded.

Connecticut in 1958; the early part of the growing season (about 3 - 4 weeks) was unusually cool and wet.

Janson G. Buchert

### 12. Separation of cytoplasmic male sterility types by chromatography.

Chromatographic analysis applied to mature anthers of cytoplasmic male steriles from nine different sources in various stages of backcrossing to WF9 shows promise as a means for classifying these cytoplasms. The chromatograms were first inspected with short-wave ultra-violet light and later dipped in ninhydrin solution. Root tissues showed no marked differences in ultra-violet light fluorescence or absorption, or in their content of ninhydrin-positive materials. Chromatograms of anthers in early stages of development were similar except for the T sterile (previously reported).

Ultra-violet light fluorescence and absorption patterns of the normal WF9 and the cytoplasmic steriles E, T and S were distinctly different from the other types examined (A, B, D, F, G and H) and from each other. The B and F sources appeared to be alike while the others fall into a separate group. Ninhydrin-positive patterns were less distinctly different.

It is hoped that with a refinement of techniques, a further separation and identification of the cytoplasms chromatographically will be possible.

Uheng Khoo Harry T. Stinson, Jr.

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Department of Plant Breeding

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### 1. Pollen viability studies.

Utilizing the bio-assay for corn pollen viability discussed previously, (MNL, Vol. 32, p. 18-19) additional experiments were undertaken in 1958. Some of the factors known to contribute to the pollen longevityviability problem were examined in greater detail. In some of the recent work, pollen kept viable for 8 days was not uncommon. The most favorable temperature for 8-day storage was +3°C., although temperatures from -8°C. to +10°C. will generally work nearly as well.

Attempts at suspending pollen in liquid diluents were entirely unsuccessful. 1.0M and 2.0M glycerol and mannitol and 100% glycerol failed to retain any viability in corn pollen for periods of time as short as 1 minute.

Fresh corn pollen was successfully "diluted" with previously killed corn pollen. Dilutions of from 1:1 to 100:1 were used effectively in viability studies. The mechanical mixing did not appear to have any deleterious effects on the fresh pollen longevity.

Additional experiments were conducted to determine optimal pollen collection, optimal storage, and the changes that take place in the pollen during storage. These will be reported in detail in a thesis in preparation by the junior author.

H. L. Everett D. B. Walden

### 2. Oxygen utilization by fresh pollen.

Some of the pollen treatments observed in the longevity-viability studies have been subjected to elementary physiological analyses. The results can be summarized as follows:

- a) The O<sub>2</sub> uptake of fresh pollen can be measured with appropriate manometric techniques. Thus O<sub>2</sub> uptake as a function of time of storage, storage conditions, etc. can be determined. In our work, O.2 O.3 gm. fresh wt. of pollen was inserted into 15 ml. "Warburg" vessels and attached to manometers.
- b) The O2 uptake of fresh pollen suspended in O.05M phosphate buffer, pH 7.3 can also be measured. In such a system, the pollen homogenate can be shown to oxidize some of the organic acids of the "Krebs" cycle. It can also be shown in the case of succinate oxidation that the respiration pathway is at least partially sensitive to cyanide and azide. A general interpretation of these pollen "respiration" studies indicates that pollen respiration is not unlike the classical respiration of yeast.
- c) The preparation of an active "mitochondrial" suspension from corn pollen has not been successful with classical methods.

H. L. Everett D. B. Walden

### 3. Respiration studies with preparations from corn seedlings.

A preparation with oxygen uptake activity can be prepared from corn seedlings: Six-day old mesocotyls and cotyledons are ground for 3 minutes in a 0.05M NaHCO3 buffer in 0.25M mannitol solution at 0°C. Differential centrifugation allows sedimentation of a pellet at 15,000 g. This pellet is washed and re-suspended in 0.25M mannitol.

Utilization of some organic acids, inhibitor studies, determination of P;O ratios, have aided in the characterization of the respiration path-

way of corn seedling preparations.

H. L. Everett
D. B. Walden

# CROW'S HYBRID CORN COMPANY Milford, Illinois

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### 1. Dwarf prolific corn.

STORY OF MALE

Our work with the multiple-eared strains of corn is showing enough promise that we are going back to teosinte again to make a wide variety of crosses. In 1958, we had 105 first-generation hybrids. For the most part, these were crossed back to maize.

We are about ready to conclude that a stalk with 6 to 8 ears, five to six feet tall, would be ideal either for silage or for grain. Crosses with dwarf lines have been made to shorten the tall normal plants to a more desirable height.

-months have also were the most of the state of the J. Munn of the state of

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This past year we had a number of F2 crosses between twin-shoot and Inbred Hy. The twin-shoot character failed to reappear. We do not understand why it failed to appear, but we are going to try another approach. A characteristic of the twin-shoot we are using is a double groove in the internode of the stalk where the twin ear buds appear.

The second of th

### 3. Dwarf hybrids.

We are multiplying our inbred seed stocks this year in preparation for commercial production of intermediate dwamf hybrids. Up to now we have called them semi-dwarf. The stalks themselves are six to eight feet tall and the ears are from 18 to 30 inches above the ground. These hybrids will fit in situations where high fertility and thick planting rates causes normal hybrids to break over badly.

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#### EAST AFRICAN AGRICULTURE AND FORESTRY RESEARCH ORGANIZATION Kenya Colony, East Africa

### 1. Resistance to Puccinia polysora Underw.

Race EA.l of P.polysora still remains the only race identified in the field in Kenya, Uganda and Tanganyika. Breeding of resistant maizes has been discontinued, and will be reopened only if races appear virulent to present stocks carrying genes Rpp1 or Rpp2.

H. H. Storey

#### ESCUELA NACIONAL DE AGRICULTURA La Molina, Lima, Perú

### 1. Pollen restoration system in Peruvian Coastal Flint maize.

The "Texas" (T) source of male sterility, represented by the single cross 203 MS x 61M, was crossed with the Peruvian Coastal Flint variety Amarillo Ia Molina in 1952. F<sub>2</sub> fertile plants were selfed, and pollen fertile plants from resulting segregating lines selfed to S<sub>2</sub>, where selected pollen fertile plants were simultaneously selfed and crossed to tester male sterile plants. Genetic analysis was carried on 1958 data from 32 F<sub>3</sub> and F<sub>1</sub> families (10 S<sub>2</sub> lines, and 22 S<sub>3</sub> lines), and their respective testcrosses, yielding the following classification:

Restoration:	Complete	100	7	Part	ial	No
Phenotypic Ratios* (Fertile:Sterile)	All:None	3:1	9:7	3:5 1:3	15:1	None :Al
Gene action		lpair	2 comple pairs(ep	mentary distasis)	2 dupli- cate pair	3
No. of lines	ıı	11	4	2	4	0

<sup>\*(</sup>Semi-fertile plants were pooled with the fertile group)

Chi-square tests conducted on phenotypic ratios gave good fits (P>0.30) in the respective groups of  $F_3$  or  $F_4$  families, and their test-crosses, to the several ratios noted above.

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It may be concluded that the pollen restoration system in the variety Amarillo Ia Molina of the Peruvian Coast is made up of at least two complementary dominant factors. No restoration of male fertility is effected when the zygote carries either one of these two factors in the homozygous recessive state. There is also evidence, that two duplicate dominant factors may be also operating as a substitute pollen restoring system in this variety.

Alexander Grobman

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### 2. Pollen restoration in Caribbean and Southern U.S.A. maize.

Nine selected lines derived from parental Cuban varieties or hybrids were crossed to the "T" source of male-sterility. Of these, only one line,  $FF(MS)l_{4-2}$  proved to be a pollen restorer. Three  $F_2$  families derived by selfing the  $F_1$  of the cross  $FF(MS)l_{4-2} \times M$ . Sterile, gave the following pooled phenotypic segregation ratio:

goal of	the fat and as	Sterile	Semi-fertile	Fertile
92 As	Observed	eds 17 at to serve	(7) " 3 " N	
3:1	Calculated	15.25	пс по тем по Щ•7	5

A good fit to the one dominant restorer factor hypothesis was obtained.

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The following S<sub>1</sub> line [M.Sterile x FF(MS)14-2]-3 derived from the cross male sterile x homozygous restorer was testcrossed to a male sterile plant, giving a perfect 9 fertile: 7 sterile progeny ratio, pointing to the possibility that the basic restoring system may be also made up in this material of two complementary genes.

A study was conducted to determine the system of restoration of Ky122, furnished by the North Carolina Expt. Station in the form of MS x
(T-115xKy-122) by crossing it with the local form of male sterile AmIM x
(203 MS x 61M) and studying segregation in 4 F2 progenies. The following
pooled ratio was obtained that fits closely a 2 complementary genes
hypothesis:

Sterile Semi-fertile Fertil

Observed 32 3 35
Calculated (9:7) 29.3 37.7

Alexander Grobman

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### 3. New sources of cytoplasmic pollen sterility.

Two new sources of cytoplasmic pollen sterility have been isolated from the race Perla, being identified in breeding plots, and checked by testcrosses, with known restorer and non-restorer lines. These 2 sources have been named EM-3000 and EM-320, and their behavior is as follows:

EM-3000: (a) completely restored by FF(MS) 14-2

(b) not restored by either Cuba M11-20##-9 or Cuba M11-3##

(c) restored 3:1 by HLM-24## -3 and by MSx (T-115xKy-122)

EM-320: (a) completely restored by FF(MS) 14-7-2 and PD(MS) 9 - 32

(b) not restored by PD(MS) 9-48 derived lines.

(c) restored 3:1 by FF(MS) 14-2-## - derived lines.

Both sources behave with these lines identically as does the Texas source of cytoplasmic pollen sterility.

Alexander Grobman

### 4. Inheritance of Cap.

The simple dominant gene hypothesis has been confirmed to operate in the character starchy cap vs. no cap in crosses of Cuban dent lines x Peruvian Perla flint lines.

In the cross PC-79-## x Cuba 23-25## (no cap vs cap) a 1:1 phenotypic ratio was obtained in the  $F_2$ , while in the  $F_2$  of three families of the cross PD(MS)9-48##x CC-94## (cap vs. no cap) a 33:4 phenotypic ratio that approaches a hypothetical 7:1 was obtained. Both ratios point to a single gene segregation with two modes of xenia operation: completely dominant xenia (7:1) and incomplete dominant xenia (1:1) in the endosperm.

Alexander Grobman

### 5. Study of chromosome morphology of races of maize in Peru.

Advances have been made in the study of the number, position, size and shape of chromosome knobs of the races of maize in Peru, trying to determine patterns for differentiating races (see first report in Maize News Letter 32:25).

Differentiating features have been obtained, and are being studied further for ample confirmation, in the frequency of appearance of knobs in certain chromosome arms, the shape and size of such knobs, the frequency of presence of the abnormal -10 chromosome, and the frequency of high number of B-chromosomes. The highest number of B's per plant, found

so far, was 4. Generally speaking, B - chromosomes are found in highland races, with low number of knobs.

> Alexander Grobman Barbara McClintock

### 6. Evidence for existence of a common prehistoric race in both North and South America.

A new cache of corn in an early Paracas stratum (circa 0-200 B.C.) was found by Dr. Dwight Wallace in Ica, on the southern Peruvian coast. This material, was found in an excellent state of preservation and permitted a careful morphological study. The ears were short, ranging from 1.5 to 9.0 cms. in length, most of them with medium to strong fasciation, with brown or red pericarp, and small yellow flinty (pop) kernels. Four ears had cherry pericarp.

This corn is clearly related to a precursor of a large number of present-day Peruvian and Andean races, and the Mexican race chapalote and seems to be similar to Huaca Prieta corn, as well as to corn from Tularosa Cave, which would mean, that this prehistoric race of corn might have been grown in both North and South America, more than 2500 years ago.

Alexander Grobman Paul C. Mangelsdorf to the sample of the collection of the first will be the first the first the collection of the collect

### district in the collection of the control of the land of the property of the collection of the collect ESTAÇÃO AGRONOMICA NACIONAL Oeiras, Portugal

### 1. A persistent nucleolus in maize.

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A study of meiosis was made in 6 F<sub>1</sub> plants from a cross between a normal inbred line and a plant with the constitution abnormal 10 10<sup>B</sup> 2Bablo. In all plants a persistent nucleolus was detected at both meiotic divisions in a large number of pollen mother cells. Parallelly, production of carmine stained nucleolar-like bodies was also observed in many cells.

A large number of droplets of staining material were found in the nucleus, surrounding the chromosome threads at leptotene. At zygotene similar droplets were observed in close connection with the synizetic knot. Large, irregular, light staining spots were observed in the surrounding nuclear sap. These spots are thought to be the products of the progressive dissolution of droplets previously formed and freed by the chromosome contraction into the symizetic knot.

The fact that in this stage droplets are found close to the chromosomal bulk seems to be a good reason to believe not only that these bodies are produced by the chromosomes but also that the chromosomes are still in an active metabolic stage. It has not been possible to ascribe the production of this substance to any particular region of the chromosomes.

At pachytene the droplets are less numerous and larger, lying completely free in the nuclear sap between the bivalents. They are generally 1/5 to 1/8 smaller than the nucleolus, in diameter. Later prophase stages are characterized by a gradual disappearance of these bodies, and at diakinesis none was observed. Instead, a granular, coalescent and light staining substance was seen spread over the nucleus. At prometaphase a persistent nucleolus was observed in a large number of cells. It soon becomes free from the nucleolar organizer attachment and orientates displaying an elliptical and finally a rod shape configuration.

At metaphase I the nucleolus is normally lying at one of the poles of the spindle. Very few cases were observed in which these nucleoli orient on the equatorial plate. They then form a long rod crossing the spindle plate with their ends close to the poles. Other times a spindle shaped configuration was observed, made up of two rods convergent at the ends and directed towards the poles. These nucleoli finally break into two pieces that move to the poles. Nucleoli were also seen lying outside the spindle. They seem to be motionless.

The division cycle of nucleoli always precedes the anaphase movement of chromosomes. It seems therefore that centrifugal forces are already operative in the spindle prior to the polar movement of the chromosomes. The directional stretching of nucleoli towards the poles is probably a function of the frame-like structure of the spindle that dictates the predominant direction of the movement. The persistent nucleoli normally disappear before anaphase. In a few cases, however, nucleoli were still found at final anaphase I lying beyond the poles, apart from the chromosomal group, seeming to be displaced by them from their previous site.

Telophase and interphase are characterized by a widespread occurrence of small, dark staining droplets in the cytoplasm. They are dissolved before metaphase II. Meanwhile amorphous nucleolar substance is produced inside the nucleus, and a persistent nucleolus is again present at metaphase II lying at one of the spindle poles. Nucleolar division has never been detected at this stage. Finally cytoplasmic droplets were again observed at telophase II, interphase and prophase of the microspore first division.

From the above description it seems logical to infer that the persistent nucleoli were due to an over-production of nucleolar substance by the chromosomes. Presence of nucleoli at the metaphase stage seems

in this case due to the saturation of the nuclear sap and cytoplasm by this additional product.

Pachytene analysis of the chromosomes in five plants disclosed their constitution as follows:

Plant 1 - 10 10B 2Bablo

Plant 2 - 10 10B 2Bablo

Plant 3 - 10 abl0

Plant 4 - 10 10B Bablo

Plant 5 - 10 10B Bablo

These plants carry a large number of knobbed chromosomes. A low degree of neocentric activity was detected at both meiotic divisions in all plants.

An attempt was made to evaluate the relative amount of the droplet forming substance in PMCs of these plants. Two morphological criteria were used: the evaluation of the mean number of droplets per cell at the pachytene stage and the counting of relative number of cells with persistent nucleoli at prometaphase-metaphase I. The results are given below:

	Average No. Fer Cell of Nucleolar- like Bodies at Pachytene	Total No. of Cells	Relative No. of Cells With and Without Per- sistent Nucleoli at PrometMet. I	Total No. of Cells
Plant 1	4.5	57	76 <b>8</b> 8	84
Plant 2	4.3	65	121:4	125
Plant 3	2.1	50	63:92	155
Plant 4	1.96	51	53:73	126
Plant 5	2.3	54	57:88	139

These figures seem to confirm our idea of a direct relationship between nucleolar persistency and the amount of droplet forming substance in previous stages. However, the most important point is that the amount of this substance is considerably greater in plants 1 and 2 than in the others. Since these plants carry an extra abnormal 10 segment, it is reasonable to assume a dosage effect of this fragment on the production of nucleolar substance.

A closer comparison of the different cases presented in this table discloses that other heterochromatic portions of chromosomes seem not to affect the production of this substance. For instance the plant with no B chromosomes shows no difference in mean number of droplets per cell at pachytene and in the proportion of nucleolate cells in comparison to the plants carrying the two translocated parts of a B chromosome.

Pachytenes of plants 2 and 3 were studied in detail with regard to the knob constitution of the chromosomes. It is as follows:

d policy lines		Plant 2	Plant 3
	L	K/K	K/K
Chromosome 1	S	K/O	0
Length being	L	K/K	K/O
Chromosome 2	S	0	0
The second second	L	к/о	0
Chromosome 3	s	0	K/O
ter a settet pe		K/0	K/O
Chromosome 4	s		0
garage	L	0	K/O
Chromosome 5	S	0	0
<b>6</b> 1	L	K/O	K/O
Chromosome 6	s	organ.	organ.
	L	K/O	к/о
Chromosome 7	S	0	0
		0	0
THE PERSON AND	S	-20 -0	. 0
and in the	L	0.1	0
Unromosome 9	~	K/K	K/K
21		B frag/0	abl0/0
Chromosome 10	S	0	0

3 4 -

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plus 2B ablo

- K- Symbol for knob
- 0- Knobless
- L- Long arm of the chromosome
  - S- Short arm of the chromosome

This table shows that knobs are relatively abundant in both plants and that differences are expected to occur in plants of the same family. Knob analysis of the remaining plants could not be undertaken. Most of the bivalents at pachytene stick together by their knobs making impossible their identification. However, this, in itself may be evidence of a heavily knobbed karyotype.

Production of nucleolar substance is frequently observed in association with heterochromatin. In maize the nucleolus is normally associated with a large heterochromatic piece, the nucleolar organizer. From the facts above described, the suggestion is made that other heterochromatic parts, as for instance knobs and B fragments, can also be activated under special genotypic conditions. This particular genotype is probably provided by the abnormal 10 segment. Influence of this segment on the neocentric activity of the other chromosomes, assumed to be localized on the knobs, is already known. We believe that detection of the persistent nucleoli and of the mechanism presumably causing them to arise was only possible due to the heterochromatin charged background in the PMCs of these plants that made possible the full expression of the abnormal nucleolar activity.

Tristao J. Mello De Sampayo

### HARVARD UNIVERSITY Cambridge, Massachusetts

## 1. The effects of teosinte chromosomes on mutation rate at specific loci.

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In previous News Letters we have reported a general mutagenic effect of teosinte chromosomes which have been incorporated into A158. The present report concerns their effect on mutation rates at specific loci. A test of a homozygous chromosome-4 stock on mutation to sugary during pollen formation yielded 1 proven su mutation in 84,329. When this individual was grown out, it was found to be semi-sterile. In another test in which mutation rate was tested in the female rather than the male and in which the teosinte chromosomes were made heterozygous by outcrossing to a cytoplasmic male-sterile inbred (ClO6) no mutants were observed to either su or y in 136,227 kernels involving about 68,113 gametes bearing teosinte chromosomes. A similar test for she in the female of teosinte derivatives

also made heterozygous with the cytoplasmic male-sterile inbred produced only 3 mutations in 1,718,989 kernels. It is significant that all three of these mutations came from a single family (57,879 kernels) and that this family involved several teosinte chromosomes (3,4, and 9).

W. C. Galinat
P. C. Mangelsdorf

#### 2. Mutations at the A locus in teosinte derivatives.

Although mutations at specific loci in teosinte derivatives are rare in the controlled experiments reported immediately above they may be more common in certain other stocks. In 1956, 241 ears were grown from crosses of an inbred strain of the genotype A C R with respect to aleurone color and an inbred strain homozygous for the unstable defective endosperm mutant de and having the genotype A C r. The F<sub>1</sub> ears from this cross would be expected to segregate for colored and noncolored seeds in a ratio of 9:7 and all but two did segregate in this manner. The two exceptions had 57.0 and 53.4% of noncolored seeds. These percentages suggested a 27:37 ratio. The colored seeds from one of these ears were grown in Florida in the winter of 1957-58 and produced ears segregating for colored and noncolored seeds in ratios of 27:37, 9:7, and 3:1. Some of the colorless seeds from the second ear when grown in the summer of 1958 proved, when tested, to be of genotype Aa, showing that a mutation from A to a had occurred.

P. C. Mangelsdorf

#### 3. Peculiar behavior of the C locus in crosses of teosinte derivatives.

A third ear from the population described in the section above segregated in a ratio of 9:7 in the F2 endosperm generation but produced an ear segregating in a ratio of 27:37 in the F3 generation. Colored seeds from one of these ears produced 27:37, 9:1, and 3:1 ratios in the following generation. All selfed plants were also tested for A, C, and R. Plants producing 27:37 ratios in selfed ears proved either to be heterozygous for A, C, and R or homozygous for A and heterozygous for R, C, and an unidentified color gene. Three of the plants segregating in a 9:7 ratio proved to be homozygous for both A and R and heterozygous for C and an unidentified color gene. In test crosses on the C tester all of the plants which were segregating for the unidentified color gene produced 1:3 ratios instead of 1:1 ratios of colored and noncolored seeds. Apparently this stock which originally was heterozygous for the C factor is now heterozygous for two C factors both of which are required to produce aleurone color. The significance of this situation is not yet clear and the presently known facts are being presented here only as a matter of record.

#### 4. Further data on an unstable gametophyte mutant.

In last year's News Letter data were presented which indicated that an unstable gametophyte mutant involving preferential segregation, although usually deleterious to the gametophytes which carry it, may in some instances confer an advantage so that backcross ratios instead of being "high" sugary are "low" sugary. This possibility was tested further in the summer of 1958. Thirteen plants from a stock which originally was "high" sugary and which had become almost "normal" sugary with minor but significant fluctuations in the direction of "high" sugary were backcrossed by and on homozygous sugary with the following results:

of the company of the second s	Total Seeds	No. Sugary	Percent Sugary
Backcrosses by sugary Row 148	4816	2388 2347	49.6 50.4
n on n , Row 347		2703	48.3

are divided and a property of the state of t

The ratios in the first two series of backcrosses do not differ significantly from normal 1:1 ratios but the deviation in the third series is significant at the .Ol level. Also one of the plants in the population had 44.5% of sugary seeds when backcrossed on Row 347 and 56.3% when backcrossed on Row 148. Both deviations from 50% are significant and show that the gametophyte factor is still present in the population and indicate that its behavior is governed to some extent by the genotype of the styles in which the pollen tubes grow. Since the gametophyte factor is linked with the Su gene all populations with significantly less than 50% of sugary seeds are presumably the product of the gametophyte factor conferring an advantage upon the gametophytes which carry it.

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P. C. Mangelsdorf

# 5. Cytology of unstable mutants.

Several of the unstable mutants reported by Mangelsdorf in last year's News Letter have been studied cytologically in F<sub>1</sub> hybrids with an inbred strain of Wilbur's Flint. Nonhomologous association between the long arms of chromosomes 2 and 4 was found in the F<sub>1</sub> involving an unstable dwarf, which in tests by Galinat using B-chromosome translocations was found to have its locus on chromosome 4. In this same F<sub>1</sub> the long arms of chromosome 1 folded back on themselves in nonhomologous pairing for a length from the knob position to the end.

One of the stocks involving the unstable defective, de<sup>t5</sup>, has probably an inversion in the short arm of chromosome 9 comprising about

two-thirds of the length of the arm.

Y. C. Ting P. C. Mangelsdorf

#### 6. Cytological observations on extracted "teosinte" chromosomes from maize varieties.

In last year's News Letter, Mangelsdorf reported the extraction from Latin-American maize varieties through repeated backcrossing to the inbred, A158, of chromosomes having genetic effects similar to those of teosinte chromosomes. Some of these strains of A158 which had been modified by substituting extracted teosinte chromosomes have now been studied cytologically in F<sub>1</sub> hybrids with an inbred strain of Wilbur's Flint which is virtually knobless. The following observations have been made.

Knobs on the long arm of chromosome 1 have been introduced into A158 from Honduras 1639 and Venezuela 1536. A knob on the long arm of chromosome 2 has been introduced from Guatemala 197.

Asynapsis was observed in approximately two-thirds of the short arm of chromosome 3 and in almost the entire length of the short arm of chromosome 7 in a strain of Al58 modified by introducing chromosomes from Nicaragua 501. Asynapsis was also observed in a derivative of Honduras 1639 in about one-fifth of the long arm of chromosome 2 and in the long arm of chromosome 4 involving a segment from the regular knob position to the distal end. In the derivative of Venezuela 1536, there occurred an asynaptic loop about four chromomeres in length in the long arm of chromosome 2 adjacent to the centromere.

Nonhomologous association was found in approximately one-third of the long arm near the centromere in chromosome 2 of a derivative of Nicaragua 500. The association was with a chromosome not yet identified.

Chromatid bridges and fragments were present at anaphase 1 in the F<sub>1</sub> hybrid of Wilbur's Flint with a derivative of Cuba 398. The chromosome involved has not been identified, perhaps because the segment is too short to regularly form a loop at pachytene.

Y. C. Ting P. C. Mangelsdorf

# 7. Chromosomes in three teosinte varieties.

Florida-type teosinte from Honduras. A collection of teosinte from Honduras resembles Florida teosinte in vegetative characteristics

and photoperiodic response but differs from it quite markedly in its chromosome knobs which are largely internal.

Chromosomes 1 and 4 of the Honduras teosinte each have two large internal knobs, one on each of their arms. Chromosomes 2 and 3 have one large internal knob on the short arm and in addition chromosome 3 also has a medium-size terminal knob on the same arm. There are no knobs on chromosomes 5, 7, and 10. On chromosome 6 three knobs are present; the one on the short arm is terminal and the others on the long arm are internal and occupy the first and the second knob positions. The terminal knob on the short arm of chromosome 8 is very small, while the terminal knob on the short arm of chromosome 9 is prominent.

Mexican teosinte from Chapingo. In a Mexican teosinte from Chapingo, pachytene chromosomes 1, 2, 3, 4, 5, and 8 were found to be different from those previously reported in this variety. Chromosomes 1, 3, 4, and 8 are knobless. Chromosomes 2 and 5 each have one internal knob; that of chromosome 2 is on the short arm; that of chromosome 5 is on the long arm. Fusion of chromosome knobs in this variety is common. On the average, chromosome 6 of Chapingo teosinte is shorter than either chromosome 7 or chromosome 9.

Mexican teosinte from Xochimilco. In this variety of teosinte pachytene chromosomes are always well spread in spite of the fact that most of them have one or two knobs. Fusion of chromosome knobs was rarely observed.

Chromosomes 1, 2, and 4 each have two knobs, one on each arm. Chromosomes 3, 5, and 7 each have one knob on the long arm. There are four knobs on chromosome 6; the terminal knob on the short arm and the knob on the first knob position of the long arm are small while the knobs on the second and the third knob positions of the long arm are large. There is a terminal knob on the short arm of chromosome 9. Both chromosomes 8 and 10 are knobless.

Y. C. Ting

# 8. Telocentric chromosomes.

Telocentric chromosomes, previously reported by Rhoades for chromosome 5, have been found for chromosome 10 in a cross of our strain carrying a B-chromosome with a strain received from Dr. Rhoades which was homozygous for abnormal chromosome 10.

At pachytene stage these telocentric chromosomes, like the normal bivalent chromosome 10, were always well paired. The size of the terminal centric region was about equal to that of the bivalent chromosome 10. The telocentric bivalent was frequently associated with the bivalent chromosome 10 at the centromere regions. Whenever this hap-

pened the bivalent normal chromosome 10 appeared to have four short arms which are alike. Sometimes the short arms oriented in such a way that they formed a closely associated quadrivalent. In such cases they appeared to exchange their partners throughout their length.

In order to determine the frequency of the association between the telocentric bivalent and the chromosome 10, about 50 microsporocytes were studied. In about one-half of the cases the bivalent chromosome 10 was associated with the telocentric bivalent and in about a fourth of the cases the telocentric bivalent was left free in the cells. Whenever it was not associated with any of the chromosomes it was usually located in the periphery of the sporocytes. Less frequently this telocentric bivalent was paired with the other chromosomes rather than that of chromosome 10. Occasionally this telocentric bivalent was associated with the B-chromosome at the centric regions.

At anaphase I the telecentric bivalent always failed to divide. Instead of two, it moved to one pole only. Therefore its distribution in the subsequent divisions would be irregular.

Y. C. Ting

#### 9. Association between B-chromosome and abnormal chromosome 10.

In the plants of a cross heterozygous for an abnormal chromosome 10 and also carrying a bivalent B-chromosome, it was found that the heterochromatic part of abnormal 10 was sometimes associated with the B-chromosome. In other instances only the knob-like region of the B-chromosome was paired with the abnormal chromosome 10 at a point of the latter's extra piece of heterochromatin. A few times the paired portion of the attached heterochromatic fragment involved its entire length. More frequently, however, the attached heterochromatic fragment was fused with the knobs on various chromosomes. These observations show that the attached heterochromatic portion of the abnormal chromosome 10, the knobs of various chromosomes, and the B-chromosomes have a high degree of "homology."

Y. C. Ting

# 10. The blotching system involving the c locus.

In earlier reports it was stated that there are four genes involved in the blotching system which causes blotches of color to develop in the aleurone in A c R genotypes. This conclusion was based on populations which had ratios closely approaching 81:175, the ratio expected when four factors are segregating. In last year's News Letter, because only three different testers could be isolated, it was concluded that only three genes are involved in this system. Now it appears that the earlier reports were more nearly correct than last year's.

The inbred strain Oh45, which is not itself blotched, proved in test crosses to be homozygous for the Bh factors on chromosome 4, 6, and 9. This suggested that there must be at least one more Bh factor in the system and that this factor was absent in Oh45.

Since Ohl5 is rr and the three Bh testers are RR we should expect 9:7 ratios in the F2 generation of the crosses between these stocks if the crosses are heterozygous for only one Bh gene and 27:37 ratios if they are heterozygous for two Bh genes.

In the cross of Ohl5 with the tester for the Bh gene on chromosome 6 the segregation was 773 blotched: 1064 not blotched on three ears—almost a perfect 27:37 ratio indicating that two of the Bh genes in addition to the R gene are segregating. On a fourth ear the ratio was 261:252 which is significantly different from either a 27:37 or a 9:7 ratio.

In the F<sub>2</sub> of the cross of Ohl5 with a tester for Bh on chromosome 4, four ears segregated alike producing 621 blotched:1340 non-blotched—a perfect 81:175 ratio—indicating that this cross is segregating for four factors: R and three Bh genes. Since only one of the recessive bh genes is contributed by the Bh tester, the other two must come from Ohl5.

In the cross of Ohl5 with the tester for Bh on chromosome 9, four ears were similar in their segregation producing 780 blotched: 1304 nonblotched seeds. This ratio differs significantly from either a 27:37 or an 81:175 ratio. The results may represent modifications due to linkage or the presence of a gametophyte factor.

The results, though in some respects somewhat inconsistent, indicate that at least four and possibly five factors are involved in this Bh system. The reasons for the modified ratios remain to be determined.

Additional data on linkage relations indicate that the <u>Bh</u> gene on chromosome 4 is located on the long arm since it shows almost no linkage with <u>de<sup>tl</sup></u> which is located on the short arm. Data presented in last year's News Letter indicated that the <u>Bh</u> gene on chromosome 9 is located on the long arm. More recent data showing 45.3% crossing over between <u>Bh</u> and <u>Sh</u> and 47.5% between <u>Bh</u> and <u>Wk</u> tend to confirm this.

P. C. Mangelsdorf

# 11. Number of genes in the r-R blotching systems.

Previous data have indicated that the number of genes involved in this system might be as high as six or seven. For some reason it has not yet been possible to isolate testers for all of these. Data obtained this year, however, confirm the earlier conclusions with respect to the number of genes. An ear, known to be homozygous for one Bh factor and apparently segregating for five or six others, produced progenies segregating in ratios of 243:781, 81:175, 27:37, 9:7, and 3:1. The actual numbers were respectively for blotched and nonblotched seeds: 198:667, 203:418, 142:207, 141:103, 182:54. The results indicate that there must be at least six factors in the system. The effort to identify testers for all of these will continue.

P. C. Mangelsdorf

#### 12. Vestigial glume modifiers.

Having finally obtained homozygous Vg Vg inbred strains in a background approaching that of sweet corn inbred P39, it became apparent that two and possibly three modifying genes are essential to insure good pollen production under adverse environmental conditions. Previously we reported that the effect of a certain weak tunicate allele in restoring tassel glumes to Vg plants bearing "glumeless" ears was sufficient to permit normal pollen production. But such restored Vg tassel glumes are flattened rather than boat-shaped and consequently they do not enclose the young anthers tightly enough to prevent shriveling of the dehiscence pore under conditions of heat and drought. However, if the young anthers are colored a cherry red by a certain R-allele, then there is sufficient additional protection provided by light obstruction within the walls of the anther to permit normal pollen production. At the actual time of pollen shedding, this red color fades out to a pale shade in contrast to the purple-anthered character which remains permanently dark.

All three of these genes (Vg, tuW, Rr) are dominant to normal and this facilitates back-crossing them into a quality-acceptable inbred (P39) of sweet corn. The final selection of the homozygous condition of these dominants following inbreeding may be accomplished in F2 by the following techniques. Since one of the effects of tuW is to cause the semi-liguleless expression of the Vg gene to become recessive in our stocks, selection of the "liguleless" plants in segregating stocks identifies the Vg Vg plants. Classification as Vg Vg on a basis of ligulelessness may be accomplished in either the seedling or mature plant. The Rrar plants in segregations may be identified by progeny tests of seedlings grown in sand flats. The tuW gene is incompletely dominant so that the homozygotes may be recognized by comparison of tassel glumes for a given Vg condition determined as mentioned previously.

The possibility of a third important modifying gene for Vg exists. In some stocks which have the necessary tuw and R-allele modifiers, the filaments of Vg Vg anthers are slow to elongate and when they do lengthen they are less than one-half normal length. Sometimes these Vg Vg

anthers remain within the glumes and never do disperse their pollen. This filament trouble is peculiar to the homozygote although it can be eliminated by selection in as much as we have one line without such filament difficulties.

W. C. Galinat and the state of t

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UNIVERSITY OF ILLINOIS Urbana, Illinois Department of Agronomy

#### 1. High-oil and high-protein hybrids.

Two new corn hybrids, Ill. 6021 (R75 x R76) (R84 x K4) and Ill. 6052 (R78 x 38-11) (R84 x K4), have been developed in the Agronomy Department of the University of Illinois. Foundation single-cross seed of these two hybrids is available to seedsmen interested in producing seed in 1959. Sufficient double-cross seed for farm use will be available for the 1960 growing season. These new hybrids yield about 30 percent more oil and 10 percent more protein than present commercial hybrids. In addition, they are similar to standard hybrids in grain yield, standability, and other agronomic traits. Nationwide use of adapted high-oil hybrids would produce almost as much oil as is now received from butterfat, soybeans, cotton, and flax. These new high-oil hybrids should benefit both the starch industry and the livestock feeders.

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# 2. Inbred lines and sister-line crosses.

Sister-line crosses are combinations between sister strains of the same inbred line. Some sister-line crosses have considerably greater yield, vigor, and standability than the original inbred line, and may be practical for the commercial use of single-cross hybrids. Data on a group of inbred lines and sister-line crosses are reported in Illinois Agricultural Experiment Station Bulletin 636. Some growers are interested in producing Hy x Oh? because of its high yield and ability to yield well under high plant populations. Hy2 yielded 35 bushels an acre; whereas, a related sister-line cross R158 x CI.42A yielded 125 bushels per acre. This latter hybrid might be used as a seed parent. In addition it is resistant to leaf blight and is higher in protein content. Oh7 yielded 51 bushels an acre whereas, Oh7 x Oh7A, a sisterline cross, yielded 85 bushels an acre. This cross might be used as the pollen parent for the commercial production of a modified version of Hy x Oh7. Many of the other sister-line crosses appear to be prom-

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ising, and could be used as seed parents of single crosses.

R. W. Jugenheimer V. Trifunovic

# UNIVERSITY OF ILLINOIS Urbana, Illinois Department of Botany

#### 1. Linkage and aberrant segregation of a new Teopod locus.

A dominant mutant, apparently identical in phenotype with  $\underline{\text{Tp}}_1$  on chromosome 7, was found by Dr. J. R. Laughnan. The new Teopod locus is located on chromosome 10, proximal to the golden locus and about 13 units from  $\underline{R}$ :

Parent	tals	Single	s G-R	Singles	Tp-G	Doub	Les
Tp G R	Ngr	TpGr		Tpgr	NGR	TpgR	NGr
122	96	1)4	18	2	1	o	0

Additional data on Teopod-golden distance is given below:

1.00	Tp G	Ng	Трд	N G	Total	Percent Recomb.
Backcross data	314	305	3	8	630	1.75
F <sub>2</sub> data	736	228	7	10	981	1.25

One strain carrying the new Teopod shows aberrant ratios of Teopod and non-Teopod plants. Heterozygotes, through three generations of testing, have produced only Tp progeny on selfing, while crosses of the same plants, used as egg or pollen parents, with non-Teopod, give 1 Tp: 1 tp ratios in the progenies. Subsequent selfing of the outcross progenies gives families showing normal 3:1 segregation.

Helen Peterson

# 2. Noncrossover alpha (pale) derivatives from Ab:P.

The  $\underline{A}^{\mathbf{b}}$  complex of Peruvian origin (beta:alpha) is highly suited to an analysis of the origin of the noncrossover alpha element since the

latter occurs about twice as frequently as the crossover alpha from this complex. From the fact that the crossover and noncrossover derivatives are indistinguishable in phenotype it could be supposed that the noncrossover alpha is the result of gene mutation of the beta element to a null level  $(\beta_0)$ . This would account for the loss of purple effect and the consequent pale phenotype through an event not associated with recombination within the complex.

To test this hypothesis we have attempted to isolate the beta element from the beta:alpha complex in order to study its rate of mutation to the null level (colorless). Beta elements isolated by crossing over were put into marked heterozygotes with the parental beta:alpha complex. These marked heterozygotes may be represented as T  $\beta\alpha$  Sh/N  $\beta$  sh. Since the beta elements in this heterozygote are identical and since they occur in identical genetic background we anticipate that they will mutate to the null level with equal frequency. However, a beta mutation in the  $\beta\alpha$  complex yields  $\beta_0\alpha$  (noncrossover pale) whereas a mutation of beta in the other strand yields  $\beta_0$ . Since no alpha element is present here this event should yield a mutant with colorless phenotype.

In the table below data from two tested heterozygotes are given. The second one listed is without proximal marking.

	Total	7 1 6 7	Derivatives	
Heterozygote	gametes	T a Sh	N a Sh	Colorless
T βα Sh/N β sh	74,000	17	5	None
N βα Sh/N β sh	76,000	<u>a Sh</u>	NAT	Colorless

The absence of colorless derivatives which, on the beta mutation hypothesis, are expected with a frequency at least equal to that of noncrossover alpha occurrences strongly suggests that noncrossover alpha derivatives are not attributable to gene mutation of the adjacent beta element in the beta:alpha complex.

Mr. Sarma reports elsewhere in this number on the analysis of non-crossover alpha cases from this complex. On the gene mutation hypothesis discussed above these should be  $\beta_0\alpha$  in constitution. His findings are in agreement with those reported above in minimizing the beta mutational event as a basis for the origin of noncrossover alpha derivatives. Both lines of evidence indicate that the step in question somehow involves a loss of the beta element without recombination of marker loci.

It has been suggested that multiple exchanges within small chromosomal segments, or "conversion", whatever that may connote, would explain the noncrossover derivatives in this and other material. Several

lines of evidence seem clearly to rule out these phenomena as causes of the noncrossover alpha derivatives in maize. With regard to the hypothesis of multiple exchange, it is apparent that in the N  $\beta\alpha$  Sh/T a sh marked heterozygote two-and three-strand double exchanges with one crossover between beta and alpha and the other immediately adjacent would indeed give rise to an apparent noncrossover N  $\alpha$  Sh strand. However, this mechanism should, with equal frequency, yield alpha derivatives on strands carrying the parental markers of the homologue (T  $\alpha$  sh). As indicated in the table below not a single alpha-carrying strand of this constitution was obtained from heterozygotes which produced 126 cases of N  $\alpha$  Sh noncrossover strands. These results are equally damaging to an hypothesis of conversion based on a copy-choice mechanism since it also calls for the occurrence of the T  $\alpha$  sh strand. The most devastating evidence

Parental	Total		Alpha derivatives			
constitution	gametes	N a Sh	T a sh	T a Sh	N a sh	
N βα Sh/T a sh	570,000	126	0	46	0	

against these hypotheses comes from an analysis of alpha derivatives from beta:alpha hemizygotes in which the homologue is deficient (Df a- $x_1$ ) for at least the A and Sh loci. The data in the following table indicate that noncrossover alpha strands occur frequently even under circumstances where the homologous chromosome provides no opportunity for pairing at the A

Parental	Total	Alpha derivatives				
constitution	Ab gametes	T a Sh	Nα Sh			
T βα Sh/N Dfa-xl	69,000	41 a Sh	0 13			
N βα Sh/N Dfa-x1	58,000	54	e			

locus. The frequency of occurrence of noncrossover alpha derivatives from the hemizygote compares favorably with that of similar cases from beta: alpha/a heterozygotes. It may be noted too that crossover alpha strands are eliminated among progeny from the hemizygote, thus confirming that the deficient segment in the Df a-x1 chromosome does indeed include the A locus.

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The foregoing data indicate that the mechanism which produces the noncrossover alpha derivative from the beta:alpha complex is intrachromosomal and does not involve the homologous chromosome except perhaps indirectly.

#### 3. Shrunken-2 sweet corn hybrids.

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Over the past several years we have carried on a limited program designed to introduce the shrunken-2 factor on chromosome 3 into the standard Golden Cross and Iochief lines of sweet corn. Because the double recessive combination of sugary-1, shrunken-2 is obviously not a commercially suitable type we have substituted the sho factor for the sup factor in the converted lines.

It is apparent that hybrid combinations involving the converted lines (sh sh Su Su) retain the characteristics found to be associated with the sh factor in the original genetic background. They have a higher sugar content at picking and at maturity than the standard su material; they also have a superior sugar-holding capacity after picking. Because there is a longer period during which ears of shrunken-2 material may be picked without sacrifice of quality it is conceivable that double-cross production of sh sweet corn may be feasible ultimately.

Limited amounts of hybrid shrunken-2 seed are available at this time. Persons interested in receiving small samples of same should write Dr. Earl B. Patterson, Maize Genetics Cooperative, Department of Botany, University of Illinois, Urbana, Illinois.

Lines of shrunken-2 material will be increased this year and will be available for distribution upon request after harvesting of the 1959 summer crop.

John R. Laughnan

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#### 4. A test of the mutational hypothesis for the origin of the noncrossover alpha derivatives from AD:P and AD:Ec.

It has been shown by Dr. Laughnan that both  $A^b:P$  and  $A^b:Ec$  yield pales of noncrossover origin, besides crossover pales due to the normal separation of the a component from the  $A^b$  complexes. In heterozygotes with a,  $A^b$  yields a second type of pale of crossover origin. This pale is mutable as it carries a in the position occupied by  $\beta$  in the  $A^b$  complex when it is tested for mutability under the influence of Dt. The rate of crossover pales is about 1/2,000 in both  $A^b:P$  and  $A^b:Ec$  but the rates of noncrossover pales differ; in  $A^b:P$  the noncrossover derivatives are about twice as frequent as the crossover pales but in  $A^b:Ec$  they are only about 1/10 of the crossovers.

Among several hypotheses put forth as explanations for the origin of noncrossover alpha derivatives, mutation of the  $\beta$  element in the complexes  $\alpha\beta$  (A :Ec) and  $\beta\alpha$  (A :P) to a null level element  $\beta$ , was tested. If a  $\beta\alpha$  element is present as a component of the noncrossover alpha derivatives, this should be separable by crossing over as a colorless element from the complexes  $\alpha\beta\alpha$  and  $\beta\alpha\alpha$ . As controls in this experiment, stable and mutable alphas of crossover origin were included. The fre-

quency of colorless derivatives, designated a\*, separable by crossing over in both noncrossover and crossover mutable pales should be similar as the site of  $\beta$  is occupied by the presumed  $\beta_0$  in the case of noncrossover alpha and by standard a in the case of crossover mutable alpha. But the a\* derivatives of crossover origin from noncrossover alpha should not be mutable under the action of Dt, whereas crossover a\* from mutable a should be mutable since the crossover event in this case separates the a that went into the original heterozygote with Ab. The direction in which these crossovers are expected to occur and the mode of oblique synapsis required differ in alphas of the two sources as shown below. Finally, no a\* would be expected by crossing over from crossover stable pales.

Oblique synapsis and direction of crossing over

A <sup>b</sup> :P	A <sup>b</sup> :Ec
c/o stable alpha  T $\alpha$ Sh $a*$ not expected by crossing over c/o mutable alpha	T $\alpha$ Sh a* not expected by crossing over
$\frac{T}{N} \xrightarrow{a} \xrightarrow{\alpha: Sh} \xrightarrow{Ta*sh(Mutable \& recessive brown pericarp)}$ Non cross over alpha	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
$\frac{T  \beta_0  \alpha  Sh}{N  \beta\alpha  Sh} \rightarrow \frac{\text{Ta* sh}}{(\text{Non-mutable})}$	$ \begin{array}{ccc} T & \alpha & \beta_0 & Sh \\ \hline N & \alpha & \beta & sh \end{array} $ Na* Sh (Non-mutable)

Several separately occurring pales of each of the three types from both the sources were compounded with  $A^b:P$ ,  $A^b:Ec$  and the North American allele A in well-marked complexes. Pales of  $A^b:P$  source were more frequently compounded with  $A^b:P$  and vice versa. The  $F_1$  materials were grown in isolation plots and back crossed with a sh or a Sh. Colorless cases occurring on such ears were picked out and analysed for the constitution of markers, mutability under the influence of Dt and pericarp reaction.

Regardless of the type and source of alpha used in the experiments, a\* cases of noncrossover origin were recovered from them. The mechanism by which these arise is not clear.

Preliminary results are summarized below. Most of the a\* derivatives have been analysed for markers and mutability but the pericarp tests are not yet available.

# Alpha derivatives from Ab:P

The population of pale gametes studied under each class of alphas and the number, marker constitution and the rates of recovery of a\* per 105 pale gametes are given in table I.

# Pales from Ab: P

Table I

	Population of Pale	a* Cases			Rate of a*per 105 pale gametes		
Pale Class	gametes	Total	Non c/o		Non c/o		
Cross over stable	93,905	4	4	0	4.26	0.00	4.26
Cross over mutable	53,250	15	10	5	18.78	9.39	28.17
Non-cross over	107,980	5	5	0	4.63	0.00	4.63
Total for Ab: P	255,135	24	19	5	7.46	1.96	9.41

In this case, the crossover stable and the noncrossover alphas behaved similarly. Out of over 200,000 pales from these, there was not a single occurrence of a colorless case of crossover origin. The rate of origin of noncrossover a\* cases is similar in both. Almost all of these colorless derivatives have been tested for their mutability and are found to be stable. The absence of a\* by crossing over in a population of 108,000 gametes in the case of noncrossover alphas and 94,000 gametes in the case of crossover stable alphas shows the structural similarity of these two classes of pales which are of different origin. From mutable pales, in which the standard a element is known to be present, five a\* cases of crossover origin were obtained from a population of smaller size, 53,250. From this, it may be assumed that the mutation of  $\beta$  in the complex  $\beta\alpha$ , to  $\beta_0$  does not constitute the basis for the origin of noncrossover pales and that the B element appears to be completely eliminated during the process. Since noncrossover a\* cases are recovered from pales of all the three classes, their occurrence cannot be used in conclusions about the existence of a null level element. However, the rate of production of noncrossover colorless cases is rather high in the case of mutable pales. Whereas all a\* cases of crossover origin from this source are mutable, the noncrossover a\* derivatives appear to include both mutable and stable types. The mutability of some of these noncrossovers indicates that these colorless cases represent the a that was incorporated in the crossover event during the production of a mutable alpha and the mechanism of the separation of this may be analogous to the isolation of noncrossover pales from A. P. The stable a\* of noncrossover origin from a mutable pale may be attributed to the phenomenon similar to the production of a\* from the crossover stable and the noncrossover pales if it is also assumed that the standard a element is lost during this process. Further information on the nature of these colorless cases will be available after the pericarp tests are completed.

# Alpha derivatives from Ab:Ec

The populations of pale gametes and the details of the colorless cases obtained from them are given in table II.

Pales from Ab: Ec

Table II

	Population of Pale		a* Cases			Rate of a*per 105 pale gametes		
Pale Class	gametes	Total	Non c/o	c/o	Non c/o	c/o	Total	
Cross over stable	125,830	22#	13*	6*	10.33*	4.77*	17.48	
Crossover mutable	96,535	21	17	4	17.61	4.14	21.75	
Non-cross over	181,120	40	35	5.	19.32	2.76	22.08	
Totalfor Ab: Ec	403,485	83	65*	15*	16.11*	3.72*	20.57	

- # 3 cases unclassified
- \* likely to be revised

Pales from Ab:Ec source differ significantly from those of Ab:P as all the three classes of pales yield both crossover and noncrossover a\* derivatives. The direction of crossing over is constant in all the crossover cases, the recombination being always for the distal marker of the chromosome carrying alpha and the proximal marker of the homologous chromosome. In the mutability tests so far, only the a\* cases of crossover origin from crossover mutable pales have been found to be dottable under the influence of Dt. This is expected as the standard a is mutable. The colorless crossovers from crossover stable and noncrossover alphas have not so far been found to be mutable.

According to  $\beta$  mutation hypothesis, only noncrossover and crossover mutable alphas are expected to yield a\* by crossing over; crossover stables are not. However, 19 colorless cases were obtained from crossover stable alphas and six of these were of crossover origin. Since the T-Sh region is only about 7.25 recombination units in length, the proportion of colorless crossovers is too large to interpret that the a\* in these cases is the usual noncrossover a\* which has experienced a coincident crossover for the region. If this were the case, reciprocal strands carrying a\* would also have occurred. This was not the case and all the six cases of crossover a\* were recombinants in the same direction. Thus, in the case of crossover stable pale, it is felt that crossing over results in the separation of a pre-existing null level element located to the right of alpha. Since the postulated null element is situated between a and  $\beta$ , it cannot be separated from the Ab:Ec complex in a single cycle. This null element seems to be associated also with noncrossover alpha and it is probable that the crossover stable a\* derivatives from these constitute the separation of this element. More conclusive proof that we are not separating a mutated  $\beta$  element,  $\beta_0$  in this case, has to await further tests.

The colorless cases of noncrossover origin from noncrossover and crossover stable pales so far tested are not mutable; those from mutable pales, as in the case of mutable alphas of Ab:P, belong to both stable and mutable types.

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# Interaction of fertility-restoring genes.

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Full restoration of male-fertility in the presence of Texas sterile cytoplasm has been shown by various workers to depend on two dominant complementary genes. In midwestern dent material, a few inbreds carry both; most other inbreds carry one; WF9 carries neither. This situation can be illustrated as follows:

Ky21, K55, I153 = AABB

K4, N6, L317 = aaBB

WF9 = aabb

(The gene here shown as A is Rf1, located near Rg on Chr. 3)

From self-pollinating plants of the pedigree (WF9 x Ky21) WF9, a line has been isolated which gave on preliminary test a completely fertile progeny with L317 and a wholly sterile progeny with WF9T; there-

fore, it is presumably of the constitution AAbb. This line will be used to study gene "B" and its possible interactions with the various partial fertility-restoring genes.

It is interesting to note that the use of inbreds of the constitution AAbb would permit, without detasseling at any stage, the production of double crosses giving only fertile plants in the farmer's field:

Even if inbred (1) above were replaced by the commonly used seed parent WF9, the proportion of fertiles to steriles in the double cross would be 3:1.

# 2. Employment of Vestigial-glume in screening for sources of smut resistance.

In the process of backcrossing material carrying the gene Vg to a series of inbred lines, vestigial-glume plants were noted to be strikingly more susceptible to corn smut, and often to ear rots, than normal sibs. If this observation holds generally true, Vg should prove a useful tool to screen for better sources of resistance to smut and perhaps ear rots, as was done by LaRue, using Cg to screen for rust resistance.

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#### Preliminary biochemical studies on the action of a gene controlling meiosis in maize.

In maize a recessive gene called "ameiotic" has been found (Rhoades, MNL 30) which prevents meiosis and leads to almost complete sterility. Occasionally a few kernels may be produced, but these result from unreduced diploid eggs. Plants of the constitution Am Am and Am am (both called normal plants throughout this discussion) are phenotypically completely indistinguishable from those of the constitution am am (called "ameiotic" throughout) except at the late reproductive stage. In the ameiotic plants tassels and ears appear normal, but the anthers fail to

develop beyond a certain stage. They remain covered inside the glumes and later the whole spikelet appears chaffy. The ears either produce no seeds or a few seeds from diploid eggs.

Biochemical studies have been undertaken with a threefold objective:
(1) to distinguish the phenotypes at an early stage, (2) to detect changes leading to the failure of meiosis (3) to obtain any possible clue for inducing meiosis in the ameiotic plants.

The experimental results obtained so far can be discussed conveniently under two heads: (a) Paper chromatographic studies of amino acids and sugars etc. (b) Studies with nucleic acids and their precursors.

(a) Paper chromatographic studies for amino-acids etc:
Roots, leaves, anthers and ears have been analysed for free ninhydrin
positive substances, sugars and ultra-violet fluorescent compounds by
ascending, descending or two dimensional paper chromatography of alcohol
and water extracts. No difference has so far been observed with respect
to sugars and amino acids or other ninhydrin positive compounds in roots,
leaves and early stages of anthers and ears. But in later stages of the
anther and ear, differences have been observed in ninhydrin positive spots,
though their exact nature has not yet been determined.

A clear difference, however, has been indicated with respect to an ultraviolet fluorescent spot in all the organs examined. In chromatograms of both water and alcohol extracts of roots, leaves, anthers or ears of normal as well as ameiotic plants two distinct fluorescent spots appeared consistently along with other variable ones. One of these two spots was yellow fluorescent and had an Rr of 0.33, the other showed a blue-green fluorescence and had an R. of 0.38. (Both Re's in ascending run with Tertiary Butanol: Glacial Acetic acid: Water solvent in 3:1:1 proportion.) In the normal plants (Am Am or Am am) the yellow fluorescent spot was either very faint or absent. In the ameiotic plants this spot was quite bright. To eliminate the error due to a concentration factor the relative brightness of the two adjacent spots was taken to be a better criterion. The y.f. spot was faint compared with the bluegreen spot in the normal plants and was as bright as the other one in the ameiotic plants. However, it may be mentioned that further observations in families segregating for the am gene as well as in families without am are necessary before attempting to characterize or identify the compound.

(b) Studies with nucleic acids and their precursors:
Nucleic acids (DNA and RNA) and their precursors; such as nucleotides, nucleosides and free purine and pyrimidine bases, have been extracted from young ears in different fractions and studied with combined spectrophotometry and paper chromatography.

Of the several fractions in the extraction process a difference between normal and ameiotic plants has been observed in two fractions:

(i) fraction supposed to contain only RNA and (ii) fraction containing the pyrimidine bases obtained from the apurinic DNA.

The first fraction showed a clear absorption peak at 260 mµ and differed only in the height of the peak indicating a quantitative difference. The possibility of a qualitative difference in terms of base composition has not been explored.

The second fraction showed distinctive patterns of u.v. absorption spectra between 200-300mµ. While the extract from normal plants had a big peak of absorption at 280mµ, that from ameiotic plants showed less absorption at the same wavelength. Chromatographic separation of the bases followed by systematic elution and further spectrophotometric analysis seemed to indicate differences in the components of the fraction. These differences might be due to one or both of two causes: (1) a difference in the composition of the nucleic acid, (2) a difference in the amount or nature of proteins. That the proteins do not differ in their amino acid composition in normal and ameiotic plants has been indicated by a chromatographic study of hydrolysates of leaf proteins, though the same has not been tested in the reproductive structures.

Further studies along these lines and concerning other biochemical aspects are in progress.

S. K. Sinha

# 2. Preferential pairing.

In the last issue of the M.G.C.N.L. it was reported that in tetraploids heterozygous for a structural aberration (inversion 3a 3L .4-3L .95) preferential pairing was proved to be operating. The evidence cited was genetic. The backcross ratio of the control duplex (AAaa) was 4.03A: la and that of the structural heterozygote duplex was 7.11A: la. The inverted segment is marked with A1 and the corresponding standard segment with a1. The difference in these ratios can only be explained by assuming that preferential pairing occurs. In the event of preferential pairing when two bivalents are formed only gametes of the type Aa would be formed. Preferential pairing in a quadrivalent would also lead to an excess of Aa gametes, because firstly double reduction cannot take place and secondly the chromosomes of a quadrivalent do not disjoin at random, there being a frequency greater than 1/3 of alternate disjunction.

Now, it is possible to present some cytological evidence which indicates that preferential pairing does occur and also to make an estimate of its magnitude, something which is very difficult to do from genetic data.

Cytological observations were made on the chromatid bridge frequency of the simplex structural heterozygote as compared with that of the duplex

structural heterozygote. Since a chromatid bridge is formed after crossing over between a paired inverted and standard segment, it follows that the frequency of chromatid bridges is a function of the frequency of non-preferential or homoeologous pairing.

The use of the chromatid bridge frequency of a diploid heterozygote probably would not be legitimate since the frequency of crossingover may not be guite the same on the diploid and tetraploid levels. Also in the tetraploid there is the possibility that in the case of quadrivalent formation, two chromosomes with a potential bridge may pass to the same pole and consequently the bridge will not be resolved at the first division. The use of the simplex tetraploid should provide a fairly good control for these two possibilities. Below is a total tabulation of the number of chromatid bridges observed at anaphase one.

AV FAMILIES	No bridges	One bridge	Two bridg
Simplex	172 65 <b>.</b> 2%	92 34.8%	
Duplex	237 85.3%	36 12.9%	1.8%

If there were no preferential pairing the bridge frequency of the duplex should be twice that of the simplex times 2/3 (since 1/3 of the time the pairing would be of the preferential type by chance alone.)

Since (2 X .348 X 2/3) or .464 / .165, the frequency of pairing of the non-preferential type is not the random value 2/3 but is reduced by a factor designated by "p", the preferential pairing factor. Thus by inserting the term 2/3 - p the equation may be balanced and the value p solved for.

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This means that 76% (1/3 + p) of the time the inverted segment pairs with the inverted segment and the standard with the standard. Only 24% of the time is the pairing the other way.

The use of trisomics to study preferential pairing appears to be quite promising. Here it will be possible to examine pachynema configurations (something which is extremely difficult to do in tetraploids) and to tabulate the different types of pairing. This part of the work remains to be done. However, there is some genetic data which can be presented which indicates that preferential pairing is operative on the trisomic level.

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	Cross				Ratio A a	
(1)	In A/NA/Na XNa/Na	3	450	149	3.02:1 X2	= 6.9 <del>**</del>
	NA/NA/Na XNa/Na	3	417	108	3.86 : 1	
(2)	Na/Na X In A/Na/Na	8	421	1620	1:3.85	x <sup>2</sup> = 259**
	Na/Na X NA/Na/Na	12	1045	1966	1:1.88	
(3)	Na/Na X In A/NA/Na	3	471	270	1.74 : 1 X <sup>2</sup>	2 = 7.6**
	Na/Na X NA/NA/Na	5	843	392	2.15 : 1	

The theoretical effect of preferential pairing at the trisomic level on genetic ratios remains to be worked out in its entirety. The problem would be simple if the chromosomes always paired as a bivalent and an univalent. Thus the results in (2) above could be explained as follows:

		Gametes expected				
The preferential type	Na In A	AA Aa aa A a				
(1/3 + p*)	N a	0 .5 0 0 .5				
The non-preferential type (2/3 - p!)	In A Na	0 .25 .25 .25 .25	,			

Since n = 1 gametes are recovered with a low frequency when a trisomic is used as the pollen parent we may neglect them. Only if p! has a value of greater than 0 may the results be explained.

A difficulty arises when we try to figure out what will happen when a trivalent is formed. Will there be an excess over random of "a" gametes produced when preferential pairing occurs? This depends on the mode of disjunction of the trivalent. Do the two chromosomes which have their long arms paired, go to the same pole more often, less often or at random? Would the presence of a chromatid bridge influence disjunction?

Another difficulty which should be mentioned is the fact that deficient and duplicate-deficient chromosomes are formed following chromatid bridge breakage which may lack the A locus. This would give a higher value for the recessive class and would bias the genetic results. This is true for the tetraploid ratios as well. However, it cannot explain the different ratios obtained with the tetraploids since it acts in the other direction.

#### 3. Analysis of crossing over in haploid gametes of asynaptic plants.

Haplcid gametes produced by asynaptic plants show a higher rate of crossing over than the gametes produced by normal sibs (Rhoades and Dempsey, MNL 23). The frequency of double crossovers is especially high. Since asynaptic plants on the average produce ears with many aborted ovules, the possibility existed that the recovered gametes represented a selected portion of the total population -- namely those derived from EMC's which had the advantage of frequent chiasmata and therefore a more regular chromosome behavior. If this hypothesis is correct, it would account for the results without the need to assume higher crossing over in the EMC's of asynaptic plants. One would expect the number of crossover gametes to be the same in an equal population of ovules from asynaptic and normal plants if all could be analyzed. Since only part of the population of gametes can be tested, no conclusions can be reached unless the number of recovered crossovers per asynaptic ear actually exceeds that from a normal ear. On this hypothesis there might also be a bias in asynaptic plants toward simultaneous recovery of crossover strands from two different bivalents in the same gametes. These possibilities were tested in the following way. Backcrosses were made using asynaptic and normal sibs as female parents and the offspring were classified both for c sh wx on chromosome 9 and for ws lg gl on chromosome 2. The number of ovules on ears of both types was counted. The results are given below:

	% Sh-Wx	% C-Sh	% Doubles	₹ Seed	% Set	Corrected % Set"	Corrected
N	21.2	3.7	.18	2780	74.9	100.0	2780
8.5	21.2	6.3 2.3*	.67 .28*	2377	30.6	40.9	5811

" corrected for reduced set of normal ears \* based on corrected number of ovules

TA	% Ws-Ig	% Lg-Gl	% Doubles	Seedlings	% Set	Corrected % Set"	Corrected E Ovules
N	8.4	18.9	0.2	1362	77.7	100.0	1362
as	14.5	29.0 9.6*	2.1	1255	25.8	33.2	3785

" corrected for reduced germination and for reduced set on normal ears \* Based on corrected number of ovules

Only a part of the seeds classified for c sh wx have been tested in the seedling bench so the ws lg gl data are incomplete. However, the number of observed crossovers in the ws-lg and lg-gl regions is greater in asynaptic plants than in their normal sibs. The same plants

showed an increase in the <u>c-sh</u> region but none in the <u>sh-wx</u> region. In the case of single crossovers in both chromosomes, the frequency of crossovers based on total ovules was less in asynaptic plants. The reduced set on these ears results in recovery of fewer crossovers per ear. With double crossovers, however, asynaptic plants produced more per ear than did the normal sibs. In the chromosome 2 data, about 2 per 1000 ovules occurred in normal plants while 7 per 1000 occurred in asynaptic plants. The latter rate is certainly a minimum since 70% of the gametes were inviable. A greater number of double crossovers (for both ws lg gl and c sh wx regions) occurred among the 30% of viable zygotes on asynaptic ears than occurred on normal ears with a much greater number of viable zygotes. This indicates either a preferential segregation of double crossover strands to the basal spore or alternatively a higher rate of production of such strands.

When the data for the two chromosomes were correlated, it was found that the ratio of chromosome 2 crossovers to chromosome 2 non-crossovers was the same among chromosome 9 crossovers and chromosome 9 non-crossovers. This was true for populations from both normal and asynaptic individuals. There is no evident association of crossover strands in single gametes.

While these observations do not support the idea of selective recovery of crossover gametes on asynaptic ears, they do not rule it out altogether. It is possible to conclude that something in addition to selection is operating, at least in the case of the double crossovers, since more double crossover individuals per ear are found on asynaptic ears than occur in the larger populations from normal ears.

E. Dempsey

# 4. Aberrant segregations from T6-9b heterozygotes.

The studies to be reported are based on the descendants of a single plant of a homozygous T6-9b stock obtained from Patterson. This translocation had been studied genetically by Patterson (MNL 32) and the backcross ratios were essentially normal. Burnham (Genetics 1950) reported 50.2% aborted pollen and 26.8% adjacent-2 segregation in T6-9b heterozygotes. Since the data below show striking deviations from the normal behavior found by Patterson and Burnham, it appears likely that these results are due to some additional modification in the single plant from which my material is derived.

The first indication of unusual behavior was found in self pollination of  $\underline{T}$  wx/ $\underline{N}$  Wx and backcrosses in which the heterozygote was used as female parent. The former gave 7-12% wx and the latter 21-26% wx. Pollen from sib plants gave normal ratios. Reciprocal backcrosses of  $\underline{T}$  wx/ $\underline{N}$  Wx plants gave the following results:

Heterozygous parent	Wax	wx	%wx
\$	601	233	27.9
	375	395	51.3

The translocated chromosomes are not recovered with the expected frequency in the female backcross progeny. Apparently there is no increased sterility, however, since the frequency of abortion on one T/N ear was observed to be about 50%.

One family segregating for the T and for abnormal chromosome 10 was studied cytologically. Plants heterozygous for the T were classified for the presence or absence of the abnormal chromosome 10. The same plants were used as females in backcrosses. The results are given below:

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Female pa	rent	Wx	wx	% Wax
T Wx wd k	N 10 N 10	212	525	28.8
T Wx wd k N wx Wd KL	abn 10 N 10	482	407	54.2

It is evident that the ratios approach normal in the plants with abnormal 10. The large knob (KL) present on chromosome 9 has little effect on segregation since similar ratios were obtained in plants heterozygous for a small knob and a knobless chromosome.

A small sample of the above population was grown to seedling stage to classify for wd and to obtain root tip counts. It was found that 3.2% of the offspring of a N 10/N 10 plant were tertiary trisomes whereas 12.9% of the abn 10/N 10 progeny were tertiaries. The increased frequency of tertiary trisomes from abn 10/N-10 plants partially accounts for the altered Wx:wx ratio mentioned above since 12 of the 14 trisomes were of Wx phenotype. However, something more must be operating in abn 10/N 10 plants to cause the increase from 28.8% Wx to 54.2% Wx.

The genetic and cytological data from the limited population tested in the greenhouse may be summarized as follows:

HIT IS AND THE	% trisomes	٤	٤*	% Wx*	% wd*	%wx-wd recomb.*	% 04	% 9-11 AI seg
T Wx wd k N 10 N wx Wd KL N 10	3.2	154	149	30.9	30.2	2.0	41.4	6.0
T Wx wd k abn 10	12.9	108	94	37.2	37.2	2.1	72.1	20.2

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#### \* trisomes excluded

The wx-wd recombination value is extremely low. In sib N 10/N 10 and abn 10/N 10 plants not carrying a translocation but possessing the same k 9/KL 9 constitution, Kikudome (in press) found 12.6% recombination in the N 10/N 10 plants and 30.8% in the abn 10/N 10 plants. Apparently, in the present case, the combined reduction by the heterozygous translocation and the large knob is too great to be counter-acted by the abnormal chromosome 10 and the same low values are found in both types of plants.

The counts of rings versus chains and of 9-11 AI segregations are based on small populations of less than 200 cells. The increased frequency of 9-11 segregations in abnormal 10 heterozygotes is correlated with the increased recovery of trisomes from these plants. The increase in ring formation in abn 10/N 10 plants is difficult to explain. Presumably ring formation depends on the presence of a chiasma in the T-wd region of 9S. The T-wk distance from Patterson's data is about 1 map units and the wx-wd region in this experiment is only 2 units. Thus even the 41.4% of rings in N 10/N 10 plants cannot be accounted for if no more than 12% of the cells have chiasmata in the critical region. (This ignores another extremely short arm of the T which would not be expected to exhibit 100% chiasmata). Since both the cytological and genetic data on recombination and ring formation are based on small populations, further tests are necessary.

E. Dempsey

#### 5. Further studies on preferential segregation.

In the MNL 31, data on preferential segregation for loci in the long arm of chromosome 3 were presented. Representing abnormal 10 as K 10, normal 10 as k 10, the chromosome 3 with a large knob at position .6 as K 3 and the knobless chromosome 3 as k 3, backcross data using the heterozygous plants as the female parent were obtained for four combinations:

K 10/k 10, K 3/k 3 K 10/k 10, k 3/k 3 k 10/k 10, K 3/k 3 k 10/k 10, k 3/k 3

The data clearly showed that preferential segregation for the  $gl_6$   $lg_2$   $a_1$  loci in the long arm of chromosome 3 occurred only in the K 10/k 10, K 3/k 3 combination. Some of the  $F_1$  sibs of the backcrossed plants were self pollinated and the  $F_2$  plants examined at meiosis for their constitution with respect to K 10 and K 3. These  $F_2$  plants were backcrossed and the following data obtained which are given in summary form together with the data from the backcrosses of the  $F_1$  plants. When there was no evidence of preferential segregation, the percent of segregation is indicated as 50 but it should be indicated that the actual values varied around this mean value.

Data from every possible combination of abnormal 10 and normal 10 with knobbed and knobless chromosome 3 have been obtained with the exception of the K 10/K 10, k 3/k 3 class which should yield 1:1 ratios

, 1 a f 1 a	% G1	% Lg	% <u>A</u>
к 10/к 10, к 3/к 3	50%		50%
K 10/K 10, K 3/k 3	50	63.3	67.9
K 10/k 10, K 3/K 3	50	( <u></u> -	50, 6
K 10/k 10, K 3/k 3	50 51.7	70.2 72.5	64.2 63.6
K 10/k 10, k 3/k 3	50	50	50
k 10/k 10, K 3/K 3	50		50
k 10/k 10, K 3/k 3	50	50	50
k 10/k 10, K 3/k 3	50	50	50

for all segregating loci. The data show clearly that preferential segregation occurs only when the chromosome 3 bivalent is heterozygous for the knob and when abnormal 10 is either homozygous or heterozygous. The slightly high percentage of preferential segregation of the A locus over that of the Lg locus in the K 10/K 10, K 3/k 3 class is anomalous but is almost certainly due to the relatively small population obtained for this combination.

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M. M. Rhoades E. Dempsey

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# Level of polyploidy and size of chloroplasts.

Using the elongate gene which when homozygous results in the formation of unreduced megaspores in plants at all tested levels of ploidy (Rhoades MNL 30), a polyploid series consisting of lN, 2N, 3N, 4N, 5N, 6N and 7N plants has been obtained. Although not isogenic, the close relationship of the different polyploids permits a comparison ' of the effects of ploidy level on various characteristics such as height, vigor, etc. One of the more interesting findings is that the size of the mesophyll chloroplasts is the same throughout the range of polyploidy although the number of plastids per cell increases with level of ploidy. This independence of plastid size from nuclear constitution is further indication of plastid autonomy.

M. M. Rhoades

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# 7. On the origin of abnormal 10.

During the course of the investigation discussed above on

preferential segregation with loci in chromosome 3, a number of plants were obtained which were heterozygous for abnormal 10 and carried a single B chromosome. Although our earlier studies had indicated no homology between the extra segment of abnormal 10 and B chromosomes, it was felt that a further examination was called for in view of Ting's (Chromosoma 1958) suggestion that the abnormal 10 which he found arose from a B-10 translocation. If the extra segment of the abnormal 10 found by Longley and subsequently widely studied by others has come from a B chromosome via translocation, pairing between the distal end of abnormal 10 and homologous regions of the B chromosome should occur frequently in plants with a single B and heterozygous for abnormal 10 because of a lack of pairing competition. A large number of pachytene figures were examined in such plants and, except for an occasional adhesion of the knob-like region of abnormal 10 to the distal heterochromatic regions of the B, there was no evidence of homology between the two chromosome segments.

> M. M. Rhoades E. Dempsey

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 The effect of the independent activator of a-mutable on pale green mutable.

In a previous communication (MCNL 30) it was reported that pale-green-stable (pg<sup>S</sup>) does not respond to the activator of a<sup>m</sup>. It is now believed that in this case a non-mutable pg<sup>S</sup> (similar to adl) was being tested. Recent experiments with a known mutable pg<sup>S</sup> clearly show that pg<sup>S\*</sup> does mutate in the presence of the independent controller of a-mutable. Furthermore, this controller corresponds in every way to Enhancer and so will be designated En.

The test of similarity showing En to cause both amI\*\* and pgs to be mutable.

F<sub>1</sub> pg-segregating types +/pg<sup>SW</sup> A A x Pg/Pg a sh/a sh<sup>\*\*\*</sup>+ mutable factor -- 1/2 Pg/pg<sup>S</sup> A/a sh no mutable factor 1/2 Pg/pg<sup>S</sup> A/a sh

+ mutable factor

\* known to respond to En

\*\* a<sup>mI</sup> is mutable only in the presence of En

\*\*\* this a responds to Dt and not to En

F <sub>1</sub> s and tested on	amI**	in tol
a sob distribution	On testcross	to amI**
Gave a-mutable	Did not gi	ive a-mutable

	v		eny of D	In proge	
1958	Total No. of ears	segregates pgm & pgs	segregates only pg	pg <sup>M</sup> & pg <sup>S</sup>	segregates only pg <sup>8</sup>
1370	8	4	0	. 0	- L
1371	4	1:	Ō	0.17	3 - 5
1372	3	2	1****	O t	0
1373	12	<u>8</u>	<u>o</u>	<u>o</u>	<u>1</u>
		15	1	0	11

\*\* amI is mutable only in the presence of En

\*\*\* this a responds to Dt and not to En

\*\*\*\* only exception to correspondence of En causing pgs to be mutable
and amI to be mutable. In all other cases, the occurrence of pgm
is correlated with a-mutability.

P. A. Peterson

#### 2. Other factors associated with a-mutable.

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#### Dense factor

19.270

Among the tested progeny of one a<sup>m</sup> allele, two very distinct pattern types were observed: very dense mutable (almost full color) and fine mutable (like Dotted). The following sample data represents some of the typical segregations of the two phenotypes.

The Cross: a<sup>m</sup> Sh/a sh x a sh/a sh (Dense) x colorless shrunken

1957

#### non-shrunken kernels

# mutable

the sale of the sale of	very dense	fine
410-30 x a sh	93*	89**
31B-1 x a sh	1414	52
410-4 M	117	49
410-14 8	168	42
410-31 x a sh***	76	39

<sup>\*</sup> On further tests, Dense gives rise to 1/2 dense : 1/2 fine.

\*\* On further tests, fine mutable gives rise only to fine.

\*\*\* These off-ratios have not as yet been analyzed.

It is evident that the two phenotypes are due to the segregation of a factor, designated "D", in whose presence, a dense pattern results; in its absence, the pattern is fine. "D" can be lost in somatic tissue since large sectors of the 2 pattern types occur on the ear as well as on the kernels. "D" is not an added dosage of activator (En) since in the case of aml with D, but without En, the kernels are not mutable.

#### Pale allele

In one family of dense type mutable ('57 262) 13/41 of the testcross ears  $(a^m/a ext{ sh } x ext{ a } ext{sh/a } ext{sh})$  were segregating Dense purple: Dense pale in various ratios, some of the ratios being 1/2:1/2. Subsequent tests have shown that there is a factor P that determines purple; in its absence, the kernel is pale-mutable. The relation of the purple factor (P) to Dense factor (D) has not been determined.

In summary, the a-mutable has the following components:

- a<sup>m</sup> = autonomously mutable many patterns from dense to very fine; mutates from colorless to colored.
- amI = stable or colorless in the absence of controller of mutability En; mutable in its presence.
- En = independent controller of mutability, arises from position at the a locus; similar to En of pg-mutable.
- D = Independent Dense factor causes the fine type, autonomous or independent mutable to be very dense; is lost somatically and arises somatically; its presence causes the fine pattern to be earlier and higher in frequency of mutability.
- P = Purple factor causes a basic pale to become purple; arose in purple stocks.

P. A. Peterson

# 3. The number of cell divisions in the growing seedling leaves.

Coincident with a study of temperature effect on mutation rate of pg (Jour. of Heredity 49: 121) it was shown that cell divisions occur in the leaf blade during the germination process and that more occur in the younger leaves than in the older ones. It is estimated that 15, 31 and 47 new cells arise between the time of germination and time of counting (when 3rd leaf is 15 cm long) in the lst, 2nd and 3rd leaf, respectively.

#### 4. Summary of linkage studies with albino mutants.

Linkage data that have accumulated to date for eight different albino mutants are summarized below. Seven of these mutants (vp-5, lw-1, w-3, cl-1, vp-2, lw-2, vp-9) are characterized by pale yellow or white endosperms and albino seedlings. The eighth mutant, ps, has pink endosperms and albino seedlings with a tinge of pink. The pastel-8686 allele of w-3 has seeds with pale endosperms that give pale green (pastel) seedlings instead of white. The green mosaic allele of vp-2 undergoes frequent back mutation to normal in the endosperm and seedling, resulting in pale yellow endosperm with patches of yellow and with albino seedlings with a mosaic of green tissue. Mutants vp-2, vp-5, vp-9, ps and w-3 frequently exhibit vivipary in addition to the traits already mentioned. In the following summary, given on the next page, the mutant is listed to the left above the chromosome map along with the chromosome arm in which it is found. The linkage maps are after Rhoades (Science 120: 115-120, 1954) with linkage values given by him listed below the chromosome while the linkage values determined by these studies are given above the lines connecting the genes with which tests have been made. In the cases of lw-1, cl-1 and vp-9, it has not been determined with certainty whether the mutants are to the left or right of their closest marker gene. The position shown is the most probable one on the basis of the present data. To the right of each linkage map is a list of the translocations with which linkage has been obtained, along with the position of the translocation break point and the linkage values. A linkage map for a ninth mutant, pastel-8549, has not been included since it is an allele to y-l.

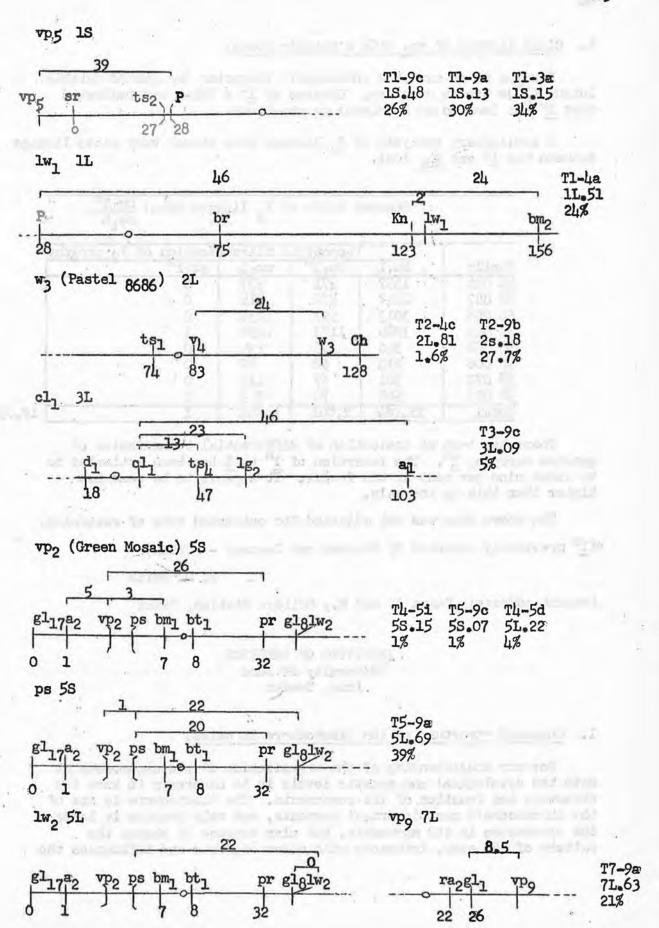
D. S. Robertson

# 5. Further allele test with albino mutants.

In addition to the albino genes listed on the above linkage map, w-8624 and w-7748 have been placed on chromosome three by a test with translocation 3-9c. Allele test with these two mutants this past summer gave positive results. Allele test with cl-1, which is also on chromosome three, gave negative results in a cross with w-8624 where both parents were known to be heterozygous.

Allele test in 1957 had established that a mutant sent me by Dr. Mumm (out of Oh7) was allelic to one sent by Dr. Braun (out of 182D). Test this year established that these were in turn, allelic to vp-5 on chromosome 1.

A new pastel mutant, pastel-4889, has proved to be non-allelic to pastel-8549 and pastel-8686. Its position on the linkage maps has not been determined as yet.



# 6. Close linkage of su, with a mutable locus.

Studies of an unstable chlorophyll character designated mutable luteus  $(1^m)$ \* are in progress. Crosses of  $1^m$  x TB4-a had indicated that  $1^m$  was located in 4S distal to the break.

A preliminary analysis of  $F_2$  linkage data showed very close linkage between the  $\underline{\mathbf{1}}^m$  and  $\underline{\mathbf{su}}_1$  loci.

•	Summary	table	of	F <sub>2</sub>	linkage	data:	Su <sub>1</sub> 1 <sup>m</sup> su <sub>1</sub> L	-
					51		1	

-	Phenotypic classification of Fo progeny							
Family	SulL	Su <sub>7</sub> 1m	sul	su <sub>7</sub> 1 <sup>m</sup>				
58 046	1357	271	537	0				
58 047	2247	478	815	0				
58 048	3013	597	1234	0				
58 049	3986	1173	1696	1				
58 058	340	95	134	0				
58 066	210	58	134	0				
58 072	301	59	113	0				
58 073	.566	. 70	.203	0				
Total	12,020	2,801	4,814	1				

19,636

There has been no indication of differential transmission of gametes carrying  $\underline{\mathbf{l}}^m$ . The reversion of  $\underline{\mathbf{l}}^m$  to  $\underline{\mathbf{L}}$  has been estimated to be about nine per cent in the female. It appears to be somewhat higher than this in the male.

The above data was not adjusted for estimated rate of reversion.  $*(\underline{1}^m \text{ previously reported by Rhoades and Dempsey - MCNL24})$ 

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# 1. Compound structure of the kinetochore in maize.

For our understanding of the organization of the chromosome at both the cytological and genetic levels it is necessary to know the structure and function of its components. The kinetochore is one of the chromosome's most important segments, not only because it leads the chromosome in its movements, but also because it shapes the pattern of the arms, interacts with other segments and influences the

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distribution of most chromosome properties.

Although excellent preparations of maize pachytene chromosomes may be readily obtained, cytologists and cytogeneticists who have studied them for the last 20-30 years have described the kinetochore in this organism as an empty region and represented it in drawings by a large circle.

This was most unfortunate since this classical organism together with Drosophila contributed to establish most of our cytological and genetic concepts. The result has been that we have looked for many years at the kinetochore as an empty region deprived of structure and subsequently of genes or any specific activity.

In the last ten years our study of the kinetochore structure in other organisms has radically changed this picture (Lima-de-Faria, Int. Rev. Cyt., 1958). But the maize chromosomes remained as a kind of exception to the now well established occurrence of a complex structure within the kinetochore.

During a study of pachytene chromosomes of maize carried out at the University of Illinois, the kinetochore of pachytene chromosomes was investigated by means of the squash technique commonly used for this organism. Anthers were fixed in propionic acid - 95% alcohol 1:3 and stored in a freezer. The P.M.C.'s were stained with aceto-carmine as in the usual procedure.

In many cells the kinetochore of pachytene chromosomes of cornexhibited with sharp clearness a quite complex structure. In maize, due to the existence of the chromomere size gradient on both sides of the kinetochore, this organelle can be very well delimited.

The kinetochore appears to be composed of chromomeres and fibrils indistinguishable in stainability and morphology from those found in other regions of the chromosome. As a rule one or two chromomere pairs are seen in each kinetochore but as many as three chromomere pairs separated by weakly stained fibrils may be observed.

The pattern is essentially the same as found in rye, Agapanthus and other organisms (Lima-de-Faria, Hereditas, 1949 and Chromosoma, 1955). Within the kinetochore of maize chromosomes there can be found as many as 7 different segments: 3 chromomeres and 4 fibrils. This reveals that the structure is sufficiently complex to permit the occurrence of rearrangements leading to the formation of kinetochores with different genetic constitutions.

A functional differentiation among kinetochores of the chromosomes of maize was found by Gurgel (MNL,1956 and X Int. Cong. Genet., 1958). At pachytene, kinetochores of nonhomologous chromosomes may associate as the kinetochores of salivary gland chromosomes of Drosophila regularly do. This association in maize is less intimate than in Drosophila

and it occurs sporadically. Of special significance is that Gurgel made a statistical analysis of the frequency of association and found that all kinetochores associated at random except the one of chromosome 5. The frequency of association was much higher for this chromosome.

This result can now be better interpreted as the compound kinetochore structure here described can be easily conceived to mutate or rearrange, leading to the formation of kinetochores with different properties.

McClintock (Genetics, 1938) has shown that chromosome 5 could be fragmented through the middle of its kinetochore, the two halves retaining their functional activity on the spindle. The functioning of one half and the structural similarity of the segments reveal that the kinetochore of maize is a repeat. The kinetochore of rye is also a functional repeat, since a kinetochore with one chromomere and two fibrils (with about one third of its elements) functions normally on the spindle, being perpetuated through mitosis and meiosis (Lima-de-Faria, Chromosoma, 1955), and further each half of the kinetochore forms a separate iso-chromosome (Lima-de-Faria, Hereditas, 1956).

When the nucleolar organizer of maize chromosomes is split into two segments both retain their functional activity, but the large proximal segment of the nucleolar organizer forms a smaller nucleolus than the small distal segment (McClintock, Z. Zellf. u. Mikr. Anat., 1934). Similarly, a kinetochore with a deletion shows higher ability to withstand elimination at meiosis and less power to influence the pattern of the arms (Lima-de-Faria, Chromosoma, 1955). The elements of both the kinetochore and the nucleolar organizer have the same essential properties but they differ from each other in their functional power.

A. Lima-De-Faria

# 2. Viability of translocated chromosomes in maize.

In the study of chromosome organization it is relevant to know whether chromosomes with new arrangements are more or less viable than those with the normal pattern. With this in view a cross was made using pollen of plants heterozygous for translocation 5-6 (T 5-6 y/N Y) and female plants carrying small y. Translocated chromosomes carry small y (white kernels) and normal chromosomes large Y (yellow kernels). The results are summarized in Table 1.

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The differential fertilization of gametophytes carrying Y and y is highly significant. Gametophytes carrying translocated chromosomes are apparently more viable than those with normal chromosomes.

In this translocation the nucleolus organizer is moved to the end of a long arm after a knob, quite far away from the kinetochore. In

TABLE 1 - Differential fertilization of gametophytes carrying Y (normal chromosome 5 and 6) and y (translocation 5-6) in maize.

Kernel		E	ar		Total
Color	1	2	3	4	
ellow Y	138	92	30	172	432
White y	192	122	27	187	528
rotal .	330	214	57	359	960

strains of maize raised outside experimental conditions the nucleolus organizer is known to occur regularly at a definite locus close to the kinetochore. Outside experimental conditions any other nucleolus location is apparently selected out. Thus, from the point of view of chromosome organization the translocated chromosome is expected to have a lower survival value.

The easy survival of the translocation under controlled culture conditions is not necessarily to be attributed to the presence of the new chromosome arrangement but to the association of the translocation with one of the many gametophytic factors known in maize.

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# Defective endosperm factors from maize-teosinte derivatives.

Additional data have been obtained on the defective endosperm types in the derivatives of the controlled introgression of teosinte in the inbred A 158. Other defective factors are turning out to be identical or allelic. Except for allelism not yet having been clearly established for detly and detl5, all the following factors should be considered allelic:

det4, det5, det10, det11, det14, det15, det17, det18, det19, det20,

An extensive study has been made of de<sup>t29</sup>, which originated in the stock carrying de<sup>t22</sup>. Since the latter gave intermediate defectives, with rather special characters, moderate reduction in endosperm development and fairly good germination, the appearance of a segregating ear with extreme defectives reduced to a simple shell, was assumed to be due to a new mutation. Test crosses demonstrated that de<sup>t29</sup>, was a stronger allele of de<sup>t22</sup> and that the latter had mutated to de<sup>t29</sup>. Moreover de<sup>t29</sup> did not keep the new character of extreme defective. In order to study the instability of the system involved, many self-pollinations have been carried out in plants obtained from normal seeds of ears segregating extreme defectives (de<sup>t29</sup> type, weight mg 0-20), intermediate defectives (de<sup>t22</sup> type, weight about mg 20-120) or both.

The results are summarized in the Tables 1, 2, 3. The available data confirm the instability both of det22 and det29: on the average, each of them, in about one case out of ten, mutates to the other type of defective; in both cases also the segregation is not too far from the theoretical 25%. When, however, the ears segregate both types of defectives the behaviour is quite puzzling. Not only, as one could expect, are cases found in which just one type of defective is detectable, but also ears are frequent where the presence of both kinds of defectives is accompanied by an abnormally high cumulative percentage of the defectives themselves.

Another curious behaviour of det22 is as follows: when kernels that show endosperm unquestionably det22/det22/det22 are germinated and plants are obtained from them, self-pollination produces ears on which is observable monohybrid segregation of normal and det22 kernels.

Table 1. Results of self-pollinated ears produced by plants originating from normal seeds of ears segregating extreme defectives.

Row and	Approximate per	rcentage of defectives	Total number of seeds
ear number	extreme type	intermediate type	
58-578- 1 - 7 - 7 bis - 7 ter - 10 - 11 - 16 - 21 - 30 - 36 - 40 - 40 bis - 50 - 51 - 57 - 61	33 28 12 28 56 21 47 32 35	0 2 0 5 0 18 1 0 0 0 0 1 4 0 0 2 0 2 0 9	148 215 139 215 193 140 159 126 129 122 198 232 194 168 227 175 217 287

Table 2. Results of self-pollinated ears produced by plants originating from normal seeds of ears segregating intermediate defectives.

Row and	Approximate pe	Total number	
ear number 58-577- 3 - 6 - 9 - 11 - 12 - 13 - 25 - 29 - 33 - 38 - 40 - 41 - 43 - 49	extreme type  1 2 0 7 28 0 2 155334 0 6 13 0 1 0 2 0	intermediate type  20 23 20 8 8 29 32 32 23 20 28 26 17 60 29 11 17 27 28 19 14 36	of seeds  129 228 203 284 106 217 160 210 124 121 193 266 87 145 164 194 379 152 103 108 238 115 359

Table 3. Results of self-pollinated ears produced by plants originating from normal seeds of ears segregating extreme and intermediate defectives.

Row and	Approximate per	Approximate percentage of defectives					
ear number	extreme type	intermediate type	of seeds				
58-579- 1	42	11	207				
- 3 - 4	22	1 24	218 156				
- 5	53 20 22 33 20 42 37 31 28 15 27 27 21 27 27 21 27 21 27 21 27 36 22 41	20	132 138				
- 7	22	27 32 0	215				
-11 -13	5 20	214 20	162 20h				
-10 -11 -13 -21 -22 -23 -24 -28 -30 -31 -37 -38 -39 -46 -46 -53	0	0 18	168 162 204 180 57 180 176 293 255 117				
-22 -23	37	18 1 25 20	180				
-24 -28	31		293				
-30 -31	15	18 26	95				
-37 -38	3	29 16	209				
-39 -39	21	22	220				
-39 bis	36	22 23 32 2 27	209 240 220 271 139 178				
-46 -53	41	2 27	178 291				

A. Bianchi

#### 2. The 'asynaptic' factor in the multiple tester.

The 'asynaptic' condition reported in the multiple tester of maize (MNL 1957) has been studied as to possible consequences on crossing-over values. In heterozygous condition such a genetic factor does not decrease the crossing-over percent in the tested yg-sh region on chromosome 9. On the contrary the recombination value seems higher, although not statistically determined, as yet.

In spite of the fact that data are available only in heterozygotes for the asynaptic condition, the results agree with the conclusions obtained by Rhoades in the study of the asynaptic factor identified by Beadle. In our material the fertility is apparently normal.

Some data on the tested region follow:

- 4							0.775	
Row No.	Family	Sh Yg	Sh yg	sh Yg	sh yg		mbination ob. error	Map units
57-427	F <sub>2</sub>	55	7	9	5	31.0	± 4.4	
-428	4 4 1	245	39	41 57	58		± 1.7	
-429	11	286	45	57	46		± 1.8	
-431	11	412	70	77	107		± 1.3	
-432	11	217	41	43	45	28.0	± 2.0	
Total		1215	202	227	261	26.0	± .8	29
		the series	co	ntrol	- 2101	94 45		130
56-506	F <sub>12</sub>	1024	150	120	153	26.0	± .9	
57-406	11	624	65	65	61		+ 1.2	
-407	n	1542	256	211	362	22.0		
-408	- 11	1330	212	246	311	24.5	± .7	
-433A		594	113	74	130	23.0	± 1.1	120
-433B	ST	459	50	32	98	14.5	± 1.0	
-434A	11	615	85	96	137		+ 1.1	
-434B	1 11	534	59	62	148		± 1.0	
Total	F <sub>2</sub>	6722	990	906	11,00	22.0	± •3	24.1
56-507/50	6 в	253	64	56	197	21.	± 1.1	23

Additional control data on the regions wx-yg and sh-wx give, in the same control stock, distances in map units quite comparable to the standard ones.

Family (pooled data)	Wx Yg	Wx yg	wx Yg	wx yg	Recombination %+prob. error	Map units
F <sub>2</sub>	5514	1/42	1833	892	41.5 ± .5	56
В	192	100	117	161	38.1 ± 1.4	48
	Sh Wx	Sh wx	sh Wx	sh wx		
F <sub>2</sub>	11750	2228	1933	2722	25.0 ± .2	28
В	254	83	55	235	22.0 ± 1.1	24

It will be noted that in all cases the recombination value calculated from backcross data is slightly lower than that from the pooled F2 data. Since backcrosses were made on the multiple recessive this may suggest that the amount of recombination is lower in microsporogenesis than in megasporogenesis.

#### A. Bianchi

#### 3. The translocation point in TB-8a.

Plants msg j, have been crossed by the TB-8a stock obtained by Dr. H. Roman. In a progeny of 18 plants, 7 showed the japonica character; 5 of these were ms, too. The japonica plants were, moreover, shorter than the normal J plants, confirming their hypoploid nature. The results suggest that the j<sub>1</sub> factor is distal to the translocation point in chromosome 8. Previously by means of deficiencies it was shown by McClintock (1933) that the j factor is in the distal portion of the long arm of chromosome 8.

#### A. Bianchi

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### JOHN INNES HORTICULTURAL INSTITUTION Hertford, England

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#### 1. Differential pollination in maize.

An attempt has been made to change the flowering times in a maize population by the method of separating seeds from the middle, bottom and top part of a single ear, which represent the earliest, medium and latest silks to emerge. The hypothesis is that a shift may take place through the differential timing of pollination. Isolation plots of each group whose seeds were separated each year were grown for three years and then the lines were combined in one large field experiment comprising three

plots. Silking times and tasselling times were recorded. It was found that there were no consistent differences in flowering times between the lines. Either the initial population had not been sufficiently heterozygous for flowering time, which is unlikely as an open-pollinated F<sub>1</sub> hybrid ear was used initially, or the differences in timing of the Silks from the three regions of the ears have not been sufficient to act as a differential sieve for separating the early and late pollen grains.

G. Haskell

#### 2. Studies with West Indian maize.

A series of six sowings at monthly intervals were made in 1957-58, commencing on 2 September, using nine varieties of West Indian maize supplied by the School of Tropical Agriculture, Trinidad. One hundred seeds (50 per seed-box) were sown of each. In this way it was hoped to utilize the differences in day-length over the 6 months period to find the best time for sowing the crop at Hertford for the promotion of plants with functional ears and tassels in the glasshouse, that would also give a satisfactory seed yield for further experimental investigations.

The number of non-normal seedlings from each sowing was recorded, and these included characteristics like dwarfness, and striped or narrow leaves. The majority of aberrants had pale leaves of varying degrees; there was an occasional albino. The graph of mean leaf number on 4 February, 1958, for plants originally separated as normal and aberrant seedlings, indicates that the controls always have more leaves than the aberrants. As the difference decreases with the lateness of sowing, this suggests that the difference in leaf number is a reflection of the difference in growth vigour of the two classes.

The plants of the six sowings indicated that the best results for pollination followed by seed setting were from particularly the second, third and fourth sowings, viz., on 28 September, 28 October and 23 November. Another advantage of these sowings was the over-lap in pollen shedding, which facilitated hand pollination. In the first sowing there was a shortage of pollen and the ears were somewhat shorter than those of later sowings. On the other hand, the fifth and sixth sowings gave a larger frequency of plants failing to reach tasselling and silking within a reasonable period, e.g. by 28 July (i.e. after 5 or 6 months in the glasshouse). The Early Caribbean family was the earliest throughout the range of sowings, although Coastal Tropical Flint was as early at the fifth sowing. Seed drying and shelling of the harvested ears was satisfactory under glasshouse conditions.

Intra-pollinations have been made between the more vigorous and the less vigorous plants in each family. The vigour of the various crosses remains to be compared.

It is concluded that for breeding purposes, the best times for sowing West Indian maize strains in the glasshouse in Britain must be related to the day-length. Sowings are best made when the days are shortening from 11 1/2 hours to 8 1/2 hours.

G. Haskell\*
W. Williams

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### MACDONALD COLLEGE OF MCGILL UNIVERSITY Province of Quebec, Canada

#### 1. Transallelic change at the C locus.

A study reported in the 1957 News Letter (p. 144) showed no invariable transallelic change of the C<sup>T</sup> component of C<sup>T</sup>C heterozygotes when used in a mating scheme similar to that developed by Brink. C<sup>T</sup> alleles from C<sup>T</sup>C homozygotes and C<sup>T</sup> alleles from C<sup>T</sup>C heterozygotes produced the same phenotype when placed on homozygous A C R pr silks in W22 inbred background.

Further studies have shown no invariable transallelic change of the C component of these same  $C^{I}C$  heterozygotes. C alleles from  $C^{I}C$  heterozygotes and C alleles from CC homozygotes produced the same phenotype within each mating whether placed on A c R Pr. Inbred Al71 (A c r Pr y) or Inbred W9 (A c r Pr Y) silks.

Robert I. Brawn

#### 2. Dark variegated.

Preliminary observations on dark variegated pericarp, a new phenotype in the mutational spectrum of the PVV allele first reported last year, has indicated that the frequency of red ears in the progeny of dark variegated kernels is considerably higher than in the progeny of the parental medium variegated. This is consistent with previous observations by Brink and his students that the amount of red striping of the pericarp is related to the frequency of self-colored offspring.

Robert I. Brawn

# UNIVERSITY OF MELBOURNE Melbourne, Australia Botany School

#### 1. Persistent nucleoli at the second pollen grain division.

In making preparations to study non-disjunction of B chromosomes at the 2nd pollen grain nuclear division, it was observed that, when B's were present, the nucleolus persisted through metaphase and disintegrated across the spindle during anaphase. From 3 to 7 quite distinct pieces of nucleolus on the spindle were noted. Non-disjunction of the B's was also observed in this case when 2 B's were present.

Margaret Blackwood\*

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world was sell on all patterns while I

#### 1. New characters.

#### teosinte branched (recessive)

The character we have been temporarily calling teosinte branched (many tillers plus slender branches at most of the nodes) has been tested with a series of interchanges marked by wx, su or pr. It shows linkage with Tl-4a (1L.5-4S.7), using su as the marker. A separate test with su shows no close linkage, hence the gene is probably in chromosome 1.

#### dwarf S-3

This character is one originally produced by irradiation by Stadler. It shows linkage with T8-9 marked with wx.

C. R. Burnham

#### 2. New linkage data.

A 3-point backcross test gave the following:

25.1% for region 1, and 33.5% for region 2.

This agrees with the order indicated earlier (News Tetter #29, p 51).

C. R. Burnham

#### 3. Big ring.

(a) Production of rings with a large number of chromosmes, progress report.

Thus far, the biggest ring induced by a homozygous line is a 010. The homozygous line, referred to as a permanent ring of 10, has five chromosomes involved in interchanges, i.e. 1-5-6-7-8, the successive steps being obtained by X-rays:

5-7 X-ray 1-5-6-7 X-ray 1-5-6-7-8. A second line which will include the other five chromosomes, 3, 2, 4, 9 and 10 is being put together by genetic crossing over. The cross of these two lines will produce 2010.

To develop a line which will induce a 620, Inman's general scheme, of having one interchange in common when two permanent rings of 6 are crossed to produce a ring of 8, two interchanges in common when a ring of 8 is increased to a ring of 10 and so on up is being used.

In addition to the permanent rings of 6 listed (News Letter #32, p. 94); plants heterozygous for the 1-9b + 1-7 (4405), 2-4b + 4-8 (5339), and 4-8 (5339) + 8-9b crossover combinations were identified.

#### (b) Crossovers in differential segments.

The first results were obtained from tests planned by Dr. Inman to determine the frequency of the complementary crossovers in the differential segments. Progeny of crosses of the two types: T1/T2 x normal and T1/T2 x T1T2 were grown and the frequency of normals determined. There is a possibility that small deficiencies at the break points may be present and yet survive the gametophyte screen. In building rings with more chromosomes by crossing single interchanges, the effect of such deficiencies might be cumulative as more interchanges are added. For T1-7 + 5-7, the values were 20.8 for the T1T2 crossover and only 3.7% for the normal complementary type. The results indicate a difference, but appear to be in the opposite direction of what might be expected. Thus far, seed set and pollen fertility appear to be normal on all 10II stocks that are homozygous for two up to four interchanges.

#### (c) Interdependent rings.

A species with an even-number of pairs and heterozygous for interchanges involving every arm will show 204 at meiosis. In these the homologous midsegments are not in the same ring (Inman, News Letter #32,

1958, p. 95). Following this a plan was set up using the following interchanges involving 4 chromosome pairs in corn: 1-6a, 1-7 (4405), 5-6c and 5-7 (5179). Of the four permanent rings needed, 1-6 + 5-6, 1-6 + 1-7, 1-7 + 5-7 and 5-6 + 5-7, the last two seem to be established and crossovers for the others will be searched for this summer in the progenies of crosses with standard normals. Of the three crosses that can be made to produce different 204 in  $F_1$ , one will have the two rings interdependent.

C. R. Burnham

THE POR LINE

4. Notes on "Breakage Points for Two Corn Translocation Series" by A. E. Longley, ARS - 34, 1958.

The following is submitted as additional information:

total days of the control of the con

2-6a - this is the one in which I originally observed extensive non-homologous pairing at pachytene. The pachytene "cross" appears more often in the long arm of 6, but the break is in the short arm, not the long arm of 6 as listed.

5-6B - this is not the same as the 5-6b I list in Genetics 35:469. My 5-6b is 580.1 - 6 sat.

5-6c - my values for this are 5L.89 6S.00. Tests in the homozygote confirm this position in the short arm of 6, not in the long arm.

6-10b - (Genetics, Ibid. p. 461). This is not the same as the 6-10b listed in ARS-34-4.

C. R. Burnham
assisted by: Paul Yagyu
J. Axtell
O. L. Miller

A STATE OF THE PARTY.

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and

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at account of confidence will be broken any two constraints and parameters

#### Conversion effects at B.

In 1953, two weak-colored plants were observed among approximately 140 plants in the otherwise uniform progeny of a single B pl individual, one from each of two selfed ears of the parent plant. The exceptions were selfed and crossed onto intense. Selfs did not segregate, and have

remained uniformly weak through 3 generations of selfing. Outcrosses to intense gave all weak, and have continued to give only weak throughout three generations of outcrossing as male and female to intense, as well as in selfs of these outcrosses. Intense lines of 4 widely different backgrounds (not necessarily different in the source of B) have been used in the crosses; all are consistent.

When weak plants (any of various individuals from selfed or outcrossed progenies) are crossed to b, the F<sub>1</sub> is weak, and segregates normally for weak: green in selfs and backcrosses. Crosses of the F<sub>1</sub> to intense segregate 1 intense:1 weak; these intense plants segregate for green (but not for weak) on selfing, while the weak plants fail to segregate for intense or green, giving only the monotonous weak type. Markers with b segregate normally in these progenies.

Using B' to designate the weak type, the pattern of this system is essentially as follows:

B B selfed gave 140 B + 2 B exceptions; new B individuals continue to arise occasionally in this B B line.

B' x B gives only B'; these selfed, or again crossed to B, give only B', et cetera.

B' x b gives weak.

B'/b selfed gives 3 B':lb, backcrossed gives 1:l.

B'/b x B gives 1 B:lB'; the B here selfed give

3B:lb, the B' give all B' in selfs and in
recrosses to B.

Only 3 exceptions to the pattern have been seen so far. One exception was a barren, male-sterile, intense plant in a progeny from B' x B, and was presumably intensified in color through injury or barrenness; another exception, from B x B', had a long, narrow intense sector; the third exception consists of two intense plants out of 41 in a progeny from B x B' which has reduced pollen fertility in some plants, including one of the intense exceptions. The exceptions do not appear to negate the pattern, but rather to support it.

It is tentatively concluded that an allele at the  $\underline{B}$  locus,  $\underline{B}$ , regularly causes  $\underline{B}$  in the same nucleus to be changed to  $\underline{B}$ , at some time or times in the life cycle, and that  $\underline{b}$  is not affected. A paper on this phenomenon is in preparation. I will be happy to send a xerox copy of a complete diagram of the sequence of pollinations, including data, to any cooperator who wants it.

E. H. Coe, Jr.

#### 2. High-haploid line.

The capacity of stock 6 for induction of haploids in a gl\_ maternal

parent is heritable. In the following, 2698 (a2 B Pl RT), stock 6, their F1, backcrosses to 6 and selfs of backcrosses are compared in maternal haploid frequencies when outcrossed to gl\_. In the "segregating" progenies only Rr B Pl plants were tested. Haploids were verified by root-tip chromosome checks.

	No. plants Tested	No. Seedlings	No. Haploids	% Haploids
2698	15017 <b>3</b> - 150 mil	1298	n n2 n2 n1 s	0.15
6 0 1361 44	5 strait	1531	35	2.29
F <sub>1</sub>	4	3109	13	0.42
F <sub>1</sub> x 6	io er <b>9</b> the of	3694	Щ	1.19
$(F_1 \times 6)$ self	9	3611	46	1.27

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#### along we proble their the pools of a life Chromosome 9 linkage.

disservery of a la The following table includes new data, sums of new data with those reported last year, and one correction, indicated by an asterisk:

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Genes X Y	Phase	XY	Ху	Yx	ху	Total	Recomb.
Ar Bkg	RS es	355	200	188	200	745	10
Ar Ms2	RS	328	167	144	Q I	639	ENCEPT 8> NEX
Ar Wx	CS	1214	87	67	310	1678	10
Bf Bk2	CS	126	34	22	8	190	46 277
IV. to due	RS	288	81	112	17.0		1 B (141 C 100
Bf Bm	RS	249	120	129	0 ×		711 < 9
Bk <sub>2</sub> Bm <sub>1</sub>	CS	752	196		80	1246	45
Bk2 Gli5	CS	209	0. 73	17	41	274	19.1 209 1250114
Bk <sub>2</sub> V	RB	2	47	37	6	92	9
Bk <sub>2</sub> Wx	RB	6	43	36	7	92	114
sign for	CS	367	39	49	72	527	20
Strong Land	RS	688	269	316	15	1268	23
Bz Sh	CB	1025	19	21	974	2039	monda 2 and Ha
Bz V	CB	771	260	240	713	1984	250 1000
Bz Wx	CB	887	157	136	859	2039	The The search
D3 Wx	CB	67	7	5	63	142	8
127	CS	964	57	125	208	1354	24
Gl <sub>15</sub> Ms <sub>2</sub>	RS	271	128	80	0	479	<11
GlicWx	CB	170	12	14	187	383	7*
Ms2 Pg12	RS	359	142	206	0	707	1 × 8
Ms2 Wx	RS	1235	488	645	0	2368	< 4
Pg12Wx	CS	797	44	31	209	1081	7
V Wix	CB	913	1146	146	891	2096	114

It should be pointed out that Bf1 is treated as recessive in the above, since it is classified only in the seedling stage.

Data from the following 3-point tests are included above.

F	Parental	Reg.1	Reg.2	1-2	Total
wx v + + + bk	36 42 78	1 5 6 6.5%	6 1 7 7.6%	1 0 1 1.1%	92
+ + + sh bz wx	870 840 1710	19 17 36 1.8%	155 134 289 14.2%	2 2 4 0.2%	2039
+ + + sh wx d <sub>3</sub>	58 48 106	15 9 24 16.9%	5 5 10 7.0%	2 0 2 1.4%	142

The following 4-point test is also included:

No improvements in the map can be made over the one presented last year; with further refinement of the data, the 6 clustered factors appear to be still more tightly disposed in relation; in fact the order of factors between Wx and Bk2 is entirely open, and will remain so until double mutants and backcross data are obtained for Ar, D3, G115, Ms2, Pg12, and V. These are all very nearly the same distance from Wx (4-14 units); all overlap in their 5% probability limits on the map.

Tests for inclusion of some of these factors between the break points of translocations 1-9a and 1-9c, in which a plant heterozygous for the two translocations will produce a female-transmissible deficiency between the break points on the long arm of the ninth chromosome, have been carried out. The test consists in crossing the 1-9a/1-9c heterozygote by the recessive. Turcotte (Maize Newsletter 30:164, 1956) has reported that Ar is included in the deficiency. This has been confirmed. Tests of d3, gl<sub>15</sub> and ±/ms, were negative. Tests of pg<sub>12</sub> were also negative, but since this is a duplicate factor system there is no assurance that positive results would have been obtained, even if the deficiency included pg<sub>12</sub>.

#### 4. Linkage tests on co.

This new aleurone factor has not yet been located. A self of c2 ±/± bz1 gave 64 colored to 67 bronze-and-colorless, suggesting close linkage, but c, is independent of wx in a large test (1309 individuals). The following linkage tests have been carried out: wx 1-9c, 52% with wx in 657 individuals; bz2, 9:3:4 in 346; lg1, more than 50% in 285; A, 308 colored to 281 colorless, consistent with about 30% recombination; wx 3-9c, 53% with wx in 393; su, 48% in 363; Pr, 9:3:4 in 279; Y, more than 50% in 339; gl1, more than 50% in 731; wx, 50% in 1309; R, 9:7 ratio in 1061. Chromosome 3 is the most likely-looking at the moment; if so, probably far out on the long arm.

E. H. Coe, Jr.

#### 5. Spontaneous mutation of CI.

An additional population of about 1.5 million gametes in the cross CICI x CC has been examined for mutants. Only one possible case turned up. Judging from the previously-reported population, this case has a 50-50 chance of being valid. Obviously the mutation rate is low.

E. H. Coe, Jr.

#### 6. Subject index to Newsletters.

An attempt to index the Newsletters by subject is in progress.

Volumes remaining to be scanned before the index is ready to assemble are Nos. 1 through 3 (not on hand here—they will be checked elsewhere), 31, 32, this issue, and any subsequent ones which come out before the rest of the job is finished. In the meantime, any cooperator wishing a moderately thorough list of vol. 4-30 references (for example: linkage notes for a given chromosome; mutability factors or mutable loci; carotinoids; centromere linkage) will be sent it on request.

E. H. Coe, Jr.

Jackson March 1985 - In Control of the Control of t

#### 7. Effect of external agents on the frequency of crossing over.

In the last Newsletter (MNL 32:100) it was reported that in a preliminary trial, treatment with a .001 M solution of the chelating compound (EDTA) gave a significant increase in the frequency of crossing over between the members of a complex  $\underline{\alpha}$  a  $\underline{sh}_2$  segment on chromosome 3. In order to check the validity of this result and also to try some other agents, a large scale experiment using the same cross ( $\underline{\alpha}$  a  $\underline{sh}/\underline{a^M}$  Sh x  $\underline{a^S}$  sh) and the same technique (leaf feeding) but with two additional agents (ribonuclease and desoxyribonuclease) was conducted.

The recording of data in this experiment was altered somewhat from last year. It was found that with the stocks used, only dilute Sh crossovers could be consistently recognized. These included the am Sh, a a Sh and a-Sh cases which could not be separated one from another. The reciprocal cl sh class including am sh a-sh, and am a sh was difficult to recognize because of poor coloration of the sh seeds. Therefore, the data listed in the table below consist of the total a Sh crossovers observed on the non-shrunken kernels.

Frequency of crossovers from the cross a a sh/a Sh x a sh.

	Total Sh seeds	Total a Sh co's	Percent
Control	56,087	96	.17 ± .017
EDTA	18,247	42	•23 ± •035
RNAase	5,884	12	.20 ± .058
DNAase	4,400	10	•23 ± •070
			-
	84,618	160	.19

From the above, it can be seen that the apparent differences between treatment and control are not significant. A re-examination of last year's results reveals that it was a mistake to consider 2/3628 as an adequate control.

M. G. Nuffer

#### 8. A dominant striped leaf character located on chromosome 3.

A striped-leaf effect has been found which is inherited as a dominant. It appeared as a single striped seedling in the  $F_1$  of a cross of a multi-Dt x A C R dt. The seedling could be described as having many medium to small, narrow, white and pale green sectors extending to all parts of the leaf and sheath. A cross of this plant by a normal plant gave progeny which segregated 1:1 for the striped phenotype. A selfed ear of the original plant produced 1/4 extreme striped plants which had mostly white tissue and very little green, 1/2 moderately striped plants and 1/4 green. Most of the extreme striped plants failed to survive, but the one that did yielded all striped progeny when crossed to normal. Several of the moderately striped plants produced 1/2 striped progeny and 1/2 normal progeny when crossed to normal individuals. One of the striped plants was crossed to the translocation waxy series and the  $F_1$  backcrossed to homozygous normal waxy. The seeds were separated for waxy and planted.

From these it was determined that the striped effect, tentatively designated Sd, was located on the long arm of chromosome 3. Its position in relation to the known markers on this chromosome is at present uncertain.

M. G. Nuffer

#### Another two-unit mutator system.

The state of the s

The above described plant was unique in another respect. It grew from a colored-colorless mosaic seed selected from an otherwise full colored ear. The mosaic pattern was transmitted to its progeny and proved to be the result of changes at the R locus. The character appears to be a mutable seed color allele (R<sup>m</sup>) which changes to r, thus producing colorless patches on an otherwise colored or mottled aleurone. These changes occur only in the presence of another factor (tentatively called M) which is located on chromosome 9 between sh and wx. The three characters Sd, R<sup>m</sup>, and M first appeared in a single plant suggesting that they have a common origin. However, they are all on separate chromosomes and a careful check of the parents of the original cross revealed that the A C R dt parent carried Sd without expressing it. Therefore, the appearance of these three characters in a single plant most likely was the result of the chance combination of a mutator factor, M, producing a mutable allele at R, and of a favorable genotype for the expression of Sd.

M. G. Nuffer

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### 1. Serological investigation with the phylogenetical relationship among inbred lines in maize.

Up to the present, many works on the serological classification of various species in the plant kingdom have been carried out by using leaves or seeds of the plant. But, within a species, data on the phylogenetical relationship among races or inbred lines have not been accumulated. Since 1952, work has been done along the latter line by using the protein extracts of maize pollen as an antigen.

Pollen grains collected from the plant were preserved in a dessicator. According to need, they were immersed in physiological saline, and centrifuged at 3,000 r.p.m.; the supernatant was used as an antigen. Rabbits were immunized with three intravenous injections of such extracts, amounting to 5 to 8.5 cc in total. At the tenth day after the last injection, bleedings were taken, and held in a refrigerator at a temperature of 2°C. At the next day, antisera were performed, and then, inactivated

by holding at 55° to 56°C for 30 minutes in a water-bath. Judgments of precipitation were made by two methods; (1) the Ring method based on visual sight and (2) the chemical method based on a comparison of the amount of nitrogen contained in precipitates of antisera analyzed by the Microkjeldahl method.

Results of experiments are summarized as follows:

- (1) Pollen extracts immersed in physiological saline, which were obtained from the inbred lines, were certainly representative of the antigenicity. The intensity of precipitation was found to be different among the inbred lines.
- (2) The protein contained in pollen extracts was composed of albumin,  $\alpha$ ,  $\beta$  and Y-globulin, of which the former two were the main constituents of protein.
- (3) Of these constituents, only two, albumin and  $\alpha$ -globulin, varied quantitatively among the inbred lines, resulting in a difference of precipitation. The related inbred lines, which were derived from a given race, showed a tendency to be similar to each other in precipitation, because of having similar amounts of the two fractions.
- (4) The antigenicity of pollen proteinwas recognized to rest mainly on the precipitation of three protein fractions, albumin,  $\beta$  and  $\Upsilon$ -globulin, but not with the remaining one,  $\alpha$ -globulin. It is thereby considered that the phylogenetical peculiarity of the serological reaction should be dependent upon the difference in the albumin fraction in various inbred lines.
- (5) A difference in precipitation between any two of the given inbred lines was closely associated with the degree of heterosis appearing in the single-crossed hybrids from their  $F_1$  combinations. The correlation coefficient between the precipitation analyzed by the chemical method and the heterotic vigor was computed to be  $r = -0.921 \pm 0.147$  and  $r = -0.753 \pm 0.245$  for the grain and green yield, respectively.
- (6) With respect to the judgment of precipitation, the chemical method was superior to the visual ring method in its precision.
- (7) For detecting the serological reaction, the three types of antigen extracts may be rated: non-heated pollen was most satisfactory, next non-heated seed, and lastly heated pollen extracts. However, heated pollen extracts were better in the case of the visual ring method although they were least satisfactory in the case of the chemical method.
- (8) A strong precipitation reaction was always found in tests of a single-crossed hybrid and its parent lines, even though it was weak between the two parent lines. When a single-crossed hybrid was backcrossed with one of its parent lines, the precipitation was greater for the test of the backcrossed hybrid and its recurrent parent line than for the single-crossed hybrid and the same parent.

- (9) From these results, it may be assumed that the serological reaction may be used as an index for detecting phylogenetical relationships among various inbred lines.
- (10) With regard to breeding methods, the precipitation reaction should make it possible to predict the degree of heterotic vigor in the F<sub>1</sub> combinations of given inbred lines, without making any crosses, because a higher heterosis usually appeared in F<sub>1</sub> hybrids between those inbred lines with the more remote relationship from a serological viewpoint.

K. Urano

# NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES Section of Physiology and Genetics Hiratsuka, Japan

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part with the bowley are sufficiently by the

### 1. Maize races native to the island Shikoku situated at the southeastern part of Japan.

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About 250 samples with 3 ears for each were collected from about 200 farmer's fields in about 50 upland localities, totaling about 750 ears. On all these ears, 20 characters were measured. In order to examine the general characteristics of the races, 57 samples were chosen and grown at three stations, Hiratsuka, Iwate and Ehime. Measurements or observations were made on 29 characters. Results obtained were the following:

- (1) All of the 250 samples were characteristic of a Caribbean type of tropical flint. In accordance with the topographic complexity of the arable land and the accompanying diversity in maize cultivation, the racial differentiation was extreme. About 60 or more local races were met with. The 250 samples were, however, identified as belonging to 28 distinct races.
- (2) The 28 races were classified into 12 types; Okuuchi, Kowase, Wada, Gojô, Abeto, Sengoku, Hiyoshi, Okawa, Kuma, Irareko, Yellow-Yamakibi and Orange-Yamakibi. Most of them had a conical ear with orange seeds, typical of the Caribbean flint, and only two, Okuuchi and Yellow-Yamakibi, had a rather cylindrical ear with yellow seeds. Some races in the 6 types, Kowase, Wada, Gojô, Abeto, Okawa, Irareko and Orange-Yamakibi, were demonstrated to be favorable as breeding material. From a genocological viewpoint, the main peculiarities are as follows:
- a) The Okuuchi type is planted as a mixed crop in sweet potato fields, and is distributed mainly over terraces on the hill-sides in the southwestern coastal region. It is used purely as a catch crop; the soft ear is boiled or roasted. The erect, short, and broad

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leaves and the smaller prolific ears with smaller yellow seeds were recognized as quite peculiar to this type. These peculiarities may trace their origin to hybridizations between a larger eared race of the Caribbean flint and a primitive race of pop corn. In fact, both races considered as the parent have from old times been native throughout the country.

- b) Two Yamakibi types, Yellow and Orange, are planted only in the high land areas with a shifting system of cultivation, alternately repeating an upland cropping and a natural vegetation in an interval of several years. These fields are widely distributed on the sharp slopes of the high mountain tops, far apart from the residence of inhabitants, as compared with the ordinary fields where races of all other types are grown. These fields are not fertilized or cared for during the growing season of maize. Both stocks may be considered representatives of the most primitive and pure types of the Caribbean races native to Japan. This maize was characterized by having the smallest ears with large seeds. The orange type is grown for human food and is best ground as a fine powder, while the yellow type is best eaten fresh.
- c) The Kowase type is native to hilly upland areas of the middle interior part. Its grains were transparent orange in color, very good in quality, rather small in size, and of considerable importance for human consumption.
- d) The Wada, late in maturity, is widely grown in upland areas on the southern side of the mountain range, which forms a backbone with many mountain branches and transverses the country from east to west. The ear with many kernel-rows was the thickest in diameter, but relatively short in length, presenting an ovate or oblong shape. Grains were medium in size, not so superior in quality, but heavy in yield. It is very important as a main crop and is used as human or cattle food in such areas.
- e) The Gojô contains only one race named "Gojo-Kei", and is distributed over upland areas in lower altitudes at the western part of the mountain range. This race was characterized by being the largest in ear-size as well as in kernel-or row-number, the latest in maturity, and by having some peculiarities of grain which was pale in color, coarser in texture, rather inferior in quality and heaviest in yield. It is an important crop grown for the same use as the previous type.
- f) The Abeto is similar to the Gojô type in its characteristics, use and distribution. But it differs from the latter in smaller size of ear, mediate maturity, good quality and lower yeild of grain, and in the somewhat higher altitudes of its growing areas.
- g) Two types, Sengoku and Hiyoshi, have a distribution area in the highlands of the western mountainous part. Both had rather small conical ears with large orange seeds. They were medium in maturity and

quality, and also rather light in grain yield. From their characteristics, they may be considered as an intermediate form between a large eared late type such as Gojô or Wada and an early highland type, Kuma, Okawa or Irareko. In the western coastal hilly area, they are an important catch-crop; for the most part they are eaten when soft before the rice or sweet potato harvest, and a proportion is stored for the winter-cake. But in the western highland area in the mountain range, they are usually used to some extent as human food and a little is fed to cattle and poultry.

- h) Two types, Okawa and Kuma, both comprising many leading mountain races, are suitable for highland cultivation in the mountain areas. The distribution range is the widest of all types; the Okawa type is widely spread over highland areas in the eastern and southern parts of the mountain range, and the other, Kuma, occurs in the northern part of the same range. Most of the races are good not only in grain yield and quality but also for human consumption as a staple food; they represent a main crop in the higher altitudes. The maturity was mediate.
- i) The Irareko, early in maturity, is distributed over ordinary fields in the highest altitudes at the central part of the mountain range. The ear was slender and cylindrical, having the fewest number of kernelrows, about 8 or 10. Grains were brilliantly orange in color, compact in texture, and good in quality and yield. Most of the races are planted as a staple crop for the mountain inhabitants.
- (3) The results obtained from the pachytene analysis of chromosomes are shown in Table 1. They indicate that there is some cytological evidence to support the preceding classification into 12 types based on a genocological viewpoint.
- a) Contrary to the data on the races native to the foot of Mt. Fuji (MGCNL, 32: 106-108), no B-chromosomes were observed in any of the samples examined.
- b) There was a striking difference regarding the knob number, according to which the types could be arranged as follows: "Okuuchi=Kowase=Wada=Gojô=Abeto>Sengoku>Hiyoshi>Okawa=Kuma>Irareko>Yamakibi".
- c) A knob constantly appeared at the 8 knob loci of the following chromosome arms 3L, 5L, 6L, 7L and 8L, its frequency being 90 or more percent for each locus in a total average. The knob occurrence at these loci may therefore be considered as a basic characteristic of the Caribbean flint, because such a high frequency for these loci agrees with that found in the races native to the foot of Mt. Fuji (see Table 1 in MGCNL 32: 107).
- d) Another peculiarity of the knob occurrence was the presence of differences in the knob frequency among the types, appearing on arms other than the 5 arms above mentioned. An examination of this point

Table 1. Number and Position of the Chromosome Knobs in 72 Races Native to the Island Shikoku.

						Race					44 1	
Chromo	somes	Okuuchi	Kowase	Wada	Gojô & Abeto	Sengoku	Hiyoshi	Okawa	Kuma	Irareko	Yamakibi	Tota
		1	3	6	9	3	2	8	15	1	4	72
1	$\left[ \begin{smallmatrix} \mathtt{S} \\ \mathtt{L} \end{smallmatrix} \right.$		0.3	0.2	0.2	1.0	0.5	0.3	0.1	-	0.3	0.1+
2	SL	1.0	0.7	0.7	0.7	=		0.3	0.1	-	0.3	0.1-
3	SL	1.0	1.0	0.5	0.3	0.7	1.0	0.9	0.9	1.0	0.8	0.1+
4	S	1.0	0.7	0.5	0.6	1.0	1.0	0.5	0.3	=	0.3	0.0
5	SL	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	0.0
6	SL	1,0	2.0	2.0	2.0	2.0	2.0	1.8	1.8	2.0	1.4	0.0 1.8+
7	$\begin{bmatrix} \mathbf{S} \\ \mathbf{L} \end{bmatrix}$	1.0	1.0	1.0	1.0	0.3 1.0	1.0	0.1	0.1	1.0	0.8	0.1-
8	SL	2.0	1.7	2.0	2.0	2.0	2.0	1.9	1.8	2.0	1,3	0.0
9	SL	1.0	:	0.3	0.1 0.2	0.3	-	0.1	0.2	-	0.3	0.2+
10	(S L		-		1	Ξ	-	-	0.1	-		0.0
M	SL	1 8 9	0.7 9.0 9.7	1.0 8.3 9.3	0.7 8.8 9.5	0.3 9.0 9.3	8.5 8.5	7.5 7.6	7.1 7.6	7.0 7.0	0.3 6.2 6.5	0.5+ 7.8- 8.3

made it possible to present a relationship of the 12 types.

(4) In order to ascertain the agro-climatic response of the given races, measurements of the 29 characters were made on 57 samples grown under different climatic conditions at Iwate in North Japan, Hiratsuka in Middle Japan and Ehime in South Japan. With regard to the average temperature during the growing season, the 3 locations can be arranged in a sequence with approximately equal intervals of temperature difference: Hiratsuka>Ehime>Iwate. Similarly, for the average precipitation they are: Ehime>Hiratsuka>Iwate. Of the 29 characters measured, 26 were affected at the three stations. The other three characters, namely leaf width, no. of kernel-rows and kernel thickness, may be considered stable attributes of the native race with a high heritability under certain environmental conditions.

Thirteen of the 26 characters, namely tasseling time, silking time, stalk diameter, shank diameter, ear length, ear width, cob weight, no. of kernels per row, kernel width, kernel length, kernel size, kernel weight per plant and weight of 100 kernels, all relating to either the organ size or time of maturity, were closely associated with temperature. At the lower temperature, the growing period of the race was longer, probably resulting in the larger organ and heavy yield.

The variability of 6 characters, no. of tillers, plant height, stalk height, leaf length, shank length, ear height and length of tassel-branches bearing axis, all of which are connected with the length of organ, was certainly associated with precipitation. In a given place, a decrease of rain-fall is accompanied by an increase of sunshine. Under such conditions, these organs tend to elongate. But there was no tendency for an increase in the grain yield under the same conditions.

The remaining 5 characters, no. of prop-rooting nodes, no. of leaves, length of tassel and tassel-branches and no. of husks, all connected with the number of nodes, were certainly affected at the 3 stations, but apparently without regard to temperature or precipitation. At present, it cannot be said whether the variability of these characters is due to the joint effect of two factors, temperature and precipitation, or to some other unknown factors.

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Further studies on breakage-fusion-bridge cycles in maize endosperm.

Previously reported investigations (Schwartz and Murray, Cytologia 1957) on anaphase configurations in young endosperm tissue indicated

that (1) single bridges are found in only a small proportion of those cells which form variegation patterns of endosperm markers known to be involved in chromatid B-F-B cycles, and (2) chromosomal B-F-B cycles as recognized by the occurrence of double crossed bridges are found in endosperm material where only the chromatid cycle is expected. The former was explained by the postulate that fusion of sister broken ends is most often weak so that the bridges break at the very early separation of the chromosomes and are thus not found in middle or late anaphase where the chromosomes are well enough separated to be scored. The latter is thought to result from non-disjunction of a chromatid bridge without breakage converting a chromatid cycle into a chromosome cycle. These studies were made with endosperms resulting from pollination with irradiated pollen and in Ac-Ds material.

Recently these experiments were repeated using pollen carrying broken chromosomes resulting from crossing over in a reverse duplication of the short arm of chromosome 9 (McClintock, Genetics 1941). A batch of pollen from a single plant (material kindly supplied by Dr. McClintock), heterozygous for the duplication which carried C and Wx on the duplicated segments and a deficient chromosome 9, was used to pollinate six c wx tester plants. Three ears were allowed to develop to maturity while the other three ears were picked and fixed 7 days after pollination. Gametes carrying the deficient chromosome 9 do not function through the male so that all fertilizations are accomplished by either gametes carrying the entire duplication or a broken chromosome resulting from breakage of the AII dicentric formed from one half the crossovers in the duplicated region. The latter gametes have a competitive advantage in fertilization over those with the large duplication.

From the proportion of variegated kernels on the mature ears it was determined that approximately one half of the endosperms received a broken chromosome 9. Since the same batch of pollen was used in all six crosses, one half of the young fixed endosperms should have had a broken chromosome 9 undergoing the chromatid B-F-B cycle. None of the endosperms should have received a dicentric chromosome. Two hundred endosperms were examined cytologically. None were found with single bridges in all or even as high as 15% of the anaphase configurations. However, occasional clusters of cells with double bridges were observed, confirming the earlier observations.

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1. Further studies on a mutable system involving chromosome 6.

This mutable system was first described in the 1957 Maize News Letter. The pale green character described in the 1957 News Letter has since been found to be allelic to piebald-l  $(\underline{pb_1})$ . In as much as the piebald allele discovered here is mutable it has been designated  $\underline{pb}^m$ .

In crossover tests made thus far, YPb/ympbm x ympbm, no crossing over between ympbm and YPb has been found. Ear sectors of germinal mutations (yellow endosperms) have been found in homozygous ympbm material. Forty one plants grown from such yellow endosperm sectors were all green. This suggests that the mutation of ym and pbm was simultaneous or coincidental. Thus, the above tests indicate that ympbm acts as a unit in inheritance affecting the expression of the plant and endosperm characters involved.

One of the homozygous ympbm plants from the original self (yPb/ympbm) was crossed by an unrelated YPb Su2 stock. Of the resulting yellow endosperms some were yellow with darker yellow spots. A number of kernels whose endosperms were yellow with darker yellow spots were planted. Several of the resulting plants (ympbm/YPb) were crossed by an unrelated white endosperm stock, (yPb), and a few by a yPb sug stock. Approximately 50% of the endosperms were yellow when the endosperm ratios from several plants were averaged. Considering the ympbm/ympbm/yPb endosperms the incidence of white endosperms with yellow mosaic areas (henceforth called yellow-mosaic endosperm) varied from plant to plant. The ratios from some plants were approximately 75% yellow mosaic: 25 white. In other cases the ratio was approximately 50% yellow mosaic: 50% white, and in others the incidence of yellow mosaic endosperms was less or more than 50%. These ratios suggested the possibility that there were two independent dominant controlling elements. However, since the incidence of yellow mosaic endosperms varied quite widely among endosperm progenies from the various plants it was also possible that mutation of the ympbm unit was either autonomous or conditioned by a linked dominant controlling element.

One of the ympbmSu\_/YPb Su\_ plants described earlier (from yellow kernel with darker yellow spots) was crossed by a yPb su\_ plant. (The same yPb su\_ stock is used throughout these experiments). Seventy one percent of the resulting ympbm Su\_/ympbm Su\_/yPb su\_ endosperms were yellow-mosaic and 29% were white. The average number of yellow spots per yellow mosaic endosperm was 18.15. A number of YPb Su\_/yPb su\_ plants (from kernels with yellow endosperms from the above cross) were grown. These plants were crossed by a homozygous ympbmsu\_ stock with a very low mutation rate for the ympbm unit. The second ears were crossed by the yPb su\_ stock. (The low mutation rate ympbmsu\_ stock was obtained from an individual selfed ympbm Su\_/yPb su\_ plant. The resulting ympbm su\_ segregates were selfed or sibbed to obtain the stock). The individual ympbm su\_ plants which were crossed to the above YPb Su\_/yPb su\_ plants were also crossed to plants of the homozygous yPb su\_ stock.

One purpose of this experiment was to see if a dominant independent controlling element (or elements) was segregating. If a dominant independent controlling element or elements were involved

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they should have been heterozygous in the original plant. In the offspring of this plant tested here, if a single dominant controlling element was segregating then approximately half of the plants would carry this controlling element and induce  $y^m p b^m$  to mutate. If two independent controlling elements were segregating then in approximately 75% of the plants the  $y^m p b^m$  unit would be induced to mutate.

Table 1. YPb Su<sub>2</sub>/yPb su<sub>2</sub> x y<sup>m</sup>pb<sup>m</sup> su<sub>2</sub>

	YPb/YPb/		Endosperm Classification ypb/yPb/y <sup>III</sup> pb <sup>III</sup>							
Plant no.	y <sup>m</sup> pb <sup>m</sup> yellow	White	Yellow- mosaic	8	Av. no. yellow spots per mosaic endosperm					
1	296	245	33	11.9	1.6					
2	192	156	46	22.8	2.2					
3	285	237	31	11.6	1.9					
2 3 4 5 6 8	258	264	18	7.8	1.7					
5	226	224	10	4.3	1.1					
6	176	183	5	2.7	1.0					
8	245	232	.3	1.3	1.0					
9	305	295	23	7.2	1.2					
10	219	172	29	14.4	1.2					
17	182	199	36	16.3	1.5					
19	226	237	10	6.8	1.1					
23	203	207	4	1.9	1.5					
25	193	182	18	9.0	1.6					
57	248	217	30	12.2	1.5					
	3254	3050	296	8.8	1.3					
Control	ı									
yPb su.	x individused above	dual y <sup>m</sup> p	b <sup>m</sup> su <sub>2</sub>	3						
11 plar		4277	2	0.05	1.0					

Examination of the data from the fourteen plants presented in Table 1 indicates that mutation in yPb/yPb/ympbm endosperms was induced in all plants. However the frequency of mutation was low and varied from 1.3% to 22.8%. Also the number of yellow mosaic areas per mutant endosperm was also quite low averaging only 1.3 mutant areas per mutant endosperm. However this represents a considerable increase in mutation rate over the control. The control is represented by endosperms from the homozygous yPb su2 x ympbm su2 (pollinator plants used for crosses in Table 1) crosses.

The second ears on seven of the plants presented in Table 1 were crossed by the homozygous yPb su2 stock. The resulting endosperm ratios were 1171 yellow and 1157 white. No mutations occurred.

In the reciprocal cross of the original plant, whose dosage of the ympbm unit in the endosperm compares with the dosage of the ympbm unit in the crosses in Table 1 (yPb/yPb/ympbm) the frequency of mutation (% of mutant yPb/yPb/ympbm endosperms) was 41.1% with an average of 8.0 mutant areas per yellow-mosaic endosperm.

It would appear that the principal cause of mutation in the original plant is controlled by the ympbm unit or some component closely linked to it. Certainly the data in Table 1 do not suggest the segregation of an independent controlling element (or elements) in these plants. However, the increased frequency of mutation of the ympbm unit in the plants (when compared with the control) is not easily explainable. It appears that each plant is capable of increasing the frequency of mutation of the ympbm unit (low mutation rate ympbm unit) when introduced into these endosperms.

Table 2. YPb Su<sub>2</sub>/yPb su<sub>2</sub> x y<sup>m</sup>pb<sup>m</sup> su<sub>2</sub>

	-		Yellow	Classific	over ty	nes	%
Plant no.	Yellow	White sugary-2	mosaic sugary2	Yellow sugary2	White	Yellow mosaic	Crossing
1	217	192	27	79	53	6.	24.1
2	155	126	30	30	37	16	21.1
3	198	179	21	87	58	10	28.0
1 2 3 3* 4 5 5* 6	64	57	- Total   1   1	25	13	232	23.9
4	203	189	6	55	75	12	26.3
5	176	173	- 8	50	51	2	22.6
5*	193	185		49	68		23.6
6	138	153	3	38	30	2	19.2
6* 8	182	169		37	51 53		20.1
8	201	179	2	44	53	1	20.4
9 9*	244	221	16	61	74	7	22.8
9*	92	97		32	74 25 25		23.2
10	163	147	20	56	25	9	21.4
17	136	162	29	46	37	7	21.6
17*	127	129	- 74	39	32	200	21.7
19	172	187	4	54	50	6	23.3
19#	103	123		24	27		18.4
23	169	174	2	34	33	2	16.7
23*	159	153		45	28	CAN TON	19.0
25	152	142	9	41	40	9	22.9
57	188	156	20	60	61	10	26.5
212	3432	3293	197	986	921	99	26.5
Contr	01 2833	2947		1242	1165		29.4
Recip	. 1787	1904 7Pb su <sub>2</sub>		962	902	-	33.6

The same plant crosses which were presented in Table 1 were analyzed for crossover frequency. The crossover data are presented in Table 2.

The yPb su2 stock was crossed to a homozygous YPb Su2 stock and the resulting heterozygotes were backcrossed by the yPb su2 stock. These data, which are used as control data, are presented at the bottom of Table 2.

It would appear that crossing over between Y locus and the su2 locus is reduced in the 14 plants. The cause of the reduced crossover ratios is not clear at this time.

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#### 1. Brachytic line from Bicol White Flint.

A brachytic inbred line was isolated from Bicol White Flint variety after a series of continuous inbreeding. The variety-source is from the white flint germplasm of the Philippine hybrids. The brachytic line is described as follows:

Color of the leafsheaths at the ground level ---- slightly reddish.

Internodes ---- shortened and the node where the ear is attached is enlarged.

Leaves --- It has 13 leaves on the average. The leaves are broad and short.

Silks ---- the color of the silks is salmon yellow.

Inflorescence ---- spreading with many spikelets.

Anthers ---- the color of the anthers is purplish. It sheds pollen profusely.

Plant height ---- the height of the plants from the ground level to the tip of the tassel is 90 centimeters on the average.

Maturity ---- Maturity refers to the number of days from seedling emergence to 50% silking. It matures from 49 to 52 days depending upon the season (wet and dry).

The identification of the brachytic line as to whether it differs from <u>br</u> is underway. If it varies genetically in many respects from the known brachytic line which is located on chromosome 1, locus 92, then probably a permanent designation may be given.

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O. Q. Ballesteros
A. C. de Mesa

### 2. Cytoplasmic male sterile and pollen restorer Philippine inbreds.

The inbred lines involved in the production of white and yellow flint hybrids approved by the Philippine Seed Board for distribution and the source of cytoplasmic male sterility (Fl44) were planted in the 1957-58 dry-season breeding nursery located at the Central Experiment Station, College, Laguna. The male sterile line was planted in rows alternately with the fertile lines. All possible crosses were made. The selected ears from each cross, i.e., disease-free plants and ears with plump kernels, were planted ear-to-row in the 1958 wet-season nursery. The recurrent parental inbreds were also planted. The emerging tassels were carefully examined and classified as follows: completely sterile (all the plants in the row were devoid of shedding pollen), partially sterile (some of the plants in the row or portions of the tassel were shedding pollen) and completely fertile (all the plants in the row were profusely shedding pollen). Microscopic examinations of the anthers were done in the laboratory field to confirm the observation.

The result indicates that one inbred line was highly homozygous for the sterile factor. Three lines were completely, uniformly and abundantly shedding pollen. The result is of paramount significance because it may pave the way to the elimination of detasseling under the tropical growing conditions of the Philippines.

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The completely sterile F<sub>1</sub> plants were backcrossed to their respective recurrent parent while the completely fertile plants were selfed. The backcrosses and selfed ears are presently grown in the 1958-59 dry-season breeding nursery.

D. L. Umali

The second secon

C. C. Jesena

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#### 1. Fertility restorer genetics.

Three progenies from the S<sub>1</sub> of the backcross: WF9(WF9 x KY21) have been obtained which when crossed to the male-sterile line SK2<sup>T</sup> give all fertile progeny but which when crossed to the male-sterile line WF9<sup>T</sup> give all male-sterile progeny. This is a confirmation of the hypothesis (Duvick, in Genetics 41:544-565, 1956) that fertility restoration in T cytoplasm depends upon the simultaneous presence of at least two dominant genes, either of which, if present as a homozygous recessive, can cause sterility. Thus, the genotypes of the various lines involved herein are presumed to be as follows:

SK2 rf1rf1Rf2Rf2

WF9 rf1rf1rf2rf2

KY21 Rf1Rf2Rf2

Selected Sof BC Rf1Rf1rf2rf2

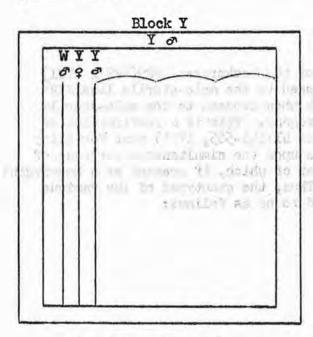
Donald N. Duvick

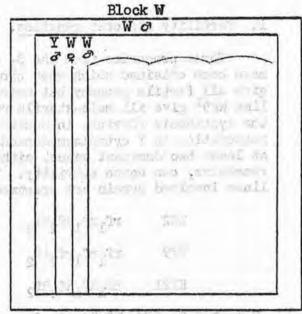
#### 2. Rapid recurrent and reciprocal selection.

By use of genetic markers displaying incomplete dominance of simply inherited kernel characteristics, it should be possible to complete cycles of selection requiring progeny tests in less time than hitherto reported (see Hull, Agron. J. 1945 and Comstock et al., Agron. J. 1949). This modification of conventional recurrent and reciprocal methods is herein outlined and designated as the rapid method.

Three crop generations are ordinarily required per cycle in conventional recurrent or reciprocal selection for yield in corn. These consist of (1) making test crosses, (2) growing test crosses, and (3) intercrossing the parent lines of the best test crosses. The rapid method hinges on the ability to separate outcrossed and intercrossed seed on single ears or groups of half sib ears (half sib ears are from sister plants pollinated by non-sister plants). This separation of seed effectively allows the combining of crop generations (1) and (3) above. The following example utilizes endosperm color as a marker in a reciprocal selection program. The rapid method for recurrent selection would be similar but requiring only one isolation plot.

Year 1. Two isolation plots (herein designated Y and W) will be necessary. Isolation may be spatial or bordered by pollen parent rows.





- Y = detasseled yellow endosperm plants, source of intercross and tester ovules in Y block.
- W ? = detasseled white endosperm plants, source of intercross and tester ovules in W block.
- Y d = normal yellow endosperm plants, source of tester pollen in W block and intercross pollen in Y block.
- W o = normal white endosperm plants, source of tester pollen in Y block and intercross pollen in W block.

The only operation necessary during the pollination season will be the detasseling of female rows. Hand pollinations will not be necessary.

Only ears from female rows will be harvested. The seed will be divided as follows:

It is necessary to label seed from each ear or from groups of half-sib ears in order to later retrieve selected remnant intercrossed seed.

Year 2. Two field trial comparisons of the progeny resulting from Year 1 will be conducted; i.e. one trial for testcross progeny (pale seed) from Block Y and a trial for test cross progeny (pale seed) from Block W. As previously indicated individual entries may consist of either seed from individual ears or bulked seed from groups of half sib ears. After scoring the test crosses for yield or other agronomic characteristics one cycle has been completed.

Year 3, 4, 5. Another cycle may be initiated by utilizing superior (on the basis of Year 2 field trials) remnant intercrossed seed from Year 1 (yellow endosperm from Block Y and white endosperm from Block W). Additional cycles would be warranted as long as sufficient variability remains in the two populations.

Selection pressure per cycle is reduced in this rapid method as compared to the conventional reciprocal method due to selection only upon the seed parents in a given cycle. Essentially the selection of the pollen parent lags one cycle behind that of the seed parent. With random pollination, the genotypes of each cycle will average midway between the mean of its preceding cycle and the mean of the selected female parents.

Ultimate gain may be favored by the rapid method due to delayed selection on the pollen parent. This delay allows a more thorough "mixing" of germ plasm and therefore more recombinations and slower fixation than the conventional recurrent and reciprocal method involving selfing at the time of outcrossing. Furthermore, the absence of selfing would allow a prescreening of maternal plants on the basis of their individual or half sib group performance in the isolation blocks. This selection would be primarily for favorable dominant and additive effects.

Pollination expenses would be reduced with the rapid method as would gross mechanical errors, due to the elimination of hand-bagged pollinations. Efficient breeding nurseries are predesigned to make the most pollinations in the least amount of time. Time is more critical during the pollinating season than any other comparable period throughout the year. In addition, the chance for gross mechanical errors (misguided pollinations) is materially reduced. The conventional reciprocal method offers appreciable chance for mechanical error.

Winter programs are in use by most organizations having programs involving reciprocal and recurrent selection. By growing a winter crop the three year program per cycle required by the conventional methods may be reduced to two years which is the minimum since the progeny test must be grown in the area of expected use of the developed hybrid. By the rapid method a cycle may be completed in one year by growing the isolation blocks for test and intercrossing during the winter.

If the breeder chooses to select on the basis of female plant or plants performance in the inter- and testerossing blocks a maximum of only two years per cycle is involved.

Preliminary to the initiation of a program utilizing the rapid method, the following projects are planned:

- 1. To observe and test reciprocal crosses of white inbreds of different origin with yellow inbreds of different origin to examine the complexities of separating intercrossed and testcrossed seed.
- To measure the combining ability of crosses within and among yellow and white lines to determine the relative merits of these sources of germ plasm.

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#### 1. Cytoplasmic factors and pollen tube growth of Zea mays, L.\*

Competition of pollen tube growth is provided by making use of pollen mixtures from yellow and white sources (M. N. L. 1958). In this investigation the reaction of cytoplasm of the well known American Inbred 33-16 was subjected to further tests. Evidence obtained from the testing of pollen originating from single crosses where 33-16 functioned respectively as maternal and pollen parent, support the assumption previously made (M. N. L. 1958), that the deficiency in the performance of 33-16 pollen tubes in pollen mixtures is due to cytoplasmic factors. A significant heterogeneity in the ratios realized was observed when different maternal parents were used suggesting a strong maternal effect. This was also apparent, but to a lesser extent within inbreds of long standing which could be regarded as having attained a high degree of homozygosity. This study has revealed that pollen tubes are very sensitive to conditions in maternal tissue and thus may be a means to study the relative homogeneity of inbreds.

Similar results were obtained in the study of the inbred Mexico 155 and a local inbred C56.

\* (In press, Proceedings of the First South African Genetic Congress, University of Pretoria, 1958).

#### 2. Competitive pollen tube growth in Zea mays, L.\*

In the previous report (M. N. L. 1958) where pollen mixtures from yellow and white sources were tested the results suggested a relation between combining ability and pollen tube growth in those cases where cytoplasmic factors apparently were not involved. The seed resulting from these pollinations were grown the following season in paired rows and the yields determined. A highly significant positive correlation was found between superior yield and the superior color class. However, in cases where the maternal parent was genetically identical to the white component of the pollen mixture used there seemed to be a preference for self pollen, in most of the cases studied, so that a negative correlation was realized. Hence, at this stage these results must be interpreted with reserve until more information becomes available with respect to the different factors which may affect pollen tube growth. It is expected that considerable light will be thrown on this aspect when the results of the present season will become available.

Additional data have supported the previous observation (M. N. L. 1958), that, with rare exceptions, varietal pollen is superior to pollen from inbreds in competition.

\* (In press, Proceedings of the First South African Congress, University of Pretoria, 1958).

J. D. J. Hofmeyr J. M. P. Geerthsen

#### 3. Quantitative genetic studies.

Maize breeding in South Africa is still in the early stage characterized by wide scale sampling of germ plasm from local open pollinated varieties as contrasted to the improvement of existing inbreds. Need for knowledge of the genetic composition of these varieties is keenly felt, therefore. Five varieties were chosen accordingly and the following investigations were carried out with them.

#### (a) Estimation of additive and dominant components of yield variance.

Non-selected full sib (biparental) and half sib (maternal) progenies were grown in two replications of plots each containing about 40 plants. Yield was expressed in lbs. of ears per plot. Additive (G) and dominant (D) components of genetic variance were determined by the intra class correlation method (first method) assuming that covariance of full sibs = 1/2G + 1/4D and covariance of half sibs = 1/4G.

For purposes of comparison a series of biparental progenies using one pollen parent on three or four ear parents was grown in two replications for three of the five varieties. (The other two are being grown

in the current season.) These were then analyzed according to the method developed by Comstock and Robinson (Biom. 4: 254). The results of both the first and this second method are given in table 1.

Table 1. Estimated additive (G) and dominant (D) components of genetic variance for yield in five South African maize varieties.

THE PERSON NAMED IN	First	Method		Second	Second Method				
Variety	No. of Progenies	G	D	No. of Progenies	G	D	Mean of All Progenies		
Robyn	48	8.20	0.12	Arry and Hi	1		8.3		
Anveld	48	3.60	12,00	60	6.84	-2.48	7.1		
Teko	52	7.72	-0-30	116	4.96	-0-11	11.1		
American white flint	58	4.16	-2.92	The state of the s			9.0		
Sahara	48	7.92	-5.64	72	4.20	0.20	8.3		
Total	254	10.60	0.48		80	-0.75	8,6		

Results differ considerably among varieties and between methods and are probably subject to a large amount of random error and some bias. The half sib progenies were expected to contain some full sibs although care was taken to pollinate plants with a mixture of a large number (ca. 30) of other plants' pollen. This should cause an underestimation of D and may partly be responsible for the negative values obtained. In general however, the results from the two methods are in fair agreement with each other and with those obtained by Robinson et.al. (Genetics 40: 45) for American varieties, giving a large amount of additive and relatively little dominant variance.

The full sibs gave an over all 12% higher yield than the half sibs. Every individual variety showed this tendency. The open pollinated varieties gave intermediate yields.

#### (b) Frequency of recessive mutants.

The number of distinct recessive seedling characters (albino, zebra, glossy, virescent, etc.) segregating in 100 S1's of each of the five varieties was as follows:

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and the second second

Robyn	21		THE THAT IS	Hell hely
Anveld	44			17
Teko	28	100		All the West
A. W. Flint	42			
Sahara	30			

#### (c) Ability to produce heterosis.

Extensive variety cross trials were carried out in South Africa in 1957. All the possible crosses among 15 varieties were tested for yielding ability in five localities. The results are in press (South African J. Agric. Sci. 1958). Crosses between the five varieties of present concern are now being tested for the fourth season in Pretoria. The amount of heterosis, expressed as yield of hybrid in percentage of parental mean, varied greatly with seasons and localities indicating marked effect of genotype-environment interaction on heterosis. The following were the mean yields of varieties, of variety crosses, and the heterosis values for each variety, over three seasons:

	Variety	All Crosses	Het. 9
Robyn	11.9	10.9	97
Anveld	9.9	11.2	109
Teko	12.2	11.8	100
A.W. Flint	10.6	11.6	105
Sahara	9.1	10.3	104

#### (d) Relation between yield variance and inbreeding coefficient.

Fairly extensive trials are being conducted in the present season to determine the effect of inbreeding (F = .125 to F = .75) on variability within and between nonselected lines obtained from these varieties. Lines from some varieties (e.g. Sahara) are commonly known to maintain variability for more generations of inbreeding than others.

Preliminary results on yield obtained from 30 S<sub>1</sub>'s and their S<sub>2</sub>'s (20 plant plots, two replications) of the variety Gobi are as follows:

	Obtained	Expected
Environmental variance	259	
Genetic variance of o. p. variety (So)	326	326
Genetic variance within lines (S1)	568	163
Genetic variance within lines (S2)	406	82
Genetic variance between lines (S1)	464	362
Genetic variance between lines (S2)	1577	489

The environmental component was obtained from a large number of nonsegregating progenies (not related to Gobi, but with comparable average yield) in an adjacent field. The So estimate was obtained from a comparison of about 150 individual noninbred plants of the variety Gobi. The expected values are based on the assumption of additive gene action for yield (see Wright, Genetics 37: 312). Deviations from the expected values could be ascribed to nonadditive gene action. The limited numbers in this preliminary experiment must be borne in mind. It is felt that genotype-environment interaction is probably a major factor in causing these discrepancies, especially since the trial included plants varying greatly in yield. As soon as more information is available on the elimination of interaction by scaling, this type of experiment should definitely be subjected to appropriate scaling.

#### (e) Genotype-environment interaction.

This phenomenon is being studied with inbred and single cross material. Statistically significant differences in variability were found between different genetically nearhomogeneous progenies grown in the same field. A partititioning of variance into environmental, genetic, and interaction components was made, giving the estimates 259, 1120 and 312 respectively when differences between hybrids and inbreds were not taken into account. A correlation coefficient of -.69 was found between mean ear weight and coefficient of variability. The mean C. V. of the inbreds was 57% as compared to 22% for the single crosses. When transformed into an antilog scale, differences in variability between progenies lacked significance and the mean C. V. of inbreds was 8% compared to 10% for the hybrids. Scaling, therefore, successfully reduced genotype-environment interaction or apparent "genetic homeostasis". More detailed results appear in "Proceedings of the First South African Genetic Congress, 1958".

In view of the extreme importance of interaction in interpreting experiments in quantitative genetics, more data is being collected at present and a greater variety of scales being tested.

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#### 1. Gene order of y, ms-si, and rg on chromosome 6.

Data from the cross y si x rg Y Si/Rg y si are 907 YSi:23 Ysi:27 ySi:102: y si which gives 2.5% recombination for the y-si region. Data from selfed ears of the triply heterozygous genotype give 2231 Y Rg:983 Y rg:1107 y Rg:8 y rg indicating 9.0 + 1.5% recombination for the y-rg region. Recovery of one rg Y Si/Rg Y Si genotype, one Rg y si/rg y si

genotype and two Rg y si/Rg y Si genotypes clearly establish that y is between rg and si. Experiments with po and Pl are in progress to determine orientation.

Herbert H. Kramer

#### 2. The location of y on chromosome 6.

Linkage tests of y-su/Y-Su, in homozygous translocation T6-10b (6L.17,10L.14) showed 65 Y Suz:110 Y suz:118 y Suz:59 y suz which gives 35.2% recombination and indicates the translocation point to be to the left of y with y on the long arm. Data presented by Patterson (1958 Newsletter p. 64) showed recombination between y and R to be 18.8% in the homozygous translocation placing the break on 6 to the right of y. These two sets of data are compatible only if the break on 6 is in the short arm as Burnham (Genetics, 1950) indicated. If the break on 6 is in fact in the short arm, the possibility of y also being on the short arm is not ruled out.

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#### 1. Intracistron recombination at the Wx/wx locus.

The dependence of the type of starch (amylopectin vs. amylopectin + amylose) produced in a microspore on the genotype at the Wx/wx locus of the microspore itself and not the parental plant allows a test in maize for the occurrence of intracistron recombination. The barrier to the investigation of such a phenomenon in higher organisms is our inability to handle populations of sufficient size to detect the infrequent recombinants if such exist. In this system, however, the requisite numbers are easily available since a maize plant produces millions of microspores and since slides containing 50,000 or more microspores can be prepared and scored in twenty to twenty-five minutes.

If two independently occurring waxy mutants at the Wx/wx locus represent changes at different mutational sites within the cistron and if recombination between such sites is a reality, it should be detectable in preparations from the pollen produced by the F<sub>1</sub> between the mutant stocks. One of the products of recombination would be a reconstituted functional locus; in this case some amylose would be formed, and a microspore carrying such a locus would stain black with a KI, I<sub>2</sub> stain in contrast to the brownish color typical of waxy microspores. Where in a cross between 2 waxy mutants the frequency of such black (normal)

microspores exceeds the mean of the frequencies of black microspores in the pollen produced by the parents, it could be an indication of such recombination. Further, the frequencies of normal microspores in the various all-combination crosses between a series of waxy mutants should be a function of the distances between the sites concerned and allow a preliminary mapping of the locus.

With such a project in mind, a number of independently occurring waxy mutants were collected. These are listed in Table 1. All possible crosses were made between the different mutants. Tassel sections were collected in 70% alcohol just prior to pollen shedding from a number of the F<sub>1</sub> plants of each cross. Subsequently slides were prepared according to a standard technique to be described later. The total population of microspores for each slide was estimated by the sum of six grid counts over the surface of the slide x a constant. Normal microspores were located and marked by a drop of Kodak Opaque.

### Table 1

Mutant	Source
0	Maize Coop; Chr. 9 tester, Rec'd. 1951.
90	Brunson; Mutation in Inbred 90.
H21	Brunson; Mutation in Inbred H21.
В	Bear; Mutation in Breeding Material.
a	Kramer; The wx isolated in Argentina.

Table 2 gives the results for the parental stocks and the crosses both for plants grown in the greenhouse in the winter of 1957-1958 and for plants grown in the field in the summer of 1958. The results are not strictly comparable since the greenhouse data consist of the results of repeated analyses of not more than 8 plants from the same cross between two mutants. The field data include a greater but variable number of plants from all crosses between two mutant stocks. Reference to Table 2 shows that for the parental stocks there are low but measurable frequencies of normal microspores. The figure for any one mutant stock presumably includes the products of back-mutation at the waxy locus, suppressor mutation, and contamination of tassel samples by the lodging of wind-blown normal pollen. Crosses between different mutant stocks may give frequencies ranging from those no higher than the parental stocks to those which are many-fold higher.

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Table 2. Estimates of Incidences of Normal Microspores

	157-158 Gre	157-158 Greenhouse		1958 Field		
Stock	Est.No.Microspores x 10	x No.Normal x 10 5 ± sx	Est.No.Microspores	x No.Normal		
a B	61	0		~		
В	503	1.6 + 0.6	472	3.8 + 1.2		
C	554	1.1 + 0.4 2.0 + 0.4	11114 559	0.7 + 0.4		
H21	509	2.0 + 0.4	559	2.7 + 0.8		
90	615	1.6 + 0.5	353	0.3 + 0.4		
H21 x B/	576	35.6 + 4.2	987	28.1 + 2.2		
H21 x 90	522	33.1 + 3.7	1168	31.8 + 2.7		
C x H21/	1174	54.3 + 3.9	1218	46.0 + 2.7		
90 x B	537	1.9 + 1.2	717	1.4 + 0.6		
90 x C	1165	75.3 + 4.0	596	88.0 + 5.7		
C x B	550	23.6 7 2.0	1077	29.5 + 2.9		
Rec. C x C*			252	29.5 ± 2.9 1.3 ± 0.9		
Cxa	289	4.5				
a x H21	385	29.4				
a x 90	287	2.4		-		
a x B	354	0.3				

<sup>\*</sup> Recovered C = {[(C x Inbred Tr ) 5 x Tr] 2 x Tr} 2 with sh wx segregates being selected after each selfing.

1958 field data includes also the reciprocal cross.

Where a number of crosses between two mutant stocks were made, the progeny from each cross was sampled. In all cases there was good agreement between the different progenies within a cross. In some cases, reciprocal crosses were available. Data again showed good agreement. The results for two sets of progenies are reported in Table 3.

Table 3. Normal Microspores in Individual Progenies

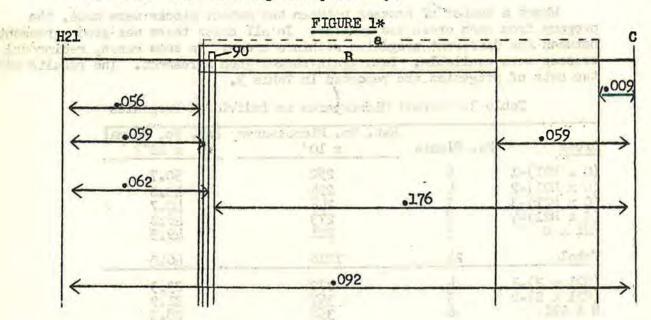
Cross	No. Plants	Est. No. Microspores x 10	Est. No. Normal x 10-5
(C x H21)-1 (C x H21)-2 (C x H21)-3 (C x H21)-4 H21 x C	6 4353	252 224 248 273 221	50.7 40.0 40.7 46.2 49.3
Total	21	1216	46.0
(H21 x B)-1 (H21 x B)-2 B x H21	456	272 357 358	23.3 31.9 28.3
Total	15	987	28.1

For most crosses which had a high incidence of normal microspores, a number of individual anther preparations were scored. In all cases the distribution of numbers of normal microspores per anther showed good agreement with a Poisson distribution. Thus the bulk preparations for any cross give a good estimate of the probability that any microspore will be normal in phenotype.

Results from the 1958-59 greenhouse planting of the BC progeny (90 x C)x C show 5 plants with no normal microspores and 4 plants with a mean of 63 x 10-5 normal microspores per plant.

Considering the above data, it seems justifiable to conclude; (1) that each of the alleles is different from any other allele, (2) that there is a characteristic frequency of normal microspores for each cross between two mutants, and this frequency is obtained every time the cross is made, (3) heterosis, per se, cannot account for the normal microspores as witness the frequencies in 90 x B and Rec. C x C, and (4) the normal microspores are the result of recombination within the locus.

If this last hypothesis is correct, it should be possible to use the data from the crosses to establish a linear order for the mutants within the locus. This can be done since only one arrangement appears to satisfy the data. This arrangement is shown in Figure 1. Note, however, that the criterion of additivity is not satisfied. This may be a consequence of the heterogeneous backgrounds in which the mutations occurred. Unquestionably, for a study of genetic fine structure, it would be desirable to induce a series of mutants in the same stock where one could be sure that the critical locus prior to mutation was the same in every case. This is being done presently.



<sup>\*</sup> The frequencies of normal microspores observed have been doubled for the purpose of map construction. The figures given are percentages.

The fact that several mutants (B and a) appear to have some size may also be due to the heterogeneous backgrounds of the mutants. One cannot discount, however, the possibility that it is actual. If the genesis of a mutant were functional locus + gene controlling element within the locus, then the mutant would appear as a block in studies of this type. Several waxy mutants which are known to have had such an origin have been included in our crosses, and data should soon be available on this point.

Functional complementation if it occurs should be revealed in the endosperm of the seed resulting from the cross between two mutants. Analyses are not complete as yet, but interactions of a magnitude which would suggest that the two mutants crossed are located in different cistrons have not been observed. Still measurable interactions are present. The percentage of amylose in all crosses involving wx, for example, is substantially greater (100%) than the percentage of amylose in either parent. More data on amylose percentage in various crosses are being obtained.

The details of the technique used may be pertinent. The tassel samples are collected as mentioned in 70% alcohol. A "curing" period of several weeks is desirable since newly collected microspores do not stain as readily with a standard strength stain as do those which have been collected longer. The standard stain formulation is 25 ml. of water, 250 mg. of potassium iodide, and 45 mg. of iodine. The stain is mixed approximately 20 hours before use and placed on a shaker over night. One hour before use, 1 drop of "Tween 80" is added and then 0.5 grams of Baker's gelatin. The mixture is heated for 5 minutes on a warm hot plate.

In preparing the slides, 24 anthers are selected—the 3 anthers from the more mature floret of 8 glumes which are just beginning to open. These are placed in the small stainless steel cup of a Virtis Microhomogenizer together with 0.8 ml. of the stain. The mixture is homogenized for 2 minutes after which it is strained through cheesecloth onto the surface of a lantern slide. The microspores are dispersed as evenly as possible and covered with a 50 x 75 mm cover slip. After the mixture has set, the edges of the cover slip are coated with colorless nail polish. Such preparations will keep for several days without desiccation and can be scored at any time in that period.

O. E. Nelson, Jr.

### 2. Gene controlling elements of the an pm system.

Notes dealing with a mutable alp allele have appeared in the News-Letter for the past several years. By and large they have been concerned with the analysis of Al locus components through the use of patterns of mutation presented by this mutable locus. This letter, on the other hand, will deal with the gene controlling elements involved.

### Variegated Phenotypes

Producing basically pale aleurone color, this unstable allele is capable of mutating to a higher level resulting in the production of deep aleurone tissue, or to a lower level giving colorless tissue. These mutations may occur at any time during the development of the tissue, hence, the deep sectors may be large if they occur early, small if they occur late, or they may be non-existent. Similarly the colorless areas may be small, large, or may, in fact, render the majority of the aleurone tissue colorless. Different combinations of direction of mutation and time of mutation result in a number of different variegated patterns.

One of the most common mutable types in this material is called "coarse pale mosaic". In this form colorless mutations occur fairly early giving moderately large colorless interruptions in the pale background. A tendency for few and late deep mutations results in infrequent deep dotting. In a second common mosaic type, aleurone tissue which is mostly colorless results from very early mutations to the null level. Changes to the deep level are more common here, so that the resulting pattern has been called "white plus dots". A third form which resembles stable pale alleles frequently appears. This state, called "apparent pale self" is considered to be a mutable type because its progeny mutate at high rates to other mosaic forms instead of breeding true for the uniformly pigmented condition. In a fourth state, designated "light pale plus dots", rare mutations to the colorless level result in an almost uniform pale background. In addition, a large number of deep dots arise from frequent late changes to the higher level. A number of other mosaic forms arise, but these will not be considered here.

### A Closely Linked Controlling Element

Several cases of variegated phenotypic expressions in maize have been attributed to gene controlling elements. Since mosaicism in the present case may occur in the plant tissue and extend into the sporogenous tissue, it can be shown that this mosaicism results from mutation at the Allocus. In crosses variegation segregates with the pale allele with which it was introduced, but if this variegation is separated from all, the frequency of the event is quite low. Since the mosaic effect comes about by mutation at all, and since the effect has been shown to be linked closely with all, it seems logical to conclude that this unstable allele, like others described earlier, results from a gene controlling element present at all and acting upon it.

The effect of this mutable locus on crossing-over in the A-Sh<sub>2</sub> region has been studied (M. N. L., 1956). Stocks carrying the mutability factor show recombination rates which are significantly different from the control rates. Both increases and decreases in the rate of recombination within the A-Sh<sub>2</sub> region were brought about by the influence of the mutability factor. In addition, in stocks carrying the gene controlling element high rates of somatic losses of the linked Sh<sub>2</sub> gene were observed (M. N. L. 1956). That a<sub>1</sub> pm can influence the rate of crossing-over in an adjacent

region as well as the somatic loss of a linked gene, two characteristics which have been shown to hold for other gene controlling elements, supports the idea that a typical mutability factor is present at the ap locus.

### Other Controlling Elements

Changes from one mutable form to another occur both at "low" rates typical of mutation and at high rates which could be explained only by segregation of an independent but influential factor. Table 1 illustrates the types of progeny the various mosaic types produce. It is apparent from the first group of four ears that the coarse pale mosaic form often mutates to other mutable types. There is, with a few exceptions, in each of the mutable forms the capability of mutating to the other states. Superimposed upon these mutational events, are changes of a much higher frequency. Ears of this type are illustrated in the second group under each of the kernel classes. Ears which show the 1 to 1 ratio between two mutant forms probably result from the segregation of a controlling element, the presence of which is necessary for the expression of one form, while the other form appears only in its absence. If this is the case, then controlling elements responsible for the following changes in form must exist.

Coarse pale mosaic --- Apparent pale self
White plus dots --- Light pale plus dots
Coarse pale mosaic --- Light pale plus dots
Apparent pale self ---- Light pale plus dots

Ears which illustrate 3 to 1 ratios could result from either the segregation of two independent but similar controlling elements, or from linkage of the controller to a pm. The 1 to 3 ratios can also be explained by linkage, if the controlling element in these cases is located on the homologous chromosome. Under the linkage explanation ratios which vary significantly from 3:1 and 1:3 can be explained by different degrees of linkage.

On the other hand, a linkage hypothesis is not the only possible explanation for the 1:3 ratios seen in Table 1, since they could also arise from the segregation of an element which suppresses the gene controller. In a pm a consistent but low proportion of the apparent pale self types breeds true. More commonly, however, the apparent pale self form gives rise to other mutable forms by mutation and segregation. If the true breeding self types result from loss of the gene controller from the genome, the mutating and segregating apparent pale selfs might arise from the presence of a gene controller suppressor. A unit of this type would allow the apm locus to be unaffected by the gene controlling element so that the kernel would appear self colored, and yet retain the potentialities to produce other mutable forms. Postulating different numbers of gene controlling elements and gene controller suppressors, one can explain an array of different ratios.

Similar interpretations of shifts between mutable forms could be postulated for the other cases presented in Table 1. Differences in the

coarse pale mosaic - apparent pale self and the white plus dots - light pale plus dot changes could be due merely to different states of the gene controllers, while the rest of the mechanism is essentially the same.

It is apparent that this mutable system is similar but not identical to the and main system investigated by McClintock. A brief comparison of the two systems is presented below.

- 1. Originated from a allele.
- 2. Self-colored pale kernels arise: 2. Self-colored kernels arise when a few of these are stable, but most of them subsequently give rise to mutable forms.
- 3. Segregation of the gene controlling element may result in two variegated classes or in one self-colored and one variegated class. (Pale self-colorless plus dots segregations are rare if they ever occur.)
  - 4. The segregating classes form poor 1:1, 3:1, and 1:3 ratios which may vary from 1:6 to 5:1.
  - 5. Ears sectored for coarse pale 5. mosaic and white plus dot phenotypes are common.
  - 6. Two ears produced on the same plant may differ in the ratios of their segregating classes, due to gain or loss of a gene controlling element.
  - 7. Germinal mutations give rise to uniformly pigmented alleles some of which may be very stable, and others which mutate at rather high

- l. Originated from A allele.
  - Spm is lost from genome and are stable as long as Spm is absent.
  - 3. Segregation of Spm results in production of a self-colored and a variegated kernel class. (Pale self-colorless with dots segregation is common.)
  - The segregating classes form very good 1:1 or 3:1 ratios depending upon whether one or two Spm units is involved.
  - Ear sectors of self colored areas (loss of Spm) on a variegated ear occur.
    - Two ears produced on the same plant may differ in their Spm constitution, hence differ in the ratios of their segregating classes.
  - Germinal mutations occur in the presence of Spm and result in stable alleles.

Table 1. Ear types produced by various mutable states

Cross: apm Sh2 a sh2	x a sh <sub>2</sub> a sh <sub>2</sub>		ly Sh <sub>2</sub> Ternel		counted
Type of Ear	Frequency	Pale mosaic	Pale self	Wh.+	Lt.pale + dots
	Plante	d Pale Mosaid	1		
Most kernels of	57% of 35 ears		-		
the parental	classified	252 163	2	3	Ö O
type		305	18	3 2	2
		388	0	2	23
Many of the	43% of the 35	:1 .88	102	0	4
kernels of the	ears classi-	TIT	94	0	17
parental type	fied 3	:1 146 77	71 29	3	6
		1.0	125	ő	o
	1	13 66	151	ı	8
	Plant	ed Pale Self			
Most kernels of	53% of the 28	0	177	0	0
the parental	ears classified	. 0	122	0	0
type		4	200	0	1
		1	1/15	0	14_
Many of the	47% of the 28	:1 87	99	0	7
kernels of the parental type	ears classi-	113	103 57	0	4
paremar type	3 Tred	:1 109	22	o	ĭ
		-2 34	122	0	0
	-	33	206	0	1
	Planted	White + Dots			
Most kernels of	12% of the 34	5	4	146	8
the parental	ears classified		ग्रां	192	7
type		0	6	188	26
Many of the	62% of the 34	1:1 0	5	1.30	174
kernels of the	ears classi-	U	0	129	142
parental type	fied	3:1 0	3	127	44 58
	7	1:3 0	9	45	183
		0	2	111	186
		0	46	31	142
20020 30 3000		0	56	27	138
Few kernels of	26% of the 34	131	3	3	73
the parental type	ears classified	116 159	20	3 2 6	108
		79	28	ĭ	2

1	1		Kernel '	Type Wh.+	Lt.pale
Type of Ear	Frequency	Pale mosaic		dots	+ dots
1051 -	Planted 1	Light Pale +	Dots	,	10.70 ==
Most of the kernels of the parental type	2% of 54 ears classified	107 ) = (102)	0	5/1	169
Many of the kernels of the parental type	74% of 54 ears classified	1:1 0 3:1 0 1:3 0 1:3 0 163 174 21 64	28 0 1 4 0 2 13 0 81 95	102 168 172 183 58 85 8 1	144 132 45 62 200 225 49 62 60 94
Few kernels of the parental type	23% of 54 ears classified	212 53 46	26 181 96	0 0	0 11 30
F 111	0.00				

D. L. Richardson

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MEXICAN AGRICULTURAL PROGRAM
Mexico City, Mexico

### 1. Studies among races of corn in Mexico.

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In 1958 a reserach project was initiated having as its goal a more intensive study of crosses among the twenty-five well defined Mexican races of corn. As seed stocks for this project it was decided that it would be better to go back to the original area of collection for each race rather than attempting to use seed stocks available in the corn bank. This decision was reached because: 1) The original collections might have been changed by mixture or selection since some of them had been increased by hand pollinations in small plots as many as five different times; and 2) The actual cost of obtaining the seventy-five pound seed stock for each race would be much less by re-collection than by hand-pollinated increases. The collections which served as basis for the original race definitions were collected from 10 to 15 years ago. Even so, it was not difficult to find corns representative of each of the

twenty-five races by looking among the smaller farms in less progressive areas. The races of corn now being grown have apparently changed relatively little except in those areas in which the National Corn Commission has established corn seed production (hybrids and/or varieties). With the rapidly expanding net-work of good roads, the growth of the production program of the National Corn Commission, the growth of the Extensive Service plus the growing interest on the part of farmers themselves, a change in the corn race distribution will probably take place at a greatly accelerated pace. This change is already evident in areas where crop improvement programs have been initiated.

R. D. Osler E. C. Johnson

### 2. Resistance to ear and tassel smut in Mexico.

Data from 1958 corn plantings in the Bajio region of west central Mexico show distinct differences of reaction among several hybrids and varieties of corn to the ear and tassel smut incited by the organism Sphacelotheca reiliana (Kuhn) Clinton.

Plantings of 12 varieties were made at 4 different planting dates. Highly significant differences were found among varieties and among dates of planting in reaction to the fungus. The most susceptible hybrids were those that included lines introduced from tropical corns. Of plantings made March 15, March 31, April 15, and May 1, the highest percentages of infection were obtained in the May 1 planting.

Commercial corn plantings in the area ranged from no infection to individual fields with 40% or more of the plants infected. Literature reports of the disease indicate it to be of minor importance, but experience in Mexico suggests the desirability of incorporating genetic resistance to the disease in corns for the Bajio region of the country.

E. C. Johnson R. D. Osler

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### 1. Relation of root rot and root type on yield and maturity of maize.

Inbred lines derived from two ears of Fulton's yellow dent have been shown to differ in the expression of root rot. The evaluation of the importance of root rot and other morphological characteristics was estimated from a series of multiple measurements involving the roots of corn

plants dug in August and September. These measurements include scores for root type, root abundance, apparent disease resistance, brace roots and fine roots. Correlations between these measurements and yield and moisture at harvest, taken on top-cross progenies of these inbreds in 1957 are presented in Table 1.

Table 1. Correlation coefficients between various root and yield measurements obtained from three-way hybrids involving inbred lines and two single cross testers grown in 1957.

, N	Туре	Abundance	Disease	Fine Roots	Brace Roots
Yield	+•403	+•300	+.232	+.356	+.282
Moisture Per Cent	+.433	+•304	+•313	+.418	+.344
Fine Roots Significant	+.656	+.776	+.708	1,000	+.268

The corn roots were dug with a mechanical digger which enabled the removal of a definite portion of the root system free of soil. The roots harvested by this procedure made it possible to carefully remove for observation and scoring the main and fine branches of the secondary root system. The 1957 results suggest "fine roots" as one of the most useful morphological characters in selecting for root rot resistance in corn.

Diallel crosses between the four most resistant and the four most susceptible lines developed in this work have now been made, along with outcrosses to an early and a late tester. This material, when grown in 1959 and 1960, should furnish information on the heritability of root rot resistance.

The 1957 data show that top cross progeny root rot scores have little correlation to similar scores taken on inbreds. The possibility of heterosis or overdominance effects is indicated.

C. M. Nagel

D. B. Shank

V. A. Dirks

D. E. Kratochvil

### Modification of cold resistance and combining ability of corn inbreds by Cobalt 60 treatment.

Dormant dry seeds of two long time inbred lines of maize, S.D. 5 and B8, were treated with 4750r units of gamma irradiation from a Cobalt 60 source in 1957, immediately prior to planting. Plants grown from the irradiated seeds of each inbred were selfed and outcrossed to check

plants of the other inbred.

Standard cold germination studies using soil from a continuous corn rotation were run on seed of the selfed irradiated inbreds in comparison with untreated checks. Highly significant differences among irradiated inbred progenies in cold resistance were obtained for both S.D.5 and B8. The array of inbred cold resistance scores, based on four replications is given in Table 1.

Table 1. Frequency Array of Cold Resistance Scores of Two Irradiated Corn Inbreds, S. D. Experiment Station, 1958.

Score	Class	Number of Irradiated S.D.5 Selfs	Number of Irradiated B8 Selfs
0-19	1	0	1
20-39	2	4	2
40-59	3	214	6
60-79	4	20	15
80-99	5	10	19
100-119	6	3	10
120-139	7	1	1
140-160	8	0	0

Population performances from the cold tests were:

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Irradiated S.D. 5 selfs	17.14	57.32	1.56**
Check S.D. 5 selfs	23.99	36.72	
Irradiated B8 selfs	20.27	67.47	1.85**
Check B8 selfs	24.42	36.45	

Yield tests of single crosses each involving one irradiated and one check parent, were run in a 12 x 12 lattice in 1958. Possibly because of the dry season, no significant differences between crosses involving an irradiated parent were obtained, nor did these singles differ as a group from the non-irradiated check single cross.

The 1958 results suggest that irradiation of seeds reduced the average cold resistance of both S.D.5 and B8 selfed progenies in comparison to the checks, although the range of variation indicates that some lines exceeding the cold resistance of the parent inbreds might be selected from the irradiated and selfed population.

It had been hoped that dominance effects in the single cross might limit unfavorable or deleterious mutation effects in the irradiated parent. The 1958 yield test which showed no significant differences among 136 single crosses involving an irradiated inbred, or of the inbreds in comparison with the yield of the check single cross, indicates that this has not been disproved. Cold tests and the appearance of visible mutants suggest that irradiation had been effective, and if yield were largely due to additive gene action, differences between checks and irradiated singles might have been expected.

This work is being continued with one additional inbred, so that three single crosses will be tested in 1959.

D. B. Shank

V. A. Dirks

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### 1. "Tassel ear" mutation.

A mutant which produces only staminate flowers in the lateral inflorescences was found in selection F735 in 1955 by Mr. B. Stead in the Union of South Africa who sent me seed of the stock. Out of 21 plants grown in 1956, one plant developed a chlorophyll deficiency in the upper half of the plant following tasseling, two plants had japonica striping and one plant had the lateral inflorescences made up entirely of staminate flowers. All abnormal plants were late flowering and were pollen sterile. A cross of a late inbred on a heterozygous plant segregated 8 "tassel ear" plants to 29 normal plants in F2 in 1958. "Tassel ear" plants tillered profusely and five or six lateral inflorescences developed on the more vigorous tillers. Normal length shanks with husk leaves from the nodes were produced but fully developed tassels replaced the ears. Usually the central spike, and often one or two branches, emerged beyond the husks. These staminate flowers produced an abundance of fully viable pollen, as did the terminal tassel.

In 1949 Eyster reported (News Letter 23:4) a "tassel-like" ear mutant that had a framework which was similar to the framework of a tassel. Limited numbers of seeds were formed on the basal portions of the branches. In 1951 he reported (News Letter 25:10) the character to be associated with bright green color and that homozygous strains were available.

The present mutant has so far proved to be completely staminate.

L. M. Josephson

### 2. New "Teopod" mutations.

Four new independent sources of Teopod or similar characters have occurred in different locations the past eight years. The first occurrence was in a nursery row of the double cross (T8xCI.21E)(L317xCh7B) growing at the Kentucky Agricultural Experiment Station in 1951. An abnormal plant in T8xCI.21E that tillered and had pod-type ears had served as the seed parent of this progeny the year previous. This source was not grown again until 1957 following the finding of other sources. It differs from the teopod of Lindstrom (Maize Coop. stock) in tillering more profusely and having longer and more slender culms. The two were crossed in 1958 to determine whether they are the same mutation.

In 1954 a farmer found a typical teopod plant growing in a field planted with second-generation seed of the topcross KólxPotchefstroom Pearl in the Union of South Africa. Crosses with Tp (Maize Coop stock) have shown it to be a repeat mutation. This source has segregated plants with all the variations described for Cg, as has also the Tp obtained from the Maize Coop. stock. No crosses for associations with other characters have been made.

A mutation related to teopod occurred in a nursery row of inbred Khl at Knoxville in 1955. The main culms of mutant plants are considerably shortened with numerous brace roots developed at the lower nodes, while tillers are usually of normal length. No seed has been produced on the main culms and only occasionally will they terminate in a single spike tassel. Tillers may have a normal tassel, may terminate in an ear, a combination of both, or may be similar to the main culm. Ears produced on normal tillers have been normal and only occasionally produce mutant plants. Axillary ears produced on tillers that terminate in an ear are generally podded at the base. Seed from either the podded axillary ear or the terminal ear will generally produce half normal plants and half mutant plants. No apparently homozygous plants have been obtained.

A typical teoped plant occurred in hybrid Funk G-711 growing in a yield trial on the Jackson, Tennessee Experiment Station in 1956. Only 11 open-pollinated seed were obtained from the plant, none of which produced plants the following year.

Only original Tp (Jour. Hered. 16: 135-140. 1925), Tp2 (Newsletter 22: 41) and the Tp Cg complex have previously been described. Apparently teopod or teopod-like mutations occur more often than indicated by reports.

L. M. Josephson

### UNITED STATES DEPARTMENT OF AGRICULTURE Beltsville, Maryland

An aleurone color pattern factor was found in segregating populations about three years ago. Testcross results suggest that a new R allele is involved. This allele has been tentatively designated as R. The extent and distribution of color is quite variable depending upon the genetic background. In some stocks the allele appears to behave primarily as a dilution factor. In others it simulates R. except basal coloration also is usually involved. In many stocks classification is difficult.

In the transfer of cytoplasmic sterility to one of the Helminthosporium resistant strains of WF9, ratios of 1 fertile to 3 steriles have been obtained. Tests are underway to determine the identity of the second factor involved in fertility restoration.

G. F. Sprague

### WEST AFRICAN MAIZE RESEARCH UNIT Ibadan, Nigeria

### 1. Resistance to the field population of stemborers in West Africa.

A number of inbred lines were introduced into West Africa from Minnesota to investigate if their resistance to the European corn borer did correlate with a possible resistance to West African stemborers. The original lines are highly susceptible to major leaf diseases in the West African environment. Therefore, crosses and backcrosses of these to the Minnesota parent were established with an adapted maize variety. These were compared, in field trials, with a local variety.

The analysis of field trials on basis of the number of bored plants indicates a possibility that recessive genes for resistance are present in the lines Minnesota A42.645 and Minnesota A404.

C. L. M. van Eijnatten

# WESTERN REGION DEPARTMENT OF AGRICULTURE AND NATURAL RESOURCES Tbadan, Nigeria

### 1. Maize breeding program.

The present program of maize breeding in the Western Region of Nigeria began in March, 1958, as a result of a cooperative scheme between the Western Region and ICA.

Observation and limited trials of local maize types grown by farmers of the Region indicate that these types are low in productivity, yielding up to 1 ton of dry grain per acre. Among the factors which appear to be responsible for low yields are (1) low soil fertility, (2) losses due to disease or insect damage, and (3) inherent inability to produce high yields even when grown under favorable conditions. The principal diseases of maize in this area are rust caused by Puccinia polysora, and various leaf blights caused by Helminthosporium turcicum, H. carbonum and Cochliobolus heterostrophus. Insect damage to the growing crop is due chiefly to stalk borers, Agrotidae, whose life cycle is similar to that of the European corn borer, Pyrausta nubilalis.

Trials of more than 600 West African maize collections are being grown at Ibadan. In addition, 78 seed introductions have been received through the kindness of the Rockefeller Foundation centers in Mexico and Colombia, the University of the Philippines, and the United States Department of Agriculture. The 43 South American acquisitions were requested on the basis of their respective origins from latitudes and altitudes similar to those of the maize growing areas of Western Nigeria. Some of these acquisitions originated in areas of high rainfall; these are being put in trials in Delta Province near the mouth of the Niger River, where the rainfall is high during the growing seasons. Small seed samples from most introductions are being grown during the dry season (Dec. - Mar.) under irrigation for seed increase to permit the growing of replicated trials in the First Season (Mar. - July). Two of the Mexican introductions have shown promise as open-pollinated varieties in both the First and Second Seasons, 1958. It is questionable whether the development of hybrid maize adapted to this area is feasible at present. There is no agency for regulating the production of crop seeds, and there is a shortage of native personnel who are qualified to undertake the necessary rigid inspection and control of hybrid maize seed production. However, some Philippine hybrids are being assessed for this area. Also, a considerable program of inbreeding is now in progress, which may be useful first for varietal improvement and for synthetics, but some of the inbreds may be useful for the development of adapted hybrids when the need arises.

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Arnold A. Wellwood

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### 1. Paramutation at the R locus in maize plants trisomic for chromosome 10.

A comparison was made of the aleurone phenotypes resulting from pollination of an rgrg inbred strain (W23) with moderately inbred W22 sib plants of the following genotypes: Rrg (control), Rrgst (disomic) and Rr/Rst/rg (trisomic). The object of the experiment was to test the hypothesis that paramutation of Rr to a weakly pigmenting allele, Rt, which was known from previous tests to occur in 100% of the cases in Rrgst plants, takes place at the zygotene stage of meiosis, when the Rr and Rst alleles are synapsed. The rg allele present in the Rrg and Rr/Rst/rg male parents had earlier been shown to be non-paramutagenic. Assuming 2 by 2 pairing at zygotene at any given chromosome level (Newton and Darlington, 1929) only the Rr male gametes that carry Rr ex-conjugants from Rr-Rst zygotene pairings in Rr/Rst/rg trisomic plants should be paramutant, and the rest should be normal, if the hypothesis in question is valid. Not more than 50%, and perhaps as few as 33%, of the Rr gametes formed by Rr/Rst/rg trisomic plants should be paramutant on this basis. The average scores for aleurone pigmentation of the Rrgs kernels, on an arbitrary scale of 0-40, for the three classes of matings was found to be as follows:

rgrgo x Rrgd 39.23±0.16
" x RrRst (disomic) 5.07±1.23
" x Rr/Rst/rg (trisomic) 6.37±1.13

Aside from a few seeds that could have resulted from pollen contamination, the  $R^r r^g r^g$  kernels resulting from the application to  $r^g r^g$  individuals of pollen from the trisomic  $R^r / R^{st} / r^g$  plants, as well as from the disomic  $R^r R^{st}$  individuals, were of the paramutant phenotype throughout. Thus the results do not support the hypothesis that  $R^r$  is changed to the paramutant form,  $R^t$ , in  $R^r R^{st}$  plants, when the  $R^r$  and  $R^{st}$  alleles are conjugated at zygotene.

R. A. Brink

2. "Enhancement" of Rr action associated with two reciprocal translocations involving breaks in chromosome 10 proximal to the R locus.

Evidence was obtained in 1957 indicating that the aleurone pigment-producing action of the standard R<sup>r</sup> allele was significantly increased (from dark mottling to near-self-color, in single dose) if R<sup>r</sup> was introduced into either the T2-10a or the T4-10b translocation. Both translocations involve breaks approximately 9 crossover units proximal to the R locus. Furthermore, it appeared from other tests that TR<sup>r</sup> (read translocated R<sup>r</sup>) was less paramutable in heterozygotes with the stippled allele (TR<sup>r</sup>/R<sup>st</sup>) than was R<sup>r</sup> in ordinary R<sup>r</sup>R<sup>st</sup> plants. More comprehensive experiments with

this material were carried out in 1958, the results of which may be summarized as follows:

- (a) T2-10a R<sup>r</sup> and T4-10b R<sup>r</sup> are, in fact, significantly stronger in aleurone pigment-producing action than standard R<sup>r</sup> in a normal chromosome 10.
- (b) On reincorporation into a normal chromosome 10 from a T chromosome, R<sup>r</sup> retains its enhanced pigment-producing action. This observation excludes an explanation of the phenomenon in terms of position effect of the conventional kind.
- (c) Enhancement of  $R^r$  action does not appear in the offspring of plants carrying a T chromosome bearing an r (colorless aleurone) allele, with standard  $R^r$  present in a normal chromosome 10  $(Tr/R^r)$ . Evidently the original change to enhanced  $R^r$  action requires that  $R^r$  be in coupling, not in repulsion, with T, in the translocation heterozygote.
- (d) Testcrosses on rr plants of TRr/TRr homozygotes yield the same enhanced Rr phenotype as results when pollen from TRr/r plants is used. Seemingly, "pairing stress" at meiosis is not a factor in the enhancement process.
- (e) Partial reversion of the enhanced pigment-producing action of  $R^r$  in a  $TR^r$  chromosome toward the level of standard  $R^r$  is found among the offspring of  $TR^r/R^r$  plants.
- (f) Paramutability of  $TR^r$  in  $TR^r/R^{st}$  heterozygotes (and also of  $R^r$  extracted from a  $TR^r$  chromosome) is markedly lower than that of standard  $R^r$  in ordinary  $R^rR^{st}$  individuals.
- (g) The partial reversion of enhanced R<sup>r</sup> toward standard R<sup>r</sup>, observed among the offspring of TR<sup>r</sup>/R<sup>r</sup> plants, is paralleled by an increase in paramutability when an R<sup>r</sup> allele with this history is made heterozygous with stippled.

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### 3. Basis of the light stippled phenotype.

A few stippled aleurone kernels with a much reduced frequency of spotting were regularly observed in a series of matings of RrRst and Rstr with rgrg. When such kernels were planted, and the resulting individuals were selfed, ears were formed that showed an Rst (light) phenotype. The frequency of such germinal changes to Rst (light) was found to be 58.7/1000 and 50.3/1000 Rst gametes when tested in RrRst and Rstr heterozygotes, respectively. A population of 13,084 Rst

gametes was tested. The frequency of germinal changes to Rst (light) in homozygous Rst stocks was found to be only 0.3/1000, based on a population of 18,586 Rst gametes.

The difference between the frequency of changes to Rst (light) in Rst heterozygotes with Rr and rr and in Rst homozygotes suggested that such changes are either 1) associated with heterozygosity, per se, at the R locus, or 2) a product of crossing over between Rst and a linked modifier carried on the Rr and rr chromosomes.

A test was made using a proximal marker, golden (g), and a distal marker, a terminal heterochromatic knob (K), to test for the association of crossing over with changes of R<sup>St</sup> to R<sup>St</sup> (light). The following cross was made: g R<sup>g</sup> K/G R<sup>St</sup> k x g r k. R<sup>St</sup> (light) kernels were selected and planted; the resulting plants were scored for golden, and the ears were pollinated with rr. K was scored by making counts of the number of R<sup>St</sup> (light) and r kernels on each ear to determine whether preferential segregation for R<sup>St</sup> (light) had occurred. The results from this test showed that changes to R<sup>St</sup> (light) were always associated with crossing over between R and K.

It is hypothesized that there is a locus about 5.7 crossover units distal to  $\underline{R}$ , the alleles of which modify the expression of  $\underline{R}^{st}$ . The modifier conditioning normal stippled expression was designated  $\underline{M}^{st}$ , and the one conditioning  $\underline{R}^{st}$  (light) expression was designated  $\underline{M}^{st}$ .

The R<sup>r</sup> and r<sup>r</sup> chromosomes in the first test carried m<sup>st</sup>, and the crosses made may now be diagrammed as follows: R<sup>r</sup> m<sup>st</sup>/R<sup>st</sup> M<sup>st</sup> x rg m<sup>st</sup>. Crossing over produced an R<sup>st</sup> m<sup>st</sup> chromosome which conditions R<sup>st</sup> (light). The complementary crossover class would be R<sup>r</sup> M<sup>st</sup> in the R<sup>r</sup>R<sup>st</sup> heterozygotes, and r<sup>r</sup> M<sup>st</sup> in the R<sup>st</sup>r heterozygotes. Both of these complementary crossover classes have been identified, and they occur with the same frequency as R<sup>st</sup> (light).

The changes of Rst to Rst (light) in Rst homozygotes cannot be ascribed to recombination between Rst and a linked modifier. The few mutants obtained from these matings have been interpreted as mutations of Mst to mst or transpositions of Mst (see below).

R. B. Ashman

### 4. Transposability of Mst, a modifier of stippled aleurone.

Numerous self-colored kernels were selected after the following cross: R<sup>St</sup>rg x rgrg. These kernels were grown out to verify the presumed mutations of R<sup>St</sup> to self-color. The ears produced by the resulting plants were pollinated with r<sup>r</sup>r. As observed in an earlier test, less than half of the phenotypically self-colored kernels gave self-colored (R<sup>SC</sup>) off-spring. Fifty plants, in fact, grown from 64 self-colored kernels did not

give germinally transmissible  $\mathbb{R}^{SC}$  mutants, but segregated stippled and colorless kernels. Among these plants two were found which segregated 1/4  $\mathbb{R}^{St}$ , 1/4  $\mathbb{R}^{St}$  (light), and 1/2  $\mathbb{r}$ , instead of the expected 1/2  $\mathbb{R}^{St}$ , and 1/2  $\mathbb{r}$  kernels.

It has been shown that Rst differs from Rst (light) only in a modifier located about 5.7 crossover units distal to R (see above). An explanation which satisfactorily accounts for the ratio observed on the ears from the two exceptional plants would assume that the linked modifier (Mst) which conditions the Rst phenotype is a transposable unit. On this basis it could be assumed that the Rst (light) phenotype results from the absence of Mst, and that in the two exceptional ears Mst has shifted from its standard position, 5.7 crossover units distal to R, to a new position which assorts independently of R. Verification of the transposition hypothesis requires progeny tests of the three classes of kernels on the ears from the two exceptional plants.

R. B . Ashman

### 5. Mutability of Ret.

Tests were made of the mutability of Rst and Rst (light) in homozygous and in several heterozygous combinations.

Rst and Rst (light) in homozygotes were observed to mutate to self-color (Rsc) at the respective rates of 17.0 and 19.9/104 gametes tested. A total of 19.920 Rst and 24.599 Rst (light) gametes were scored. When Rst and Rst (light) were heterozygous with rr, they were observed to mutate to Rsc at the respective rates of 4.9 and 4.3/104 gametes tested. A total of 2,055 Rst and 4,623 Rst (light) gametes were scored from heterozygotes with rr. The basis for the difference in rate of mutation of Rst and Rst (light) to Rsc in homozygotes and heterozygotes with rr is not yet known. Several somatic mutations of Rst to Rsc have been found, which indicates that mutations to Rsc are probably not regularly associated with crossing over.

In Rst (light) homozygotes, one mutation to colorless or near-colorless aleurone was found in 26,805 gametes tested. No mutations to colorless or near-colorless aleurone were found in Rst homozygotes; 20,825 Rst gametes were scored. Mutations to colorless or near-colorless aleurone with either red or green plant color were observed in both Rrst and Rrst (light) heterozygotes. It was assumed that mutants with green plant color came from stippled, and mutants with red plant color from Rr. Based on this assumption, Rst and Rst (light) were observed to mutate to colorless or near-colorless aleurone in heterozygotes with Rr at the respective rates of 5.4 and 4.2/104 gametes tested. A total of 10,942 Rst and 4,720 Rst (light) gametes were scored. These data show that the frequency of mutations of Rst and Rst (light) to colorless or near-colorless is much greater when stippled is heterozygous with Rr than when it is homozygous. The basis for this effect of homozygosity

and heterozygosity on the mutability of stippled is not yet known. Allelic interaction or crossing over, or both, may be involved.

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R. B. Ashman

# 6. Paramutagenic action of colorless and near-colorless mutants from Rst.

The colorless and near-colorless mutants from Rst and Rst (light) (see above) were made heterozygous with Rr and tested for their paramutagenic action. ("Paramutagenic" is a term used to describe the action of Rst on the pigmenting capacity of Rr in RrRst heterozygotes; see Brink, Cold Spring Harbor Symp. Quant. Biol. 23, 1958.)

The one near-colorless mutant obtained from homozygous Rst (light) was found to be paramutagenic. Eight mutants with green plant color were obtained from RrRst and RrRst (light) heterozygotes, and all were found to have retained the paramutagenic action of stippled. This is additional evidence for the assumption made above that mutants with green plant color from Rr/stippled heterozygotes are mutations from the stippled allele.

Ten colorless mutants with red plant color were obtained from R<sup>T</sup>R<sup>st</sup> and R<sup>T</sup>R<sup>st</sup> (light) heterozygotes; five of these mutants were found to be paramutagenic, and five were found to be nonparamutagenic. These results suggest that at least some of the r<sup>T</sup> mutants arise from recombination between components of R<sup>T</sup> and R<sup>St</sup>. The paramutagenic r<sup>T</sup> mutants exhibit the plant color characteristic of R<sup>T</sup>, the paramutagenic action of R<sup>St</sup>, and have lost the aleurone pigmenting action of both R<sup>T</sup> and R<sup>St</sup>. It is not possible at this time to postulate a single crossover or mutational event that will satisfactorily explain all the observed changes. Tests on these mutants are being continued.

R. B. Ashman

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### 7. Dosage effect of the Rst allele on aleurone pigmentation.

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An experiment was made to determine the quantitative effect of varying doses of the R<sup>St</sup> allele on aleurone pigmentation. The matings were as follows: rgrg x Rst Rst, Rst x rgrg, and Rst Rst, selfed. The kernels were scored using a modification of the reticule method described by Brink (MGCNL, 31). The results were as follows:

Dosage of R	No. of kernels	Mean index of pigmentation per kernel
Rat rg rg	480	8.75
Ret Ret re	480	19.83
Rst Rst Rst	360	27.70

These data were tested for conformity to expectation on the basis of linear regression and were found to give a close fit. Thus the aleurone spotting effect of increasing dosages of the Rst allele appears to be additive.

H. B. Cooper

### 8. Partial restoration of paramutant Rr.

A test was made to determine the amount of restoration that paramutant R<sup>r</sup> undergoes when passed through one sporophytic generation with three different r<sup>r</sup> mutants from standard R<sup>r</sup>, and when passed through one sporophytic generation as a homozygote. The modified R<sup>r</sup> will be designated as R<sup>r:1st</sup> (Brink, MGCNL 32). The R<sup>1st</sup> allele used as the "inducer" in this test has been described by Ashman (see current MGCNL). All alleles used in this test had been previously incorporated into the highly inbred line of W22. The female test parent used was the uniform inbred line W23 r<sup>g</sup>r<sup>g</sup>. The mating plan was as follows:

	The second second		Testcrosses
ming acceptance	Rr:lst Rlst	(1)	W23 rgrg x Rr:lstRlst
W22 Rr Rlst (selfed)	Rr:lat Rr:lat	(2)	W23 rgrg x Rr:lstRr:lst
W22 rrr x Rr Rlst (mutants from standard Rr)	<sub>R</sub> rılst <sub>r</sub> r	(3)	W23 r <sup>g</sup> r <sup>g</sup> x r <sup>r</sup> R <sup>r</sup> :lst
W22 RrRr (selfed)	Rr Rr	(4)	W23 rgrg x Rr Rr

The kernels of aleurone phenotype R<sup>r:lst</sup> and standard R<sup>r</sup> mottled were scored with the aid of a head lens, magnification approximately l.k. The kernels were matched to a set of standard kernels ranging in aleurone pigmentation from colorless, through grades of mottling, to self colored. The kernels from testcross (1) above provide an estimate of the initial aleurone phenotype of the paramutant R<sup>r:lst</sup> allele. The kernels from testcross (2) provide an estimate of the phenotype of the R<sup>r:lst</sup> allele after it has passed through one sporophytic generation as a homozygote. The kernels from testcross (3) provide an estimate of the R<sup>r:lst</sup> allele after it has passed through one sporophytic generation heterozygous with the three respective r<sup>r</sup> mutants. The kernels from testcross (4) provide an estimate of the phenotype of standard R<sup>r</sup>. The scores for each class of R<sup>r:lst</sup> phenotypes and the R<sup>r</sup> phenotype were converted to percentages. The results are as follows:

Testcross	Endosperm genotype of kernel scored	No. of kernels scored	Mean percentage pigmentation per kernel
W23 rgrg x Rr:lstRlst	Rr:lst rgrg	4550	15.34
W23 rgrg x Rr:lstRr:lst	11	900	40.64
W23 rgrg x Rr:lstr	- H	700	53.09
W23 rgrg x Rr:lstr25		900	53.30
W23 r <sup>g</sup> r <sup>g</sup> x R <sup>r:1st</sup> r <sub>27</sub>	M. M. Carrier of	1250	51.35
W23 rgrg x Rr Rr	Rr rgrg	400	82.48

The results may be summarized as follows:

- (1) The Rr:lst allele extracted from Rr:lst Rr:lst rs, Rr:lst rs, and Rr:lst rs, regularly reverts in pigment-producing potential toward, but not to the levels of, standard Rr.
- (2) The Rr:lst allele extracted from the Rr:lst Rr:lst homozygote shows significantly less restoration, in this case, than the Rr:lst allele extracted from the mutant heterozygotes. This is not in agreement with certain earlier observations, and the relation requires further study.
  - (3) The r mutants tested do not differ in restoring action. Progressive restoration of the R allele has been tested. Six independent r mutants from standard R, standard r, and standard r alleles were used in the test. The R allele was made heterozygous with each of the above restoring alleles. The heterozygotes, R estored R allele and then used as males on inbred W22 r ergo. The restored R alleurone phenotypes produced by the above mating plan are as follows:
  - (1)  $R^{r:1st}$  phenotype after one and two generations of restoration with each of the r alleles.
  - (2) Rrist phenotype after one generation of restoration with one of the r alleles and then one generation with the r allele.
  - (3) R<sup>r:lst</sup> phenotype after one generation of restoration with each of the <u>r</u> alleles and then made homozygous for one generation.

The material from the progressive restoration test has not been scored in detail; however, general inspection indicates that little or no progressive restoration occurred.

### Tests to determine whether paramutation is unidirectional or reciprocal in R<sup>1</sup>R<sup>5</sup> heterozygotes.

The R<sup>r</sup> allele conditioning self colored aleurone and plant pigment is regularly changed to a weakly pigmenting form in the progeny of heterozygotes possessing the R<sup>st</sup> allele (Brink, 1956). Two tests were conducted to determine whether the stippled phenotype produced by R<sup>st</sup> is regularly altered in the R<sup>r</sup>R<sup>st</sup> heterozygote. The first test was made in the following way. A W22 R<sup>r</sup>R<sup>st</sup> heterozygote was selfed. The R<sup>st</sup>R<sup>st</sup> and R<sup>r</sup>R<sup>st</sup> progeny from this self were testcrossed on W23 r<sup>g</sup>r<sup>g</sup>q. The kernels of R<sup>st</sup> r<sup>g</sup>r<sup>g</sup> aleurone phenotype from the testcross ears were scored under a 30x binocular microscope fitted with a 20 x 20 reticule, covering an area of approximately 12 square millimeters. A predetermined area of the abgerminal side of the kernel was brought under the reticule and the number of spots delimited by one-half of the reticule were counted. The results were as follows:

Testcross	No. of kernels scored	Mean no. of spots per kernel
W23 rere x Ret Ret	360	35.22
W23 rgrg x Rr Rst	240	33.52

A test of significance gave t = 0.56, P>0.5.

The second test was made in the following way. A W22 Rst r heterozygote was selfed and used as the pollen parent on W22 RrRr. The Rst Rst and Rr Rst progeny were testcrossed on W23 rgrgqq. The kernels of Rst rgrg aleurone phenotype from the testcross ears were scored with a microscope fitted as described above. A predetermined area of the crown was brought under the reticule, and the number of spots delimited by one-twentieth of the reticule area were counted. The results were as follows:

Testcross	No. of kernels scored	Mean no. of spots per kernel
W23 rgrg x Rst Rst	240	6.08
W23 rgrg x Rr Rst	240	6.37

A test of significance gave t = 0.51, P>0.5.

These facts indicate that paramutation is unidirectional in the  $\underline{R}^{\mathbf{r}}$   $\underline{R}^{\mathbf{st}}$  heterozygote.

H. B. Cooper

## 10. The effect of dissociation (Ds) on the stability of the variegated pericarp allele, PVV.

The addition of a transposed Modulator (tr-Mp) to a PVV/PWF

0/49

heterozygote greatly reduces the frequency of somatic mutations of the variegated pericarp allele (PVV) to self-red (PTP), and thus gives the light variegated phenotype. It has been reported (Genetics 41:901-906) that the addition of an Ac (Activator) element of McClintock's Ac-Ds system has a comparable effect on the stability of the variegated allele. It was of interest, therefore, to determine if Ds (Dissociation) also has an effect on the stability of PVV.

A Ds element, in the standard position on chromosome 9, was introduced into inbred W22, and carried through three generations of backcrossing. Pollen from a plant homozygous for Ds was then placed on silks of PVV/PVV plants of inbred W22 background. The progeny from this cross were grown out, and separated into plants possessing Ds and those lacking Ds, on the basis of losses in the genome of an aleurone marker gene distal to the Ds locus. Seventy-five ears of each group were then scored for the number of somatic mutations of PVV to PTT with the aid of a set of model kernels having mutant areas (red) of graded sizes.

The data obtained are summarized in table 1.

Table 1. Distribution of the number of mutations of PVV to PTP per 1000 kernels in the two genotypes resulting from the cross of PVV/PVV x PVV/PWV, Ds/-.

Genotypes	Estimated Total Kernels	Number Ears Scored	No. of mutations per 1000 kernels distributed according to the size of the mutant area					
			1/8-1/4	1/4-1/2	1/2-	l kernel	2 or more	Total
PVV/PVV, Ds	33,355	75	5.40	8.06	3.90	1.20	0.15	18.71
PVV/PVV, no-	-Ds 32,685	75	8.11	9.15	3.76	1.19	0.34	22.55

The reduction in the frequency of somatic mutations of PVV to PTT in the group possessing Ds as compared to the group lacking Ds is statistically significant at the 5 percent level. However, the reduction in the frequency of somatic mutations due to Ds is not at all comparable to that due to a tr-Mp or an Ac. Wood and Brink (Proc. Nat'l Acad. Sci. 42:514-519) found that the addition of a tr-Mp to a PVV/PWT heterozygote in inbred W23 background reduced the total number of mutations of a size of one-eighth kernel or larger by 69.56 percent and that the relative reduction was quite uniform for mutants in each size class. Comparison of the group totals given in table 1 shows that the total number of PVV to PTT mutations of all sizes exceeding one-eighth of a kernel is only 17.03 percent lower in the group containing Ds than that in the group lacking Ds. Also, the reduction is in the one-eighth to less than one-fourth, and one-fourth to less than one-half kernel classes.

Thus, the effect of Ds in reducing the grade of variegation in PVV/PWT heterozygotes if, indeed, it is real, is much less than that of a tr-Mp or an Ac. Furthermore, the effect of Ds occurs later in ontogeny than does that of tr-Mp, since Ds caused no apparent reduction in the frequency of somatic mutations occurring sufficiently early to give rise to mutant sectors one-half kernel, or larger, in size.

Elwin R. Orton

### 11. Diffuse.

Diffuse (Df) was previously described (Jour. Hered. 45:47 - 50; M. N. L. 32) as a dominant pattern gene that partially inhibits pericarp pigment produced by the PRR allele. More recent findings indicate that this explanation is inadequate.

PRR/PRR, Df/df ears often exhibit colorless or near-colorless sectors of variable size in addition to a fine patchwork of colored, lightly colored, and colorless areas. For the initial tests of heritability of the colorless phase of Df, kernels were selected from both large (100 kernels or more) colorless and dark-diffuse areas on the same ear, and then grown separately so that levels of expression could be compared on a progeny basis. The seven colorless sectors tested all gave offspring indistinguishable from those derived from the dark-diffuse areas. In 1957 a family from a similar colorless sector gave colorless offspring, whereas the plants from the dark-diffuse kernels on the same ear yielded only dark-diffuse. This finding prompted further testing of colorless sectors for heritability of the irregularly expressed colorless phase.

An additional ten colorless sectors were tested in the same manner in 1958, but these sectors were much smaller (less than 30 kernels) than those previously tested. All families from these colorless sectors produced colorless and near-colorless offspring; the dark-diffuse areas gave only dark-diffuse ears. It seems that the plants obtained from kernels in the smaller sectors possess, or at least exhibit, the colorless phase of <u>Df</u>. In contrast, plants obtained from the kernels in the larger colorless sectors, do not show the colorless phase.

In the two cases in which the kernels from entirely colorless Diffuse ears, derived in the previous generation from colorless sectors, were progeny tested some of the resultant ears had the dark-diffuse phenotype, whereasothers were very lightly pigmented, but none were colorless, as were the immediate parents.

 $\underline{\mathrm{Df}}$  has now been found to be a partial inhibitor of aleurone pigmentation also. When pollen from  $\underline{\mathrm{Df}}/\underline{\mathrm{df}}$  plants is placed on silks of A C R plants the resultant ears contain 5 - 10% smoky kernels, with the remainder self-colored. The smoky kernels when grown all prove to be Diffuse, whereas the self-colored kernels give rise to both Diffuse and non-Diffuse plants. The Df expression in the pericarp of plants grown from these

smoky kernels is frequently of the colorless phase.

The relationship between inhibition of aleurone pigmentation, size of the colorless sectors, and the transmission of the colorless phase is yet to be determined.

I. M. Greenblatt

### 12. Removal of pericarp with HCl.

Satisfactory removal of the pericarp from dried corn kernels has been accomplished by treatment with hydrochloric acid. The kernels from which the pericarp is to be removed are placed in a 10% HCl solution, and then heated in a boiling water bath for approximately 8 minutes. They are then transferred to a fine meshed wire basket, and washed under a strong stream of cold water. The force of the water removes the pericarp loosened by the acid. Treatment with acid does not affect the aleurone markedly except to convert purple pigments, when present, to a bright red. This technique is helpful in large-scale scoring for both endosperm and aleurone characteristics when the pericarp would otherwise interfere.

J. Kermicle I. M. Greenblatt

Addendum

DEKALB AGRICULTURAL ASSOCIATION, INC.
DeKalb, Illinois

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# 1. Culture of haploid cells.

Current work of others on tissue and cell culturing suggests that it may be possible to effect a radical improvement in the monoploid method. The aim of work we now have in progress is the development of true breeding strains (homozygous diploids or the equivalent) of corn and other species directly from isolated cells of the haploid phase (either spores, gametophytic cells or gametes) freed from the parent tissue. The attempt is being made to grow these cells through the proembryonic and embryonic stages in basic synthetic media suplemented with growth stimulating substances and to induce somatic doubling of the chromosome complement at some stage prior to formation of the meiocytes.

Permission to cite above note not required.

S. S. Chase B. **Tro**tsis

#### III. REPORT ON MAIZE COOPERATIVE

As mentioned in the 1957 Maize News Letter, three separately-acquired stocks of Chromosome 6 traits have very similar phenotypes: antherless (at), silky ear-1 ( $si_1$ ), and E. G. Anderson's new trait "male sterile-silky" ("ms-si"). All were previously found to be closely linked to  $\underline{Y}_1$ .

Among several hundred plants of these traits grown here, all mutant plants have been both antherless and silky in phenotype. Intercrosses between the Cooperative's stocks of at and si\_ indicate allelism. No published description of the original stocks of at has been found. According to the published description of si\_, mutant plants have supernumerary silks on the ear and also have a few silks in the tassel, with little pollen shed. Under Illinois conditions, neither at nor si\_ plants have exserted anthers. On the assumption that there has been no past mix-up in at stocks, at and si\_ are apparently allelic. Intercrosses of si\_ and "ms-si" also indicated allelism. Testcross data involving si\_ and Y\_ gave 8 recombinants in 265 plants (3.0%). A small progeny carrying a third marker, Pl, indicates that the probable order is si\_ - Y\_ - Pl.

A trait submitted to the Coop as probably being  $ms_1$  has given 9.2% recombination with  $\underline{Y}_1$  in testcrosses (23 recombinants in 251 plants). Our collection does not include a known stock of  $ms_1$  for an allelism test. This trait may, however, be mislabelled since the  $ms_1$  reported in the 1935 Maize Linkage Summary showed about 3% recombination with  $\underline{Y}_1$ . This unidentified male-sterile is not allelic to  $\underline{si}_1$ . It has not yet been tested with po.

The gene po in Chromosome 6 has been uncovered by hemizygous tests involving transmissible duplicate-deficient complements from three translocations. These translocations, along with Dr. Longley's cytological placements, are as follows: 2-6 5419 (2L.82; 6S.79), 4-6 4341 (4S.37;6S.81), and 6-9 4778 (6S.80; 9L.30). In similar tests yg2 (Chromosome 9) was uncovered by two translocations: 8-9 4453 (8L.86; 9S.68) and 3-9 6722 (3S.66; 9S.66). Other translocations and distally-located markers are being tested.

There are still a number of older genetic traits that should be added to the Coop collection if stocks of them can be found. We would appreciate receiving verified stocks of any of the following traits:

```
Chromosome 1--ga6, zli

" 2--ts1, d5
" 3--yt
" 4--de1, de16
" 5--sf, cb, f2
" 6--ms1, w5 w6, v6, yd, at
" 9--yf, pk, Pr2, Da2, de15, v15, w11, 03
```

Chromosome 10--18, sp2, pg1, f3, xn1, l4 Unplaced--bl, bn, mi, ps A-B Translocations -- TB-6a, TB-7a.

Requests for stocks should be sent to the Botany Department, University of Illinois, Urbana, Illinois. The listing of Maize Cooperative stocks below includes the more useful combinations which are presently available. Many additional combinations not specifically listed can be supplied or may be derived from segregating progenies.

### Chromosome 1

```
a HE THERE IS NO ASSOCIATION FOR THE STATE OF THE
                                                        to the U.S. In special after Daywood and Service
         adl and bm2
        anl Kn bm2
                                Militar pullen south bider lithrain continues action, norther
         as sen
         Him a court and models that and expenses and no according to seem to be
         Kn waste , which the property are not the the state of all all and are
         lwi lore and harmonial and the lates this cale the late to
         necrotic 8147-31
          PCW
          PMO
          (POR)
         PRR adl anl PRR adl bm2
         PRR adl bm2

PRR anl gsl bm2

PRR brl fl anl gsl bm2

PRR brl fl anl gsl bm2
         PVV
         PWR bm2
                                  the fitting on the Chrysles with a best continued at the series
         PWR
                  gsl bm2
                                     prosting indistrict entitled addressed at his land
         PWW bri fi bm2

sri PWR ani bm2
          sri PWR ani gsi bm2
         sr zb, PWW
                                    PERSONAL PROPERTY (SEASON) PROPERTY OF THE PROPERTY OF THE PERSONS ASSESSMENT OF THE PERSONS ASS
         ts, PWW brl bm2
         Tsg orth task sites allower weeks to todain a title one want
bloover about no one could be expected the police than the of beine
         VPgal asharing and to gas its contra to them no tylengrated by a
         pg
         zbų ms<sub>17</sub> pWW
                 PWW bm2
         zb)
         zbl
         zb<sub>l</sub> ts<sub>2</sub> PWW
```

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### Chromosome 2

al lg1 gl2 B sk
al lg1 gl2 b sk
ba2
fl1
lg1 gl2 b
lg1 gl2 b
lg1 gl2 b
lg1 gl2 b fl1 v1
lg1 gl2 b fl1 v1
lg1 gl2 b sk fl1 v1
lg1 gl2 b sk fl1 v1
lg1 gl2 b v1
lg1 gl2 b v1
lg1 gl2 gs2 b v1
ls1 gs2 b sk
ws3 lg1 gl2 b sk
ws3 lg1 gl2 b sk

### Chromosome 3

A<sub>1</sub> ga<sub>7</sub>; A<sub>2</sub> C R
A<sub>1</sub> sh<sub>2</sub>; A<sub>2</sub> C R
Ad-31; A<sub>2</sub> C R
Ad-31 sh<sub>2</sub>; A<sub>2</sub> C R
Ad-31 sh

### Chromosome 3 (Continued)

```
d<sub>1</sub> gl<sub>6</sub> Lg<sub>3</sub> d<sub>1</sub> lg<sub>2</sub>
 dl Lg3
 dl pg2
 d<sub>1</sub> Rg
 di tsh 1g2
d_2
 g16
 gl6 lg2 al et; A2 C R Dtl
gl6 lg3
gl6 Rg
 g16 V17
 lg2 Alb et; A2 C R Dt1
 lg2 al et; A2 C R Dt1
 lg<sub>2</sub> s<sub>1</sub> sh<sub>2</sub> et; A<sub>2</sub> C R Dt<sub>1</sub> lg<sub>2</sub> s<sub>1</sub> et; A<sub>2</sub> C R Dt<sub>1</sub>
 lg2 pm
Lg3
pg2
 pm
raz lgz pm
 raz Rg
 Rg
 rt; A1 A2 CR
 tsu na
 Lda
 Primary trisome 3
```

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2 2 of poly for a

10 1 0 42 100 10 10 1 0 42 100 10

Will Night to Miller

1 D = D-6

ALL RESERVED

### Chromosome 4

bm3
bt2
de(1 or 16?)

Gal Sul
gal sul
gl3
j2
la sul gl3
la sul Tu gl3
lo Sul
lo sul
lw,; lw3
ol
spl sul

### Chromosome 4 (Continued)

st
sul bm3
sul gl3
sul gl4
sul j2 gl3
sul Tu
sul Tu gl3
sul zb6
sul zb6 gl3
sul zb6
sul zb6 gl3
sul zb6
Tu
sul
Ts5
Ts5
sul
Tu
gl3
v8

### Chromosome 5

a2; A1 C R a2 bm1 pr v2; A1 C R a2 bt1 pr; A1 C R a2 bt1 pr; seg bm1 bv1; may seg v2; A1 C R a2 pr; A1 C R bm<sub>1</sub> bt<sub>1</sub> bv<sub>1</sub> pr; a<sub>1</sub> A<sub>2</sub> C R bm<sub>1</sub> pr ys<sub>1</sub> v<sub>2</sub>; A<sub>1</sub> A<sub>2</sub> C R bml Ag1 btl pr; Al A2 C R Ga Btl ga bt1 gl<sub>5</sub> gl<sub>17</sub> a<sub>2</sub> bt<sub>1</sub> v<sub>2</sub>; A<sub>1</sub> C R gl<sub>17</sub> v<sub>2</sub> intensifier of pr closely linked to bt lw<sub>2</sub>; lw<sub>4</sub> na2
pr: A1 A2 C R
shfl = "sh1"
"sh3" = allele of bt1 v3 pr; A1 A2 CR ví2 vp<sub>2</sub> gl<sub>8</sub> vp<sub>2</sub> pr; A<sub>1</sub> A<sub>2</sub> C R vp7 pr; A1 A2 C R

### Chromosome 6

```
at = allele of si
po Y1 pl
po y1 pl
Pt
sil Yl Pl
sil Yl pl
sil yl pl
y1 110
Y1 ms(1?)
y1 ms(1?)
Y1 pb4 pl
y1 pb Pl
y<sub>1</sub> pb<sub>4</sub> pl
y<sub>1</sub> pg<sub>11</sub> pl; wx pg<sub>12</sub>
y<sub>1</sub> Pl Bh
y1 pl Bh
Y1 Pl sm py; A1 A2 b PRR
Y<sub>1</sub> pl su<sub>2</sub>
yi pl su2
Yi Pl; seg wi
Yi pl; seg wi
y1 Pl; seg w1 H D -1 F - 1 av pm 1 pm 1 mm 1 mm 2 mm 2 mm
yl bl; seg wl
"male sterile-silky" = allele of sil
                                             "orobanche" (seedling)
"ragged" (seedling)
"white 8522" (seedling)
"white 8896" (seedling)
```

All of business gladerica

20 27 181

to the ser

### Chromosome 7

bd gl<sub>l</sub> sl Bn<sub>l</sub> ij in; pr A<sub>1</sub> A<sub>2</sub> C R o2 gll sl o2 gll sl Bnl o2 ral gl oz vg gli; seg ral 02 v5 ral gl1 Hs 02 v5 ral gl1 Hs ral gl1 Tpl val Tpl vpg gly; wx

### Chromosome 8

wn v16 ms8 j1; l1 "necrotic 6697" (seedling) "sienna 7748" (seedling)

### Chromosome 9

aul au2 bk<sub>2</sub> ms<sub>20</sub> bm C sh<sub>1</sub> wx; A<sub>1</sub> A<sub>2</sub> R c sh<sub>1</sub> wx; A<sub>1</sub> A<sub>2</sub> R c sh<sub>1</sub> wx gl<sub>15</sub>; A<sub>1</sub> A<sub>2</sub> R c wx; A<sub>1</sub> A<sub>2</sub> R c wx bk2; A1 A2 R
Dt1 (See Chromosome 3 stocks) I wx; A<sub>1</sub> A<sub>2</sub> R Pr B pl I wx; A<sub>1</sub> A<sub>2</sub> R pr B pl 17 ms2 ms2 sh1; A1 A2 C R ms20 shi wx d3 shi wx 17 shi wx pg12; y pg11 pl shi wx vi wx ar wx Bf1 wx bk2 wx d<sub>3</sub>° wx dæ<sub>1</sub>; A<sub>1</sub> A<sub>2</sub> C R wx gy wx 14 wx pg12; y pg11 pl  $yg_2$  c  $sh_1$  wx;  $A_1$   $A_2$  R  $yg_2$  C  $sh_1$  bz wx;  $A_1$   $A_2$  R Primary trisome 9

### Chromosome 10

bf2 dul

### Chromosome 10 (Continued)

```
g_1
g1 12
g1 rg; A1 A2 C
                                                    THE REPORT A PROPERTY OF
gl r sr2
gī9
11; v16 ms8 j1
li g1 R; A1 A2 C
li gi r; Ai Az C
li g1 r; A1 A2 C; carries abnormal lo nl1 g1 R; A1 A2 C
Og R; A1 A2 C B P1
Rg sr2
rr sr2
Rmb; A<sub>1</sub> A<sub>2</sub> C
Rnj; A<sub>1</sub> A<sub>2</sub> C
Rst; A<sub>1</sub> A<sub>2</sub> C
v<sub>18</sub>
                                              4 Del 1
zn
"oil yellow" (seedling and plant)
Primary trisome 10
```

A the own jobs A sec-

( N 1 21)

### Unplaced genes

```
cl
ct
de<sub>17</sub>
dv
dy
fl2
gl<sub>11</sub>
g112
gl<sub>14</sub>
gl<sub>16</sub>
glg
h
13
mes
ms6
ms7
ms9
ms10
ms11
ms<sub>12</sub>
ms13
mszl
Mt
New starchy
```

### Unplaced genes (Continued)

ra3
rd
Rs1
rs2
"sh5"
tw1
tw2
v13
va2
vp6
ws1
zb2
zb3

### Multiple gene stocks

Al A2 C Rr Pr B Pl Al Az C Rg Pr B Pl A<sub>1</sub> A<sub>2</sub> C R<sup>g</sup> Pr B pl lg<sub>1</sub> y A<sub>1</sub> A<sub>2</sub> C R Pr Pr wx Pr wx Pr wx gl m pr pr sully wx pr y gl<sub>1</sub> pr y wx pr y wx gl Al A2 c R Pr sul y wx y sh<sub>l</sub> wx A<sub>1</sub> A<sub>2</sub> C r Pr sul y g<sub>1</sub> bm2 lg1 a1 su1 pr y sh1 wx g1 j1 wx g1 colored scutellum lg sul bul al gli ji sul yl wx &l A2 C Rg pr yl sul ral gli yl wx gll

### Popcorns

Amber Pearl Black Beauty Hulless

### Popcorns (Continued)

Ladyfinger Ohio Yellow Red South American Supergold

### Exotics and Varieties

Argentine Popcorn
Black Mexican Sweet Corn (with B chromosomes)
Black Mexican Sweet Corn (without B chromosomes)
Gourdseed
Maiz chapolote
Papago Flour Corn
Parker's Flint
Strawberry Popcorn
Tama Flint
Tom Thumb Popcorn
Zapaluta chica

### Chromosome rearrangements

A selected group of chromosome rearrangements, whose breakpoints mark most of the regions of the ten chromosomes, is being maintained primarily for use in determining the chromosome locations of new traits. Two inversions, Inv 2a and Inv 9a, are included. All of the rearrangements 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

lg<sub>1</sub> or gl<sub>2</sub> Inv 2a (also available with <u>Ch</u>) 2S.7; 2L.8 wx Inv 9a 9S.7; 9L.9

### Reciprocal translocations

wx 1-9e	18.48; 9L.22
wx 1-9 4995	1L.19; 9S.20
wx 2-9b	25.18; 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

## Reciprocal translocations (Continued)

	wx 4-9g	45.27; 9L.27
	wx 5-9a	5L.69; 9S.17
	wx 5-9c	5S.07; 9L.10
	wx 5-9 4817	5L.06; 9S.07
	wx 5-9 5614	5L.09; 9L.06
	wx: 6-9a	68.79; 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 <sub>1</sub> 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 1-4a (also available with PRR)	1L.51; 4S.69
5	su 1-4d-(also available with PRR)	1L.27; 4L.30
1,1	su 4-5j	4L.21; 5L.36
	su, y 4-6a	4L.37; 6L.43
	su. 4-8a	48.59; 8L.19
	su, R 4-10b	4L.15; 10L.60
	y 1-6c (also available with PRR)	1S.25; 6L.27
	gl <sub>2</sub> 2-3c	25.46; 35.52
	gl <sub>2</sub> 2-3 5304	25.62; 3L.29
	gl <sub>2</sub> · 2-6b	25.69; 6L.49
	gl <sub>2</sub> ; R 2-10b	2S.50; 10L.75
	g1 <sub>1</sub> , 6-7 4545	6L.25; 7S.73
	6-T3	

# Stocks of A-B chromosome translocations

B-la	1L.2	Proximal to Hm
B-1b	15.05	The second of th
B-3a	3L.1	April 1944
B-4a	45.25	Proximal to sun
B-7b	7L.3	Proximal to ray
B-9a	9L.5	
B-9b	95.4	Between C and wx; close to wx
B-10a	10L.35	Proximal to gl

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