MAIZE GENETICS COOPERATION

NEWS LETTER

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I. Foreword

On behalf of all maize geneticists, it is a pleasure to acknowledge our indebtedness to Miss Ellen Dempsey for editing and supervising the assembly of this volume of the Maize News Letter. Hers is an arduous and demanding task which she has performed exceedingly well and without the proper recognition. Acknowledgment should be given to Marian Beremand, Michael Freeling, Judith Wall and Margaret Walsh for their assistance in proof reading.

The cost of publishing this year's News Letter has been met from a grant by the National Science Foundation to the Maize Genetics Stock Center at the University of Illinois. We are truly grateful for this financial help which makes the News Letter possible.

Announcements:

We have been asked to call your attention to the seventh edition of the Maize Research and Breeders Manual, published by Illinois Foundation Seeds, Inc. and prepared by Clarion B. Henderson. The 1972 revision is now available upon request from Illinois Foundation Seeds, Inc., P.O. Box 722, Champaign, Illinois 61820. The next revision is scheduled for 1976.

As indicated in C. R. Burnham's report from Minnesota, reprints of his book "Discussions in Cytogenetics" can be purchased from the author.

Errata:

1) Through an unfortunate oversight, the name of the author of the first article in the 1972 Maize News Letter was omitted. This article, on page 3, was contributed by Istvan Kovacs, Head of the Plant Breeding Department, Agricultural Research Institute, Hungarian Academy of Sciences, Martonvasar, Hungary.

2) On page 100, the title of article 3 should read "Induced mutations on prolific type of maize".

M. M. Rhoades

II. OBITUARIES

Ernest G. Anderson, 1891-1973

Friends and colleagues of Professor Ernest G. Anderson were saddened to learn of his death January 30 of this year at his home in Columbia, Missouri. He would have been 82 on March 3.

A native of Nebraska and a graduate of the University of Nebraska, he took his advanced training with Professor R. A. Emerson at Cornell University. During the 1922-23 academic year while teaching at C. C. N. Y., he had the opportunity of working in the laboratory of Professor T. H. Morgan at Columbia University during an exciting era of Drosophila genetics. Dr. Anderson often remarked on his good fortune in being associated with this early period of Drosophila work. He also expressed his admiration for the rigorous scientific standards of Professor Emerson and for his leadership in establishing a tradition of cooperation among maize geneticists.

From 1923 to 1928, Dr. Anderson was a member of the Botany Department at the University of Michigan. During the 1920's he published a number of significant contributions in Drosophila genetics. In 1928, he joined the faculty of the California Institute of Technology, where he remained until his retirement in 1961. During this period, he accumulated a large collection of chromosome rearrangements in maize and conducted extensive mapping studies with reciprocal translocations.

In 1946, Dr. Anderson agreed to cooperate in the study of the biological effects of the Bikini atom bomb test by analyzing mutations induced in maize. Later, the program was extended to include mutations induced by the atom bomb test at Eniwetok and by monitored dosages of ionizing radiation applied at the Oak Ridge National Laboratory and elsewhere. Over a period of years, this work was supported in part by successive grants from the Office of Naval Research. Anderson had an active collaborator in Dr. A. E. Longley, Geneticist with the Agricultural Research Service, who determined the interchange points of more than one thousand chromosome rearrangements.

Throughout this period and for the remainder of his life, Dr. Anderson devoted most of his research effort to these hundreds of induced

gene and chromosome mutations. Both before and after his formal retirement, he spent a great deal of time in verifying and increasing the stocks and in assisting personally to ensure their orderly transfer to the Maize Cooperation Genetic Stock Collection.

As a scientist, he was dedicated, painstaking and thorough. While he sometimes professed to be lazy, his associates learned to interpret this remark as indicating his strong preference for the thoughtful, simple, incisive approach to a problem as contrasted to a frontal assault by sheer energy. Those who worked with him came to appreciate the directness and economy of his methods.

While he disliked crowds and was impatient with pretense or superficiality, he genuinely enjoyed people as individuals and was uncommonly patient, helpful and encouraging to those who sincerely sought his assistance or counsel. Even to those who were aware of his wide-ranging curiosity, the breadth of his knowledge and interests was a continuing source of surprise. Stimulating in conversation, appreciative of humor, he had an unassuming, gracious, warm personality; one could feel totally comfortable with him whether sharing conversation or silence.

Scientists have lost a respected colleague and humanity a humanitarian. To members of Dr. Anderson's family I offer my sincere sympathy. In knowing him more intimately, they must feel still more deeply the full dimensions of the loss.

E. B. Patterson

Avraham Shlomi - Israel's corn man

One week before Avraham Shlomi was to celebrate his retirement after 25 years of leadership in Israel's corn breeding research program, he was tragically killed in an automobile accident on his way to work. Shlomi was for years the only individual in Israel working in corn breeding. His deication to his work was an example for many scientists in Israel. He was called by many "Mr. Corn".

Shlomi was born in Austria in 1904. In 1921, he resigned from his studies at the University of Prague in order to fulfill his dream - to leave

and work in the Jewish homeland. From the day of his arrival in Israel, Shlomi gave his all to agricultural development. In 1946 he went to the U.S. to study the new methods of hybrid corn breeding. On his return to Israel in 1948 he established the department of corn breeding. Until his death, he headed this department. During his life's work he developed 15 different commercial hybrids, some of which are in use in other countries. In the last several years of his life, he put most of his energy and knowledge into the development of maize varieties suited for use in underdeveloped nations.

Shlomi's goal in life was corn development and not personal achievements. Therefore, theoretical research and subsequent publication of results were considered, by him, as a waste of time. One of his greatest contributions was the development of variety x inbred line commercial hybrids. These hybrids are especially adapted to marginal farming areas and underdeveloped countries.

Y. Efron.

III. REPORTS FROM COOPERATORS

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1. A proposition for a new classification of maize lines according to their cytoplasm and rf-factors.

Classification of this type is not known to us so far. On the basis of a study on the genotypes in 150 fertile lines, 70 sterile analogues and 33 fertility restorer analogue lines obtained on the basis of sterile T- or S-type of cytoplasm (T. Dankov, Dissertation, 1972) we propose the following classification, designation and short practical indication of all maize lines.

Table 1

Proposed designation of maize lines with various $\underline{rf}_1$, $\underline{rf}_2$ and $\underline{rf}_3$ genotypes.

Name of the group	Genotype	Designation of cytoplasm		
		N f st	T f st	S f st
Universal maintainer of Ts and Ss	$rf_1rf_1rf_2rf_2rf_3rf_3$	Num f	Tum st	Sum st
First semi-restorer of Ts and maintainer of Ss	$Rf_1Rf_1rf_2rf_2rf_3rf_3$	NR1 f	TR1 st	SR1 st
Second semi-restorer of Ts and maintainer of Ss	$rf_1rf_1Rf_2Rf_2rf_3rf_3$	NR2 f	TR2 st	SR2 st
Full maintainer of Ts and restorer of Ss	$rf_1rf_1rf_2rf_2Rf_3Rf_3$	NR3 f	TR3 st	SR3 f
Full restorer of Ts and maintainer of Ss	$Rf_1Rf_1Rf_2Rf_2rf_3rf_3$	NR1-2 f	TR1-2 f	SR1-2 st
First semi-restorer of Ts and restorer of Ss	$Rf_1Rf_1rf_2rf_2Rf_3Rf_3$	NR1-3 f	TR1-3 st	SR1-3 f
Second semi-restorer of Ts and restorer of Ss	$rf_1rf_1Rf_2Rf_2Rf_3Rf_3$	NR2-3 f	TR2-3 st	SR2-3 f
Universal restorer of Ts and Ss	$Rf_1Rf_1Rf_2Rf_2Rf_3Rf_3$	NR1-2-3 f =NUR	TR1-2-3 f =TUR	SR1-2-3 f =SUR

Eight principal genotypes according to rf-factors are the basis of the proposed classification. They may be on N-, T- and S-type of cytoplasm and they would show fertility or sterility depending on the relationships between the cytoplasm and the nucleus. The factors not designated in this short classification occur in recessive state.

This classification provides an indication of the type of cytoplasm and the state of rf-factors with every line and shows for what purpose it could be used in hybrid seed production on sterile basis.

Toma Dankov

2. Elimination of hidden isolated heterozygous states of Rf-factors in sterile lines and single cross hybrids with the genotype $Trf_1rf_1rf_2rf_2$.

During the maintenance of the sterile lines 171, 144g, 0266a and Wf9 with the genotype $Trf_1rf_1rf_2rf_2$, fertile plants without vigorous growth appear systematically. The latter are due to mutation of the recessive factors rf_1 and rf_2 to the dominant factors Rf_1 and Rf_2.

The occurrence of fertile plants could not be eliminated by the method of analyzing crosses between individual plants of the sterile and the fertile analogue. In these analyses, not only the ideal genotypes $Trf_1rf_1rf_2rf_2 \times Nrf_1rf_1rf_2rf_2$ are considered pure but also the genotypes having single dominant factors are considered likewise since sterility occurs in them too, namely, $Trf_1rf_1rf_2rf_2 \times NRf_1rf_1rf_2rf_2$, $Trf_1rf_1rf_2rf_2 \times Nrf_1rf_1Rf_2rf_2$, $TRf_1rf_1rf_2rf_2 \times Nrf_1rf_1rf_2rf_2$, $Trf_1rf_1Rf_2rf_2 \times Nrf_1rf_1rf_2rf_2$, $TRf_1rf_1rf_2rf_2 \times NRf_1rf_1rf_2rf_2$ and $Trf_1rf_1Rf_2rf_2 \times Nrf_1rf_1Rf_2rf_2$.

However, in the next stage of the maintenance of the line, crosses between the following genotypes are possible also: $TRf_1rf_1rf_2rf_2 \times Nrf_1rf_1Rf_2rf_2$ and $Trf_1rf_1Rf_2rf_2 \times NRf_1rf_1rf_2rf_2$, and they produce fertile plants (25%) with the genotype $TRf_1rf_1Rf_2rf_2$ in the sterile analogue during the next year.

We succeeded in eliminating this undesirable phenomenon by selection of pure genotypes through analyzing crosses using two well differentiated testers with the genotypes $Rf_1Rf_1rf_2rf_2$ and $rf_1rf_1Rf_2Rf_2$, namely:

1. for cleaning of the fertile analogue:

$TRf_1Rf_1rf_2rf_2 \times 1Nrf_1rf_1rf_2rf_2$ = 100% sterility

$Trf_1rf_1Rf_2Rf_2 \times 1Nrf_1rf_1rf_2rf_2$ = 100% sterility

2. for cleaning of the sterile analogue after it has been pollinated with pollen from the already cleaned fertile analogue:

$$1aT\underline{rf_1}\,\underline{rf_1}\,\underline{rf_2}\,\underline{rf_2} \times N\underline{Rf_1}\,\underline{Rf_1}\,\underline{rf_2}\,\underline{rf_2} = 100\%\ \text{sterility}$$

$$1bT\underline{rf_1}\,\underline{rf_1}\,\underline{rf_2}\,\underline{rf_2} \times N\underline{rf_1}\,\underline{rf_1}\,\underline{Rf_2}\,\underline{Rf_2} = 100\%\ \text{sterility}$$

This method of cleaning applies also to sterile single cross hybrids with the same genotype when they produce undesirable fertile plants.

Toma Dankov

3. A new method for determination of the degree of fertility in hybrids on sterile cytoplasm.

The methods used so far for determination of the degree of fertility determine it as percentage of fertile pollen against the total amount of pollen shed. The sterile stamens which were not extending outside the floweret were not taken into account. From a practical point of view it is more correct that fertility degree should be expressed as percentage of fertile pollen against the total amount of pollen which would be produced by plants with a normal cytoplasm. The only method meeting to a certain extent this requirement is the method of Galleev (CMS v selekcij i semenovodstve kukuruziy, Kiev, 1962).

The method utilized in our studies employs the following procedures: samples from 1000 flowerets are taken several days before or at the time of flowering of the tassels. The flowerets should be chosen from different plants and different parts of the tassels. Cross sections are made on 40 flowerets. The low part of the floweret held with a microscopic needle is observed under a stereo-microscope (25 times magnification). Data are taken on fertile, sterile and semi-sterile stamens. Assuming that the latter contain 50% of sterile pollen, the degree of fertility is determined by the percentage of fertile pollen in relation to the amount of pollen which would be produced by the tassels if all the stamens produce pollen normally.

Toma Dankov

4. Degree of fertility in single cross hybrids with sterile cytoplasm.

The determination of the degree of fertility in single cross hybrids with sterile cytoplasm is important for the establishment of the proportion between male and female components when these hybrids are used as pollinators.

Using the above reported method, we have found the following degrees of fertility in single cross hybrids with T- and S-type of cytoplasm differing in genotype and mode of development.

Table 1

Percentage of fertile pollen in single cross hybrids with T or S cytoplasm differing in genotype and mode of development.

No.	Genotype	Mode of development	No. of hybrids	Fertile pollen %	m %
1a.	$TRf_1rf_1Rf_2rf_2$	$TRf_1Rf_1rf_2rf_2$ x $Nrf_1rf_1Rf_2Rf_2$	14	58.9	4.95
1b.	- " -	$Trf_1rf_1Rf_2Rf_2$ x $NRf_1Rf_1rf_2rf_2$	5	74.5	6.06
1c.	- " -	$Trf_1rf_1rf_2rf_2$ x $NRf_1Rf_1Rf_2Rf_2$	8	91.8	2.12
2.	$TRf_1Rf_1Rf_2rf_2$	$TRf_1Rf_1rf_2rf_2$ x - " -	14	93.9	2.07
3.	$TRf_1rf_1Rf_2Rf_2$	$Trf_1rf_1Rf_2Rf_2$ x - " -	14	93.9	2.11
4.	SRf_3rf_3	Srf_3rf_3 x NRf_3Rf_3	24	63.1	2.45

The decreased degree of fertility in variants 1a and 1b in comparison to 1c is probably due to the weaker complementary action of the factors $\underline{Rf_1}$ and $\underline{Rf_2}$ when they originate from the two parents. The decreased degree of fertility in variant 4 is explained by the fact that the male gametes with recessive factors-$\underline{Srf_2}$ (Buchert, Genetics 44, 4, 1959) lose their vitality.

The fact that some single cross hybrids with sterile cytoplasm and restored fertility give significantly lower percentages of fertile pollen should be taken into account when they are used as pollinators.

Toma Dankov

5. Restoration of fertility in double cross hybrids on sterile basis.

A theoretical possibility for development of double cross hybrids with two sterile lines has been reported by Duvick (Proc. 14th hybrid Corn Industry Research Conf. 1959, 42-52) and Galleev (Vestnik sel'skohoz. nauk, 1959, No. 6). These hybrids have the advantage of eliminating the need of detasseling during the development of their parent single cross hybrids also. However, we do not know anyone having observed so far to what extent their fertility becomes restored.

Making use of the large diversity of sterile and fertile analogues, maintainer and restorer lines (Dankov, T., Dissertation, 1972) we have developed all possible hybrids according to genotype (Table 1.). Restoration of fertility in 4 to 58 combinations has been studied in each genotype, and 50 to 60 plants from each combination were examined once or twice.

There are 108 possible genotypes and 88 of them are expected to give a certain percentage of fertile plants. They are presented in Table 1. All hybrids can be classified into two types according to the type of cytoplasm of the first mother line, namely T-type and S-type. The hybrids of the T-type are divided into two groups according to the type of cytoplasm in the second mother line, namely T- and S-type, and into 6 sub-groups according to the expected percentages of fertile plants, namely 0, 25, 37.5, 50, 75 and 100%. The S-type hybrids are also divided into two groups according to the type of cytoplasm of the second mother line, namely T- and S-type, and into 3 sub-groups according to the expected percentages of fertile plants, namely 0, 50 and 100%.

In hybrids with two mother sterile lines of the S-type, 91% of the plants are fertile although 50% is to be expected. This is due to the inviability of the male gametes with recessive rf-factors, namely $\underline{Srf}_3$ (Buchert, Genetics, 44, 4, 1959). Besides this regularity, some other peculiarities and trends are observed which may be explained by the relationship between the cytoplasm and the nucleus. The environmental conditions have greater influence on the manifestation of fertility in double cross hybrids with two sterile lines than in single cross hybrids and F_2 generations on sterile basis. Taking into consideration these peculiarities,

Table 1

Restoration of fertility in double cross hybrids with two sterile mother lines (in % fertile plants).

♀ sterile single cross \ ♂ single cross		$TRf_1rf_1Rf_2rf_2rf_3rf_3$	" Rf_3rf_3	" Rf_3Rf_3	$TRf_1Rf_1Rf_2rf_2rf_3rf_3$	" Rf_3rf_3	" Rf_3Rf_3	$TRf_1rf_1Rf_2Rf_2rf_3rf_3$	" Rf_3rf_3	" Rf_3Rf_3	$Srf_1rf_1rf_2Rf_3rf_3$	" $SRf_1rf_1rf_2rf_2$	" $Srf_1rf_1Rf_2rf_2$	" $SRf_1Rf_1rf_2rf_2$	" $Srf_1rf_1Rf_2Rf_2$	" $SRf_1rf_1Rf_2rf_2$	" $SRf_1Rf_1Rf_2rf_2$	" $SRf_1rf_1Rf_2Rf_2$	" $SRf_1Rf_1Rf_2Rf_2$
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1. $Trf_1rf_1rf_2rf_2$	V.	10	13	9	10	9	11	10	9	10	-	-	-	-	-	10	12	12	12
	Ex.%	25.0	25.0	25.0	50.0	50.0	50.0	50.0	50.0	50.0	0	0	0	0	0	25.0	50.0	50.0	100
	Ob.%	24.7	41.6	37.4	56.1	60.1	53.1	49.3	55.3	57.0	-	-	-	-	-	31.0	53.4	54.4	96.9
2. $TRf_1rf_1rf_2rf_2$	V.	14	16	9	8	12	10	11	12	8	-	-	12	-	14	10	11	12	9
	Ex.%	37.5	37.5	37.5	50.0	50.0	50.0	75.0	75.0	75.0	0	0	25.0	0	50.0	37.5	50.0	75.0	100
	Ob.%	42.7	51.5	56.2	52.4	63.7	63.1	55.4	58.2	58.4	-	-	18.2	-	36.9	50.6	53.9	57.3	78.1
3. $Trf_1rf_1Rf_2rf_2$	V.	15	19	7	10	18	11	13	8	10	-	9	-	6	-	14	15	14	13
	Ex.%	37.5	37.5	37.5	75.0	75.0	75.0	100	100	100	0	25.0	0	50.0	0	37.5	75.0	50.0	100
	Ob.%	39.4	41.1	48.6	61.2	65.4	61.2	51.8	52.3	58.3	-	37.3	-	52.9	-	40.1	64.4	50.5	89.7
4. $Trf_1rf_1Rf_2Rf_2$	V.	8	6	7	4	5	9	7	5	7	-	-	9	-	10	8	5	9	10
	Ex.%	50.0	50.0	50.0	50.0	50.0	50.0	100	100	100	0	0	50.0	0	100	50.0	50.0	100	100
	Ob.%	60.8	50.3	51.8	51.3	60.2	54.3	57.6	53.5	71.3	-	-	36.0	-	58.5	50.2	50.5	58.0	81.6
5. $TRf_1Rf_1rf_2rf_2$	V.	12	22	8	10	13	10	14	13	11	-	10	-	8	-	11	13	17	14
	Ex.%	50.0	50.0	50.0	100	100	100	50.0	50.0	50.0	0	50.0	0	100	0	50.0	100	50.0	100
	Ob.%	53.7	52.6	54.5	61.3	66.4	63.4	54.4	58.9	59.0	-	30.3	-	72.3	-	51.5	66.5	54.2	82.5
6. Srf_3rf_3	V.	-	58	26	-	28	22	-	31	20	19	15	30	17	26	30	25	41	29
	Ex.%	0	50.0	100	0	50.0	100	0	50.0	100	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
	Ob.%	-	58.2	94.5	-	57.0	86.4	-	54.9	87.1	93.6	93.4	89.9	94.0	84.5	90.8	91.6	88.0	92.6

restoration of fertility in double cross hybrids on sterile basis with two sterile lines may be predicted with a relative accuracy.

Toma Dankov

6. An attempt for induction of mutation of normal cytoplasm into sterile cytoplasm by treatment with streptomycin.

According to Sager (Scient. Amer. 1965, 212, 1), Petrov et al. (Refer. jurnal, Rasteniev. 1969, 10, 55, 10), Yehuda and Dlana (Planta 1970, 91, 195) streptomycin appears to be a specific mutagen for cytoplasmic factors. The mutation of normal cytoplasm into a sterile one is of importance to the creation of new sources of sterile cytoplasm and for shortening of the period of development of sterile analogues.

We repeated the experiment of Petrov. 100 germinating seeds were taken from each line containing the genotypes Wf9-N$\underline{rf_1rf_1}\,\underline{rf_2rf_2}\,\underline{rf_3rf_3}$, VIR-75-N$\underline{rf_1rf_1}\,\underline{rf_2rf_2}\,\underline{Rf_3Rf_3}$, A-344-N$\underline{Rf_1Rf_1}\,\underline{Rf_2Rf_2}\,\underline{rf_3rf_3}$ and 0570a-N$\underline{Rf_1Rf_1}\,\underline{Rf_2Rf_2}\,\underline{Rf_3Rf_3}$. The following concentrations of streptomycin in distilled water were used: 0.001 γ/ml, 0.01 γ/ml, 1 γ/ml, 10 γ/ml., 100 γ/ml, 1 mg/ml and 10 mg/ml. The germinating seeds were soaked in this solution for 24 hours at temperature 22-24° C. Dry and soaked seeds served as controls in each experiment. Two progenies were observed after the treatment.

Male sterility was not found in any of the variants.

Toma Dankov
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1. Physiological investigations of the stature mutant nana-1.

Coleoptile elongation, seedling elongation, isoenzymes of peroxidase and peroxidase activity, respiration of coleoptiles and mesocotyls, protein synthesis, and changes in ribonucleic acid in seeds and seedlings of the stature mutant nana-1 were investigated.

Seedlings were treated with indoleacetic acid in various concentrations. Measurement showed a significant increase in growth by the treated

plants when compared to untreated control groups. Coleoptile sections were treated with IAA and showed a significant increase in growth over intact controls.

Coleoptile sections were treated with various concentrations of tryptophan and tryptamine. Those treated with tryptophan exhibited no significant difference between treated and untreated groups. The length of coleoptiles treated with tryptamine increased a significant amount over the intact control groups.

Peroxidase isoenzymes were studied by means of electrophoretic and colorimetric techniques. Mesocotyls of dwarf and normal seedlings were examined for differences in electrophoretic banding patterns of peroxidases. No differences were determined by this method. There were also no differences in the peroxidase activity between the dwarf and its normal sib.

The respiratory activity of the dwarf and normal coleoptiles appeared to be both aerobic and anaerobic. Aerobic activity of nana-1 and normal was equal. Anaerobic activity of nana-1 and normal was also equal. The respiratory activity of the dwarf and normal mesocotyls was different with the normal exhibiting greater respiratory activity than the dwarf.

The uptake of ^{3}H-leucine and its utilization in protein synthesis was different in the dwarf and normal mesocotyls. Both dwarf and normal mesocotyls took up ^{3}H-leucine at approximately the same rate, but pooling effect was noted in that the dwarf was not as efficient as the normal in extracting leucine from the amino acid pool and utilizing it in protein synthesis. A similar effect, though not as severe, was found in the coleoptiles.

Whole seedlings were treated with IAA and the total RNA was determined. Over the five day treatment period, a general increase was exhibited until the fifth day when a decrease was noted. Treatment with IAA generally increased the amount of RNA synthesis in the dwarf and decreased RNA synthesis in the normal.

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1. Mutation studies at the $\underline{Sh_1}$ locus in maize.

The $\underline{Sh_1}$ locus is particularly suited to a study of the nature of induced mutations. It has an easily recognizable phenotype, closely linked flanking markers, and a protein product which is easily analyzable by electrophoretic and immunochemical criteria. Furthermore, positive evidence of interallelic complementation among previously analyzed $\underline{sh_1}$ mutants suggests that newly induced mutants can also be put to the functional test.

A project to induce $\underline{sh_1}$ mutants by gamma irradiation and to investigate them with regard to the above characteristics has been initiated. Kernels and plants of the $\underline{C}\ \underline{Sh_1}$ genotype ($\underline{A}\ \underline{C}\ \underline{R}$ stock) were irradiated in two different experimental lots. Acute doses of 10 and 15 kR were given to the first and chronic doses of 0.8, 1.1 and 2.5 kR were applied to the second lot. M_1 plants were used mainly as female parents in crosses with pollen from a $\underline{c}\ \underline{sh_1}$ tester stock. Ten ears showing kernels of colored $\underline{sh_1}$ type in a total population of 1145 ears were obtained. Electrophoretic analysis with nine of these mutants shows that neither the $\underline{Sh_1}$ protein nor any other new protein band is visible in their endosperm extracts. The single mutant kernel born on the tenth ear gave rise to a plant which did not produce any seeds on selfing and the mutant is consequently lost. Electrophoretic results such as these are most likely to be caused by the loss of the $\underline{Sh_1}$ locus. Efforts to further characterize these mutants and to obtain a larger number of $\underline{sh_1}$ mutants by gamma irradiation are in progress.

P. S. Chourey

2. Unusual property of the $\underline{C_1}$ locus.

A derivative of $\underline{I}^{Trombay}$ (Inhibitor of aleurone color) was apparently not transmitted through the male gametes (Chandra Mouli et al., Can. J. Genet. Cytol. 12:259-263, 1970). During the course of further analysis of this line, it was observed that even through the female its

Table 1

Types of kernels obtained in reciprocal crosses of colorless individuals (I Sh Bz wx x C sh bz wx) and C sh bz wx plants.

Cross	Kernel types						
	IShBzwx	Cshbzwx	Ishbzwx	CShBzwx		CshBzwx	CShbzwx
				Light	Dark		
♀ IShBzwx x Cshbzwx	51	158	19	5	86	4	0
	55	116	13	4	101	1	0
♂ Cshbzwx	10	66	10	8	50	3	1
	47	121	11	34	42	2	0
	49	187	12	33	111	2	0
	28	138	15	23	75	4	0
	3	102	5	17	110	0	0
	18	99	9	9	72	1	0
	32	112	15	18	85	0	0
	23	124	16	18	79	4	1
Total				169	811		
	316	1223	125	980		23	2
Exact Reciprocals		76			60	1	2
♀ Cshbzwx x		96			89	2	0
		89			89	2	0
♂ IShBzwx Cshbzwx		74			72	3	0
		42			53	0	0
		75			75	0	3
		80			86	0	0
		73			79	2	2
		71			76	2	1
		143			153	2	2
Total		822			832	14	10

Table 2

Types of kernels obtained in reciprocal crosses of colorless individuals (I Sh Bz wx x C sh bz wx) and c sh Bz wx plants.

Cross	Kernel type			
	IShBzwx*	CshBzwx	Ishbzwx	CShBzwx
♀ IShBzwx / Cshbzwx x cshBzwx ♂	94	96	5	6
	124	123	8	10
	110	106	6	12
	134	140	14	9
	78	65	6	3
	44	44	2	3
	98	94	9	12
	124	131	8	8
	113	119	9	15
	91	96	5	5
Total	1010	1014	72	83
Exact Reciprocal	138	148	4	4
♀ cshBzwx x IShBzwx / Cshbzwx ♂	84	74	2	4
	80	74	2	5
	80	72	5	5
	108	116	6	12
	57	55	1	3
	84	89	4	2
	112	104	5	4
	89	98	2	2
	71	62	4	2
Total	903	892	35	43

*Colorless.

transmission is apparently subnormal. However, the closely linked markers Sh and Bz segregate as expected (see Table given below), suggesting an alternative possibility that I is not expressed.

Cob no.	Genotype	Kernel type				
		I Sh Bz	C sh bz	I sh bz	C Sh Bz	C sh Bz
B70-40-4 1-40	I Sh Bz x C sh bz / C sh bz wx	60	164	22	100	7

Colorless kernels were reciprocally crossed with C sh bz wx and C sh Bz wx stocks. Tables 1 and 2 show the types of kernels obtained in the crosses.

The findings are:

1. In crosses involving the C tester (Table 1) I was expressed in 3.7% to 28.6% of the kernels in the observed cobs and the average frequency through the female parent was only 16.5%.
2. Through the pollen, I was not expressed in more than 80 cobs studied.
3. Both the linked markers, Sh_1 and Bz, had equal and normal transmission indicating that only I is affected.
4. There is a normal proportion and segregation of I (colorless), Sh and Bz in reciprocal crosses involving a c tester (Table 2). But, colorless individuals as pollen parents on C and c gave colored and colorless kernels, suggesting lack of transmission of I, complete inactivation, or mutation of I to i in the male and a partial change in the female parent.
5. Therefore, there is a difference in the mutational event of I in both male and female parents.
6. There is a high proportion of I sh bz wx kernels (Table 1). Further studies are in progress to clarify this type of behavior.

S. E. Pawar
Chandra Mouli

3. EMS induced dominant mutation.

A dominant mutation was observed in a progeny when A C R (Brink stock) plants arising from seeds treated with ethylmethane sulphonate (EMS) were crossed with a c_1 sh Bz wx stock. A large mutant sector of 45 kernels was obtained on a cob containing 245 seeds. The phenotypic expression of the mutation is dominant over the wild plant type. The expression starts 7 days after sowing in both homozygous and heterozygous mutant plants in the progenies. The main character noted is that a curling of all the leaves takes place from both sides of the leaf margin lengthwise and the leaf appears as if it is rolled. Curling of the leaves in turn causes entangling at the leaf tips during the growth period. The leaf width is reduced to more than half the size of the normal leaf type. The leaf surface appears completely inverted, as the hair and rough surface were observed on the lower side of the leaf as against the upper side of the normal wild type. The hairs on the lower surface of the mutant leaf are more prominent. It has been observed that, due to curling and entangling of the leaves, the position of the leaf sheath is affected. The stress created on the leaf sheath produces abnormal apparent phylotaxy of the leaves on the stem. Four types of variations were noted (1) leaves are one sided, (2) leaves perpendicular to each other, (3) irregular, and (4) normal distichous. These appear in different frequencies and may be of secondary origin. The expression of the characters varies according to the nature of the wild type plants used in the crosses.

Homozygous (possible) plants have stunted growth and the size is reduced by half in comparison with heterozygous plants, in which some of the plants were stunted. Highly stunted plants did not produce silk but occasionally a few plants produced pollen. The mutant plants grow up to the sexual period. Selfing, sibbing and crosses to wild type plants revealed that the trait is maintained in a heterozygous condition only. So far, it has not been possible to obtain plants carrying the trait in a homozygous condition.

Table 3 shows the types of plants in progenies of selfed or sibbed mutant plants and in progenies from crosses to a few tester stocks. In the inbred progenies there is a significant deviation from 3:1 and 2:1 ratios. Progenies No. 1 and 4 clearly show a monogenic ratio. Crosses

Table 3

Types of plants in selfed and sibbed progenies of curled entangled (Ce/N) plants and in progenies from crosses to marker stocks (♀)

Progeny	Seeds sown	Plant type			Marker stock as female	Plant type	
		Normal	Mutant			Normal	Mutant
			Stunted	Not stunted			
1) A70-34f-40	19	4	8	6	cShWx	-	-
2) B71-Ce-3/7	40	4	4	3	"	96	80
3) B71-Ce-4/5	50	9	8	11	"	60	55
4) B71-Ce-6/5	50	10	12	15	"	115	135
5) B71-Ce-7 x	18	1	3	4	"	75	81
6) B71-140C-3/4	152	35	48	30	su	62	72
7) B71-140C-5/6	47	11	10	16	"	93	103
8) B71-140C-7/8	81	14	16	22	sh_2	57	56
9) B71-31e-10/2	225	51	62	63	"	88	92
Total	682	139	171	170		646	674
			341				

Table 4

Observed types of plants in reciprocal cross of curled entangled plants with multiple markers (MM) $\underline{bm_2}$, $\underline{lg_1}$, $\underline{a_1}$, $\underline{su_1}$, $\underline{pr}$, $\underline{y_1}$, $\underline{gl_1}$, $\underline{j_1}$, $\underline{wx}$, $\underline{g_1}$.

Pedigree No.	Seeds sown	Plant type		Multiple marker stock as ♀	
				Plant type	
		Normal	Mutant	Normal	Mutant
1) B71-Ce-9/MM	65	28	26	44	48
2) B71-31f-4/MM	78	39	32	91	74
				48	54
3) B71-31C-4/MM	60	27	31	99	86
				25	25
4) B71-31C-8/MM	68	30	28	78	81
				69	74
5) B71-31f-3/MM	68	32	28	68	81
				24	18
				49	34
6) B71-31f-6/MM	112	57	48	51	37
				68	68
				88	61
7) B71-6C-2/MM	142	62	75	119	104
8) B71-11a-4/MM	69	24	32	116	129
9) B71-11b-2/MM	81	28	31	65	75
10) B71-11J-8/MM	104	44	49	86	84
Total	847	371	380	1188	1133

to tester stocks as female had equal proportion and segregation of mutant and normal plants.

Table 4 shows the exact reciprocal crosses between a multiple marker stock and the mutant plant. Mutant plants as either male or female parents showed equal proportions of mutant and wild type plants. Expression of the mutant phenotype in the F_1 generation indicates dominance and the equal proportions and segregation of both types in the reciprocal crosses suggests a monogenic behavior. The mutant gene responsible has been designated curled entangled (Ce).

S. E. Pawar
Chandra Mouli

4. Silk and pollen treatment.

Paraffin oil is found to be an extremely useful medium in the treatment of corn pollen with chemical mutagens (EMS) (Neuffer MNL 42-124). In view of this, pollen treatment was compared with a new method of treatment, i.e., silk treatment. A 0.1% EMS emulsion was made in paraffin oil. Pollen carrying the dominant markers R^r, A_1, Su_1 and Sh_1 was thoroughly mixed with paraffin oil containing EMS and immediately smeared on the silks of recessive stocks. In another set of experiments, the silks of recessive marker stocks were smeared with the above emulsion and then pollinated with the dominant marker stock. The frequencies of whole and partial losses of R^r, A_1, Sh_1, and Su_1 in the silk treatment were found to be 2, 1, 0.8 and 0.5 percent, whereas in the pollen treatment the frequencies were 2.1, 1.2, 0.8 and 0.6%, respectively. The frequency of marker losses seems to be almost the same in both treatments. Treatment of silks permits easy pollinations and favors good seed set as compared to the pollen treatment where some pollen is killed.

S. E. Pawar
Chandra Mouli

5. Genetic behavior of induced floury and opaque mutations.

Allelic tests between the standard fl_2 type and two newly obtained floury mutations showed that the new floury mutations are allelic to fl_2. The opaque type, when crossed with standard o_2, did not show allelic

behavior. The opaque type and the wrinkled mutants have also been crossed with a multiple marker stock to study the location of the new mutants.

S. E. Pawar
Chandra Mouli

BLANDY EXPERIMENTAL FARM
University of Virginia
Boyce, Virginia

1. Blandy Experimental Farm of the University of Virginia reactivated.

The Blandy Farm again became an active research institution with the appointment of a new director on 1 July, 1972. He is Thomas Ewert, who came to Virginia from the Longwood Gardens near Philadelphia. His research will involve plants in the Orland E. White Arboretum, the most extensive in Virginia.

2. Pollen irradiation studies begun.

In 1972, pollen of an inbred strain of maize, B14, was irradiated with 1300 r of gamma rays from a large Co^{60} source (70,000 curies) stored in the pool of the reactor at the University of Virginia. The irradiated pollen was placed on silks of the B14 inbreds growing at Blandy. In 1973, a large number of R_1 plants will be self-pollinated. The resulting seeds will be grown, ear to row, in greenhouses at Blandy and at Charlottesville and seedlings examined for mutations. In seed irradiation experiments conducted previously, it was found that 3 to 4 percent of the populations tested were segregating for some seedling mutation.[i]

[i] Singleton, W. R. 1969. Induced Mutations in Plants, 479-483. International Atomic Energy Agency, Vienna, 748 pp.

W. Ralph Singleton

BOSTON COLLEGE
Chestnut Hill, Massachusetts
Department of Biology

1. Maize and teosinte relationship.

Reeves and Mangelsdorf (Reeves and Mangelsdorf, 1942) first proposed that maize and teosinte should be congeneric. During the last decade, I have studied the relationship between these two species and have found additional evidence to support their proposal (Ting, 1964; Ting, 1967). However, the controversy on the relationship between these species has recently arisen again. It seems to me that this is completely unwarranted.

For the past two years, I have employed new techniques, such as electron microscopy and DNA-binding fluorochrome staining, to investigate further the relationship between these species at a subchromosomal level. The data obtained through these studies also agree with the previous conclusion that maize and teosinte should be congeneric. I think that this should be accepted.

Y. C. Ting

2. Additional studies on the synaptonemal complex of haploid maize.

During the last year, studies on the synaptonemal complex of haploid maize have continued. It was observed that the lateral elements of the complex are undoubtedly bipartite in structure. Based on four randomly chosen samples, the average width of the central element was 499 Å, while that of the lateral elements was 524 Å. Flanking both sides of the central element, the space between the central and lateral elements was 419 Å crosswise (Table 1). Hence, the average width of a single complex is about 2385 Å, which is close to that of single complex in diploid maize.

In certain thin sections, it was shown that the component fibrils of the central elements were clearly two in number. However, central elements with three longitudinal components, as reported in Gryllus, were never found, nor were the ladder-like central elements as in Philaenus. Unfortunately, great difficulties were encountered in trying to definitely identify transverse fibers between the central and the lateral elements

Table 1

Diameters (width) of the components of the synaptonemal complex in different haploid maize plants (in Å).

Components	Plant No.				$\bar{X}$
	1	2	3	4	
Lateral element	519	513	577	487	524
Central element	519	513	577	390	499
Distance between lateral element and central element	346	385	461	487	419

such as those found in mice and quails. As the division advanced to early diplotene stage, the central element appeared first to undergo disintegration. Short fibrils in the center of the complex became evident. These fibrils were, however, only lightly stained in contrast to the chromatin regions next to the lateral elements.

Differing from the behavior of the synaptonemal complex of certain animal meiocytes, the complex of haploid maize was never observed to be attached terminally to the nuclear envelope. It was difficult to ascertain the relationship between the nuclear envelope and the chromatin fibers around the complex.

At diakinesis, even though the axial elements of some chromosomes remained clear, those of most chromosomes disappeared. In the areas presumed to be chromosomal, only the densely stained chromatin was present. By acetocarmine squash technique, it was found that in the same stage a majority of the cells had 10 univalents. Approximately 26 percent of the cells had from one to two bivalents. They were frequently formed by end-to-end associations. It is likely that they were brought about by stickiness of heterochromatin rather than by previous exchange of chromatids. In other words, no convincing chiasmata were observed. Table 2 shows the percent of cells having 10 univalents as well as those having eight, seven and six univalents in a total of 674 randomly selected cells. In addition, bridges and fragments were rarely seen at both anaphases I and II. Hence,

it is reasonable to conclude that no cytological evidence of crossing over was obtained.

Table 2

Chromosome associations at diakinesis in five haploid maize plants.

Type of associations	Number of cells	Percent of total
10 I*	494	73.3
8 I, 1 II	160	23.7
7 I, 1 III	2	0.3
6 I, 2 II	18	2.6
Total	674	

*I, II, and III designate univalent, bivalent and trivalent, respectively.

Furthermore, ears born on F_1 progeny from the cross between various haploids and an inbred diploid were also examined at harvest to determine whether ovule abortion was sufficiently high to indicate the presence of inversions or reciprocal translocations. Ovule abortion of all ears examined was no greater than five percent. Therefore, it may be concluded that no inversions or translocations were present in the F_1 plants employed. This again indicates that no crossing over occurred in the parental haploids.

These data support my previous conclusion that the synaptonemal complex does not lead to crossing over.

Y. C. Ting

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1. Methods for electron microscopic study of maize pachytene chromosomes.

In the course of making serial electron micrographs of maize pachytene nuclei, a number of different techniques were tried in an effort to obtain maximum contrast and ease of reconstruction of the synaptonemal complexes which hold together the pachytene bivalents. These various fixation and staining techniques may be useful to workers who contemplate ultrastructural studies of various features of the meiotic prophase nucleus.

Fixation of Anthers.

A. Aldehydes (1) 4% formaldehyde in 0.1 M cacodylate buffer + 4% sucrose; (2) 6% glutaraldehyde in 0.1 M cacodylate buffer + 4% sucrose.

Fix for ½ to 1 hour in (1), then add an equal volume of (2) to give a concentration of 2% formaldehyde + 3% glutaraldehyde. Fix a further 2 to 2½ hours. Total fixation time - 2½ to 3 hours.

0.067 M phosphate buffer has also been used with success. Sucrose may be omitted. It is important to keep the anthers beneath the surface of the fixative as they have a tendency to float, and uneven fixation results. Holding them down with a piece of tissue (Kimwipes) or fine cloth, or shaking in a rotary shaker usually improves the uniformity of fixation within and between anthers. Physical damage to the anthers should be avoided, although cutting anthers in half with a fine clean cut (e.g. a new, degreased razor blade) improves fixation with only marginal damage. Anthers are usually dissected out in buffer or the first fixative.

B. Wash 3 times ½ hour in buffer + sucrose (or buffer only).

C. OsO_4 - 2% in buffer. Fix for 1½ hours.

D. Wash 3 times ½ hour in distilled water.

E. Stain for 3 hours at 60° C in 2% aqueous uranyl acetate (Locke et al., 1971).

F. Wash thoroughly 3 times 1 hour in distilled water.

Dehydration and Embedding.

Alcoholic dehydration has been used. Infiltration and embedding with Spurr's (1969) low viscosity resin can be carried out directly from the dry absolute alcohol stage. Luft's (1961) Epon or Araldite mixtures may also be used after propylene oxide. Flat embedding of anthers allows orientation so that sectioning is carried out perpendicular to the longitudinal axis of the anther and all four locules can be examined.

Staining.

After sectioning and picking up the sections on grids, only lead staining is necessary, such as the method of Reynolds (1963).

Two techniques which give various degrees of preferential staining of the synaptonemal complex have been tried.

(1) Bernhard's (1969) EDTA Technique destains selectively the DNA but not RNA and results in the synaptonemal complex being stained but the surrounding chromatin remaining unstained. Osmium fixation is omitted; therefore omit steps B through E in the above schedule. After picking up the sections, the grids are stained in saturated uranyl acetate for 20 minutes, floated on 0.2 M EDTA for 1 to 2 hours, washed and stained with lead citrate as above. The length of time in uranyl acetate and EDTA has to be varied according to the thickness of the sections. This method gives good contrast of the lateral elements of the synaptonemal complex. Chromatin in centromeres and the nucleolus organizer also appears to stain. Care is necessary to avoid contamination of the sections by precipitation during the long EDTA step. Step E above may be included but staining with uranyl acetate after sectioning is usually still necessary.

(2) Positive Phosphotungstic Acid (PTA) - Sheridan and Barrnett (1969). This technique results in staining of basic protein residues in the absence of osmium staining. Hence steps B through E are again omitted in the fixation. After dehydration, the anthers are stained overnight in alcoholic 1% PTA at ice temperature (or in refrigerator). The time of staining appears to have some effect on the specificity as 15 hours resulted in the lateral elements of the synaptonemal complex staining much more intensely than the chromatin, whereas 19 hours found the chromatin almost as electron dense as the lateral elements. The nucleolus organizer and the centromeric chromatin are also differentially stained.

After staining, the anthers are washed several times in absolute alcohol, infiltrated, embedded and sectioned as usual. No further post-sectioning stain is required.

Both the EDTA and the PTA methods omit OsO_4 fixation and hence the preservation of membranes is not always perfect. Both are useful for allowing the tracing of synaptonemal complexes through serial sections without the hindrance of obscuring chromatin, yet they allow chromatic knobs and in particular the centromeres and nucleolus organizer to be identified. Using the PTA method, I have been able to reconstruct and identify the synaptonemal complex of entire pachytene bivalents from maize microsporocytes, including inversion heterozygote bivalents. The reconstruction technique is essentially the same as I had previously used for Neurospora crassa (Gillies, 1972).

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C. B. Gillies

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1. Characterization of DNA from maize lines of different heterochromatic constitution.

Maize is a suitable organism with which to investigate the nature and functional significance of heterochromatin, since in this organism well characterized genetic stocks possessing different heterochromatic constitutions are available. We have used the following lines:

A. Lines with large heterochromatic knobs or chromosomal segments

K_{10} - a large heterochromatic segment on chromosome 10

K_9 - " " " knob " " 9

B chromosome line - possesses 2-4 B chromosomes/plant

F_1 - bulk seed of the hybrid C121E x C103A

B. Lines without large heterochromatic knobs

Wilbur's Flint knobless

DNA was isolated using a modified version of the Marmur technique described by Rinehart (M.N.L. 40: 1966). Etiolated epicotyls from five day seedlings were ground in liquid nitrogen and then made into a slurry with an equal weight of 0.15 M NaCl - 0.1 M EDTA pH 8.0 buffer. Sodium lauryl sulfate was added to a final concentration of 2% and the mixture was heated at 60° C for 10 minutes. Pronase (2 mg/ml final) was added followed by incubation at 45° C for 3 hours. An equal volume of buffer saturated phenol was added and after shaking for 20 minutes the emulsion was centrifuged to separate the phases. The aqueous supernatant was removed and the interphase was re-extracted with a ½ volume of buffer, recentrifuged to produce a second aqueous supernatant which was pooled with the first. The DNA was wound out after layering with two volumes of cold ethanol, washed sequentially in 70%, 80% and 95% ethanol and finally taken up in 1/10 SSC (SSC is 0.15 M NaCl + 0.015 M sodium citrate). After adjusting the ionic strength to SSC, pancreatic RNase (200 ug/ml) and T_1 RNase (20 units/ml) were added and incubated at 37° for 45 minutes. Self digested Pronase (100 ug/ml final) was added and the solution was incubated at 37° C overnight. The solution was deproteinized by shaking for 15 minutes with an equal volume of chloroform:isoamyl alcohol (24:1). The interphase was

Fig. 1 CsCl Gradients

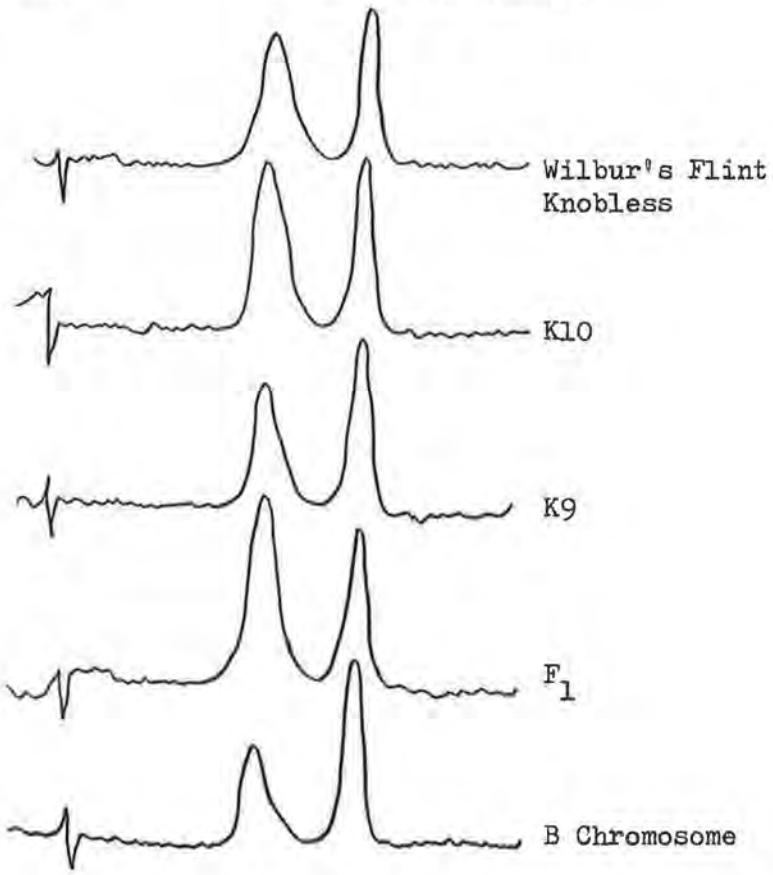
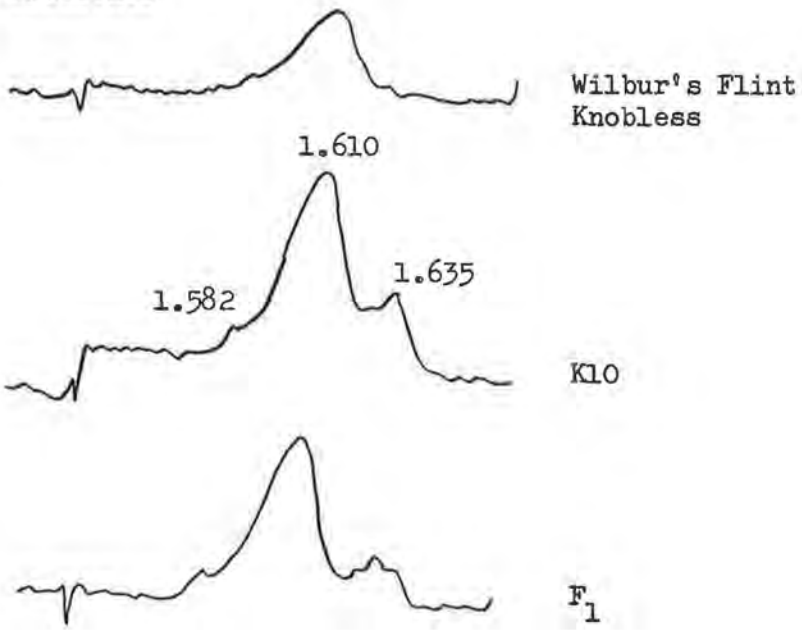


Fig. 2 Act.D-CsCl Gradients



re-extracted with a ½ volume of buffer (SSC) and aqueous supernatants were pooled. This deproteinization was repeated until no protein was seen at the interface. 3M Na Acetate (pH 6.0) was added (1/10 volume). The DNA was wound out after layering with two volumes of cold ethanol, again washed in 70%, 80%, and 95% ethanol and finally taken up in 0.01 M tris-HCl, 0.001 M EDTA pH 8.0.

Buoyant Density in CsCl

Buoyant densities were determined in a Beckman Model E ultracentrifuge. Within the limits of our measurements all 5 lines have an identical buoyant density of 1.700 (Fig. 1). In all lines there is a heavy shoulder, which is perhaps more pronounced in the B chromosome line. Rinehart (MNL 40: 1966) also found identical densities of the DNA from lines with and without B chromosomes, but measured a slightly heavier density (1.7015) than we have observed here. This difference, if significant, could be due to such factors as slightly different techniques, different marker DNA's or to different maize lines.

Fractionation using Actinomycin D

Act D is known to bind to guanine bases in DNA thus causing a reduction in the buoyant density. We would like to report here the preliminary results of analytical CsCl centrifugation of DNA in the presence of Act D at a molarity approximately equivalent to the molar phosphate of the DNA. Striking differences have been repeatedly observed between the DNA's of the three lines thus far tested (Fig. 2). All three DNA's appear to contain both light and heavy satellites (with respect to the main band) but differ widely in the relative content, particularly of the heavy satellites (those sequences binding less Act D). At present we are purifying these various satellite fractions by preparative centrifugation and then hope to be able to quantitate the relative amounts present in different lines by filter hybridization.

Tony Pryor

DEKALB AGRESEARCH, INC.
Thomasboro, Illinois

1. Genetic resistance to race T Helminthosporium maydis (Nisk and Miyaki).

Following the severe southern corn leaf blight epiphytotic in 1970, we began a program of screening for genetic resistance to race T within a number of heterogeneous composite populations, ostensibly in T cytoplasm and carrying the Rf_1 and Rf_2 restorer genes. These populations had been assembled around 1960, principally by Basil Tsotsis, originally for the purpose of the extraction of male-sterile restoring lines and had been maintained by open-pollination in isolation while being subjected to mass selection for resistance to race O of H. maydis, among other diseases.

The composites were planted in our 1970-71 Homestead, Fla., winter nursery and inoculated with ground leaf tissue obtained from severely diseased fields (all ensuing nurseries were similarily inoculated). Seed from ears of about 500 relatively resistant plants was bulked and planted in the Thomasboro, Ill., nursery in the summer of 1971. Resistant plants were again selfed and selected ears were shelled individually, and the S_2 progeny were planted, ear-to-row, in the fall of 1971 at Homestead. Resistant lines were again selfed and also crossed by rf_1rf_1 rf_2rf_2 normal male plants. Bulked S_3 and BC_0 progeny of selected S_2 lines were planted in a second winter nursery at Homestead in early 1972; resistant lines were again selfed and the BC_0 crosses were again crossed by normal males, and the resulting S_4 line and BC_1 progeny were planted in the 1972 Thomasboro summer nursery.

Most of the BC_1 lines were completely fertile, indicating that the corresponding S_4 lines were probably in normal cytoplasm. However, nine out of the total 146 BC_1 lines did segregate for sterility, confirming that the corresponding S_4 lines were actually in T cytoplasm (Table 1). In general, the disease reaction of the backcrosses was more severe than that of the S_4 lines which indicates that the resistance is genetic and not cytoplasmic in nature. Disease reaction varied among the S_4 lines; however, lines with scores of 2.0 or less appeared to have a very high degree of resistance. In these lines, both the number and size of lesions was drastically curtailed. The line x backcross interaction evident in the

Table 1

Disease reaction scored on a scale of increasing severity from 1.0 to 9.0 of several S_4 lines and their corresponding backcrosses to normal males. Sterility-fertility reaction of the backcrosses also included.

Pedigree	Disease reaction	No. of plants		
		Fertile	Partial	Sterile
800LMTR-4-S4 800LMTR-4-S2 x n^2	2.0 5.0	11	2	8
66AMSC-10-S4 66AMSC-10-S2 x n^2	1.5 7.0	10	1	10
70AC-10-S4 70AC-10-S2 x n^2	4.5 5.0	9	2	7
400FTR-1-S4 400FTR-1-S2 x n^2	3.0 7.5	8	0	15
400FTR-5-S4 400FTR-5-S2 x n^2	4.0 8.0	9	3	7
800FTR-7-S4 800FTR-7-S2 x n^2	3.0 5.0	16	2	2
800LFTR-5-S4 800LFTR-5-S2 x n^2	1.5 5.0	12	0	8
800LFTR-6-S4 800LFTR-6-S2 x n^2	5.0 5.0	12	0	5
1000FTR-7-S4 1000FTR-7-S2 x n^2	2.0 5.0	14	0	5

data may have been caused from the use of normal males which varied in maturity and possibly in genetic resistance to race T. Our preliminary observation is that the inheritance of resistance is quantitative and mostly additive, though we plan to use this material in further experiments designed to provide more precise genetic information.

G. R. Johnson

ESTACION EXPERIMENTAL REGIONAL AGROPECUARIA PERGAMINO - INTA
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1. "Flower-pot technique" as a new method for mutation induction on maize pollen.*

Use of induced mutations in corn breeding has been rather limited mainly because of:

a) enormous genetic variability already present in this allogamous plant and

b) scarcity of efficient mutagenic treatments giving clear-cut results.

Ethyl methane sulfonate (EMS), a powerful and very efficient mutagenic agent on cultivated plants, has been usually employed in many species by soaking seeds in an aqueous solution and then applying a genetic analysis to the M_2 or M_3 generations, originating from M_1 plants produced by treated seeds, in order to pick up eventual mutants.

We tested a new method which we named "flower-pot technique". Immature male inflorescences from an inbred line (flint type) were kept at room temperature in the laboratory. Peduncles were put into Erlenmeyer flasks and immersed either in bidistilled water ("control") or in freshly prepared EMS solution (0.2 per cent in volume) ("treated"). About three days later, and after anthesis, pollen was collected.

At the beginning of treatment, the pollen stage varied from interphase between first and second mitosis in tassel branches to nearly mature pollen grains in the central axis and at the end of treatment after thirty-six hours, from late second mitosis to mature pollen grains, respectively.

Crosses were made with the two types of pollen ("control" and "treated") using another inbred line ("opaque-2") as female. Seeds produced were considered as the M_0 generation from the treatment.

In the M_1 generation arising from "treated" pollen a wide range of types, colors, sizes and patterns of grains were recorded, completely different from those of parental types or those found with "control" pollen. The M_2 observations confirmed previous results.

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*Received March 28, 1972.

Though the data are incomplete and the results are only partially analyzed, it can be stated that this is an interesting technique because of its simplicity and efficiency. Its apparent advantage over other methods could be due to its operation during differentiation of the pollen grain (haploid phase) as opposed to many conventional techniques which perform during the diploid phase, under diplontic selection pressure.

Guillermo S. Ryan

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1. Possible nontunicate to Tunicate mutations.

Mangelsdorf and Galinat in their paper on "The tunicate locus in maize dissected and constituted" (PNAS 51:147-150, 1964) state that if their genetic analysis of the Tu locus is valid, Tu "cannot occur as a mutant in modern commercial nontunicate maize".

This note is to record four cases in which Tunicate ears have appeared in commercial corn. The first to come to my attention was sent to me by Midwest Research Associates about a decade ago. Dr. Bruce Ashman, Purdue University, found a Tunicate ear near Madison, Wisconsin about 1960. A third Tunicate ear was found at Macdonald College, near Montreal, in 1968 in a five acre increase block of the open pollinated land-variety Quebec No. 28. The most recent find occurred as two ears in a Foundation seed increase of Funk Seeds International near Bloomington, Ill. in 1972.

There is no sure way of knowing if these Tunicate ears resulted from mutation of tu to Tu or whether they resulted from "blow-in" pollen the previous generation. All of the mutants except that from Ashman still exist in my cultures and they will be analyzed to determine if they are different in some way from the standard Tu allele. Should they be different, this would suggest a mutant origin. Seed is available for distribution.

Robert I. Brawn

2. Cytoplasmic male sterility research.

Previous research using EMS to induce cytoplasmic male sterility in corn has been reported (1). More recently Petrov and Zheleznova reported that streptomycin produces cytoplasmic male sterility (U.S. Pat. Applic. #3,594,152).

Petrov and Zheleznova used doses of 0.0005 - 100 micrograms/ml or 0.00000005 - 0.01% on germinated seed for 24 hours. In our research using streptomycin, doses of .001, .005, .01, .05, .10, .150%, and control were used. Seeds of an inbred line of corn were germinated for 30 hours at 27°C; at the end of this time some radicles had emerged. Subsets of experiments were performed; in one set the germinated seeds were placed embryo down in petri dishes on Kimpak that was saturated with the streptomycin solution. In the second set germinated seeds were completely submerged in flasks of the streptomycin solution. In another experiment dry seeds (ungerminated) were placed embryo down in Petri dishes on Kimpak that was saturated with the streptomycin solution. All these experiments were conducted for 24 hours at 25°C.

The treated material was planted by digging trenches with a hoe and placing the sprouted seeds in them, after which they were covered with soil. The procedure of putting treated seed in trenches worked quite well; one contributing factor to this success was good soil moisture.

Shortly after emergence it was noted that some of the seedlings were albino. In fact, all seedlings were albino in material that had been germinated and then completely submerged in the 0.10 and 0.150% streptomycin solution (Table 1). The albino plants did not turn green and subsequently died.

Affecting the chlorophyll was encouraging, since we are undoubtedly doing something in the cytoplasm. If we are doing something genetic remains to be determined. Apparently there have been a few genetic studies to induce cytoplasmic mutants in higher plants (1). However, the effect of streptomycin on chloroplast development was discovered by vonEuler, who found that seedlings watered with a streptomycin solution developed colorless leaves. Studies with Euglena established that growth on streptomycin led to irreversible loss of chloroplast-forming ability. It has also been reported that in algae, streptomycin is a specific mutagen for chloroplast

DNA's and that streptomycin is an effective mutagen for cytoplasmic genes (see 2).

A rather good dose response, recorded as "% of planted stand" was obtained with the various treatment procedures (Table 1). However, no sterile tassels were noted in the M_1 generation in any treatment, nor could any sterile sectors be found in the tassels. Also no observable differences were noted in the mature plants among the treatments; in fact, the surviving plants appeared quite normal. The material was self-pollinated and will be planted ear-to-row in 1973.

Table 1

Total plants, number and percent of albino plants from three streptomycin experiments (germinated seeds planted directly in field).

	Germinated seeds on Kimpak (100 planted)				Germinated seeds submerged (42 planted)				Dry seeds on Kimpak (30 planted)			
	1*	2	3	4	1*	2	3	4	1*	2	3	4
Control	82	--	--	82	38	--	--	90.5	26	--	--	86.7
.001%	78	--	--	78	35	--	--	83.3	24	--	--	80.0
.005%	90	--	--	90	27	--	--	64.3	24	--	--	80.0
.01%	87	--	--	87	30	2	6.7	71.4	23	--	--	76.7
.05%	34	--	--	34	8	4	50.0	19.0	23	--	--	76.7
.10%	29	3	10.3	29	10	10	100.0	23.8	14	3	21.4	46.7
.150%	19	9	47.4	19	16	16	100.0	38.1	13	3	23.1	43.3

*1. No. surviving plants
2. No. chlorotic plants
3. % chlorotic plants of survivors
4. % of planted stand

Research to induce cytoplasmic male sterility with EMS is continuing. As previously reported (1), male sterile plants were detected in progeny of inbred lines that had been treated with EMS. However, after crossing these sterile plants with the untreated controls the plants became fertile in the subsequent generation. This is indicative that a recessive gene for male

sterility was causing the sterility. Also a "state" of the cytoplasm (dauermodification) may have been induced by the mutagen treatment. Another possibility under investigation is that cytoplasmic mutations for sterility were induced but when they were crossed to the untreated controls restorer genes were brought in leading to fertility. This assumes that the inbred line is segregating for restorer genes. To examine this possibility, remnant seed from each treatment that showed male sterility was planted and outcrossed with one of two unrelated inbred lines. These F_1's have been self-pollinated and will be planted in order to examine this theory. This approach may be feasible since Edwardson (3) reported that genes which restore fertility to cytoplasmic male sterile corn occurred in 59.6% of Latin American varieties and that the frequency of such genes in U.S. inbreds is 10.5% and that 2.81% were segregating for restorer genes. Also the variety Golden June, the source of Texas male sterile cytoplasm, was segregating for restorer genes (4).

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1. Unusual reaction of N and T cytoplasms to H. maydis, Race T.

One maize hybrid with N cytoplasm in a 1971 experiment segregated into a 3 resistant:1 susceptible phenotypic ratio. The same hybrid with T cms had one resistant plant. Paired entries were planted with hand planters. It is possible, but not likely, that this plant resulted from a kernel intended for the adjacent plot.

A. A. Fleming

2. Normal vs. male-sterile cytoplasms in maize.

In interregional and regional experiments with inbreds and hybrids at Athens in 1971, differential reactions of cytoplasms and genotypes, in addition to that for H. maydis, Race T, were observed. Inbreds Va 35 N and 33-16 N showed more red anthocyanin in leaves than their T cms counterparts. NY X65 N had what appeared to be a physiological yellowing of leaves in comparison to green leaves for NY X65 T cms. M14 S had yellow striping of leaves while M14 N had green leaves. Pa 33 N, Pa 70 N, and M14 S had a greater intensity of apparent corn stunt than their counter sources of cytoplasm.

Under the prevalence of H. maydis, Race T, a comparison of N and T cytoplasms showed, in many of the hybrids, reduced plant height, yield, number of ears per plant, number of erect plants, and grain quality. In general, T cytoplasm decreased ear height. However, one hybrid with T cms averaged 43 inches in ear height; its N counterpart averaged only 38 inches (13 cm difference). The T cytoplasm decreased number of days to midsilk in Dixie 18 while it increased the days to midsilk in NC 222.

Helminthosporium lesions on F44 N were especially small. Lesions on GA 156 (Ga cms) looked as if they might be H. turcicum instead of H. maydis lesions.

A. A. Fleming

3. Mineral-deficient maize inbreds.

In 1972, extreme purpling occurred in the leaves of young plants of the yellow-kerneled inbred, Cl 21, on Appling soil in the nurseries at the Plant Science Farm, Athens. Under prevailing cool weather the purple color remained until the plants were 18 or more inches high before disappearing.

Plant analyses at the University of Georgia Soil Testing Laboratory pinpointed a suspected P deficiency. Phosphorus content in the lower leaves was 0.23% instead of the normal level of 0.30%, although adequate fertilizer had been applied by broadcast and in the drill.

Another inbred, GA 153, could be spotted easily in the nursery both in 1971 and 1972 due to the yellowing of its leaves. Plant analyses showed that this white-kerneled inbred is deficient in Mg (magnesium) and also N

(nitrogen). GA 153 is probably a low accumulator of Mg and therefore sensitive to a lack of this element in its tissues. The lower leaves only had .10% Mg. The normal amount is .20% or more.

The lower leaves of GA 153 also had only 1.77% N when they should have had 3.00%. The N deficiency is probably due to an inability of the plants to take up the applied N fertilizer.

The stocks should be of use in future genetic and fertility experiments.

A. A. Fleming
J. B. Jones

4. Viability in long-stored seeds of maize.

Seeds of 200 entries of S_0 - S_3 lines were produced in 1965. They were stored in filing cabinets under ordinary conditions of room temperature and humidity of the Southeast at Athens for seven years and then tested for viability in a germinator in 1972. A total of 21% of the entries germinated, ranging from 2 - 88% in germination. Resistance to Rhizopus sp. in the germinator was noted in 3.5% of all the entries, the range being from very resistant to moderately resistant.

The variation in viability and also resistance to Rhizopus appeared to be hereditary. Seedlings were transplanted to the field to obtain germplasm for future studies and breeding programs.

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1. Seed viability of maize (Zea mays L.).*

In the last issue of MGCNL 45:94-95 Dr. Walton C. Galinat has reported the oldest seed viability of sweet-corn "Chuspillo" from Bolivia,

*Articles 1-6 were received March 21, 1972.

which was kept under cold-storage conditions at below 40° F, stored in small screw cap bottles. This report has aroused our interest to check the seed viability of maize kept at room-temperature. Fortunately, we were able to procure 10 screw cap bottles containing maize seeds found in the course of renovating a laboratory in the Biological Laboratories of Harvard University. The maize seeds were collected 30 years ago (from 1942 crop year) by Dr. James W. Cameron, who at that time was working with Professor Paul C. Mangelsdorf.

Randomly selected seeds from each bottle were soaked in distilled water for about 6 hrs., then kept in paper cups with moist paper-towels at room temperature (70 to 72° F). The score for germination was made on the 12th and 15th days, and the number of germinated kernels for each day was added to represent the total percentage for each bottle. Of the 10 bottles of seeds, five showed no germination, while the percentage of germination in bottles with collection number 429 was 28% (slow growth, represented by the size of the seedlings); Coll. #1168-5-11 was 36% (medium growth); Coll. #353 had 44% (slow growth); Coll. #351 was 60% (most vigorous growth); and Coll. #354 had 68% germination (next most vigorous growth).

Using these data for viability in 30 year old maize seed stored at room temperature, we suggest that different races of maize may have a different capacity to retain seed viability. We also suggest that it would be useful to select those genetic races of maize which may retain higher percentages of seed viability without resorting to expensive cold-storage methods often not available in other parts of the world.

Umesh C. Banerjee
Elso S. Barghoorn

2. Feminization in teosinte (Euchlaena mexicana Schrad.).

Most of the races of teosinte, having originated in Mexico and Central America, are short-day (SD) plants. They fail to flower, when grown outdoors in the North Eastern United States during summer, due to prolonged day length. Under natural conditions of their habitat, the vegetative phase of teosinte is terminated, and plants flower and fruit, when the day length becomes short. But in the New England climate, such SD conditions arise very late in the growing season and plants are killed by early frosts before

they initiate flowering. Early flowering in teosinte may be induced by SD treatment, that is, by artificially reducing the day length. For this purpose, seedlings at the 6 to 7 leaf stage are used for SD induction, and the treatment is terminated at the first sign of tassel emergence. It has been observed that very young seedlings (at the 5 or less than 5 leaf stage) are not responsive to this SD treatment.

In the late winter of 1970 (1st week of February), we have grown seedlings of several races of teosinte under greenhouse conditions (regulated temperature at 60 to 70°F). The purpose was to obtain pollen grains for detailed palynological studies. The following races were used: (1) Chalco; (2) Amecameca; (3) Los Reyes; (4) Guanajuato #45121; (5) Huehuetenango, Tzisbaj (Guatemala); (6) Jutiapa #51186 (Guatemala); (7) Huehuetenango Huista, (Guatemala); (8) Michoacan #45320; (9) Guanajuato #46452; (10) Guerrero #47259; (11) Guerrero #47269; (12) Guerrero #47335; and (13) Chihuahua, Nobogame (for the accuracy of the collection numbers or localities, see Wilkes, H. G., 1967). The seeds of the above named races were first soaked in glass-distilled water for three hours, then kept in paper cups with moist paper-towels, covered with Saranwrap to retain moisture. After seeds had germinated, the seedlings were transplanted to soil in 6 inch pots and grown under natural light in the greenhouse. When a few seedlings reached the 6 to 7 leaf stage, the SD induction was started by placing a thick, black cloth around the greenhouse bench on which these plants were placed. Since we had very limited greenhouse space, we were forced to keep the remaining young seedlings on the same bench on which we were inducing SD treatment at the 6 to 7 leaf stage. After 70 days of SD induction, we observed that only a few plants showed signs of tassel formation. However, the treatment was continued. After 100 days of SD induction, it was found that most of the plants from various races were producing silks (feminized). After careful separation of these plants three major categories were noted, namely (A) plants with normal tassel (male flowers) and silk formation (female flowers), the percentage of these plants being low, (B) plants with a poorly developed tassel (aborted male flower) and normal silk formation, and (C) extreme cases in which the plants were entirely feminized, producing only silks; in the latter category even the

position of the tassels was completely taken over by silk producing female flowers. The percentage of these feminized plants was high. Unfortunately, we were unable to produce seeds of such feminized plants due to lack of pollen grains for pollination.

Since the factors for soil, greenhouse temperature, and watering were similar for all the plants, the only variable was the leaf stage of the seedlings when SD treatment was started. Thus, the seedlings which were too young (with less than 6 to 7 leaves) received a prolonged SD induction, which may have caused the transformation from a monoecious condition, as in maize, to completely feminized plants.

We are thankful to Dr. H. G. Wilkes of the University of Massachusetts at Boston for the supply of seeds used in this investigation.

Umesh C. Banerjee
Elso S. Barghoorn

3. Misuse of the term "vivipary".

The term "vivipary" was first used by Linnaeus (1737, 1759) for the vegetative shoots developing on the inflorescences in place of spikelets, in Polygonum viviparum and some grass species. Collins (1909) noticed in maize production of small, vegetative bulblike structures forming on the tassels instead of the staminate spikelets. Harris (1912), working with teosinte, raised a question about the correct use of the term "vivipary". He applied a new term "chloranthy" for the situation in which floral parts are transformed into foliar organs. Eyster (1931) has used the term "vivipary" in maize to indicate the continuous development of a plant body from its unicellular inception to maturity, without the intervention of a period of dormancy. Later, Arber in 1934 emphasized the use of these terms in a more restricted sense. According to her, only the germination of the seeds on the parent plant should be regarded as "true vivipary" and the phenomenon described by Linnaeus as "vivipary", by Collins (1909) as "bulblike structures" and by Harris as "chloranthy" should be designated as "proliferation". Further, it has been found (Harris, 1912; Reeves and Stansel, 1940; Ullstrup, 1952) that proliferations are physiologically initiated by incomplete floral induction or by fungal infection. But in "true vivipary" the zygotic embryo grows directly into the seedling without

cessation of growth, while still attached to the parent plants. The factors involved in this phenomenon are still not adequately known.

In maize (Zea mays L.) both "true vivipary" and "proliferation" may occur. The case in this species is much more interesting because the plant is monoecious, male and female sexes being present at different locations on the plant body. In maize "proliferation" is expressed only at the location of male flowers in tassels and "true vivipary" occurs only in the cobs, where female flowers are formed.

Umesh C. Banerjee
Elso S. Barghoorn

4. Factors controlling "true-vivipary" in maize (Zea mays L.).

As we have indicated above, "true-vivipary" in maize is restricted to the cobs or female flowers. It has been recorded in the earlier literature that the sprouting of kernels occurs while they are still attached on the ear enclosed inside the husk. This condition has been considered as a "primitive character". But such premature sprouting of the kernels under natural conditions proves disadvantageous because of an insufficient water supply to maintain growth during unfavorable periods.

Various causative factors were suggested for such sprouting in maize. Weatherwax (1923) reported that environmental conditions such as warm, moist weather are responsible for premature germination. Lindstrom (1923) and Mangelsdorf (1923) considered that this phenomenon is associated with defective endosperm. Eyster (1924) suggested that a "primitive sporophyte" in maize occurs when the fertilized egg continues to grow into the new plant without going through a period of dormancy. He also proposed that the character is inherited as a simple Mendelian recessive. Further, he indicated that it appears to be associated with factors for pale yellow endosperm and albino seedlings. Mangelsdorf (1926) again reported that a number of genetic factors are involved in the inheritance of premature germination and these factors operate at various stages of endosperm development and differ in some of their effects. In a recent publication on maize by Neuffer et al. (1968), chromosome numbers 1, 2, 3, 5, and 7 are assigned for the viviparous condition. But it is still not clearly established whether vivipary in maize is entirely controlled by environmental

factors, by the genetic make-up of the sporophyte, or by both.

During early September 1970, unusually warm, humid weather conditions prevailed in Cambridge, Massachusetts, for over 10 days. A corn type (#16 (UCB); a dent corn variety with the 4th chromosome from Nobogame teosinte) was found producing viviparous seedlings in several cobs. On close examination of these viviparous cobs, a few sprouted kernels were also found associated with fungus growth, but no albino seedlings were observed in these propagules. The association of fungus growth with viviparous kernels was also recorded by Dr. Walton C. Galinat (personal conversation with UCB). He believes that the breakdown of endosperm by fungal growth initiates viviparous growth in maize. We have also examined plants of the other maize types grown during the 1970 crop year and found no sign of vivipary.

During the 1971 crop year, we have replanted a few seeds of the maize type #16 (UCB) along with the other types. No viviparous cobs were observed on these plants this year, a situation correlated with the absence of the prolonged warm humid weather of the preceding year. However, when we enclosed a few cobs of type #16 within plastic bags, while the cobs were still enclosed in the husks and attached to the parent plants, we found that most of the kernels on such cobs germinated, simulating the case of "true-vivipary". This experiment was further extended by using the other corn types. We have also found that juvenile kernels, with endosperm at a milky-stage, fail to germinate in plastic bags; hence it seems that complete maturation of the endosperm (cellular stage) is required for such sprouting. Apparently the endosperm must retain sufficient moisture in the cells to help sprouting when cobs are covered with plastic bags.

In conclusion, it may be suggested that environmental factors are more important or at least dominate under natural conditions in inducing vivipary in maize.

We are thankful to Dr. W. C. Galinat of the University of Massachusetts, Waltham Field Station and to Dr. H. G. Wilkes of the University of Massachusetts, Boston Campus, for their help in locating several of the older references on vivipary.

Umesh C. Banerjee
Elso S. Barghoorn

5. The membrane structure of spore-galls of corn smut (Ustilago maydis (DC.) Corda).

Investigators working with maize and teosinte plants are well aware of the formation of smut galls (Ustilago maydis (DC.) Corda). The fungus may occur in any part of the host and produces tumor-like swellings. However, the infection is always localized; the shape and size of the swellings (galls) varies according to the plant parts involved. The smut galls when young appear to be covered with a glistening silvery membrane, which, as it matures, becomes dry and papery. Finally, the membrane ruptures and exposes large masses of powdery dark spores.

The structure of this gall membrane is still inadequately known. It is interesting to establish whether the membrane is formed by the host tissues or by the fungus itself. According to Clinton (1905, p. 20), the membrane is composed largely of semigelatinized fungus threads. Duggar (1909) has also reported that the membrane is made up of modified fungus threads mingled together with dried host cells.

In the present investigation, we have closely observed the development of the smut galls in kernels while still attached to the cobs. At initial stages, the gall tissues are entirely composed of large masses of abnormally active host tissues. Later, with the exception of a few outer cell layers, most of the internal tissues of the galls were invaded and consumed by the fungal hyphae. Finally, large masses of dark spores are formed. The gall membrane, which is normally more than one layer thick, becomes desiccated and papery. Eventually, the gall membrane ruptures due to internal pressure exerted by the large masses of chlamydospores. We have further studied the structure of the mature gall membranes at the ruptured points using scanning electron microscopy (SEM). SEM observations show that the mature gall membrane is composed of at least 1 to 3 layers of hypertrophied parenchyma cells of host tissue. From the outside this membrane is very smooth and shows a clear cellular organization, while the inner surface of the membrane is entirely covered with loose chlamydospores. We were unable to observe any indications of attachment of the spores to the membrane.

Umesh C. Banerjee
Elso S. Barghoorn

6. Scanning electron microscopy of chlamydospores of teosinte smut (Ustilago maydis (DC.) Corda).

In our earlier reports in MGCNL 44:42(1970) and 45:237(1971), we have described the microstructures of the chlamydospores of maize smut (Ustilago maydis (DC.) Corda). This species of fungus most commonly attacks maize plants (Zea mays L.). But in Mexico and Central America various races of teosinte (Euchlaena mexicana Schrad.) are also susceptible to the same fungus species. However, differences, if any, between the chlamydospores obtained from the two hosts have not been reported.

The following report presents our preliminary observations on the microstructures of mature chlamydospores of teosinte smut, obtained from different races of teosinte. The plants were grown at Waltham, Massachusetts, during the 1971 crop year. The mature chlamydospores from different smut galls from various locations in the host tissues were isolated and studied with the scanning electron microscope (SEM). The spore samples were prepared following the methods described by us previously in MGCNL 45:237(1971). The chlamydospores from smut galls were obtained from the following races of teosinte (1) Chalco, stem infection; (2) Guerrero (Wilkes, teosinte Coll. #47711), stem infection; (3) Michoacan (Wilkes, teosinte Coll. #47890), stem infection; (4) Guerrero (Galinat, teosinte Coll.), tassel (male flower) infection; (5) Chalco, tassel infection; and (6) Chalco teosinte and maize hybrid (Galinat Coll.), kernel infection.

The gross micro-morphology of the mature chlamydospores of teosinte smut under SEM shows a close similarity with corn smut. But the spore size, shape, spine distribution, and diameter of the spines at the base show slight differences from corn smut, depending on the type of infection and the race of the host species involved. The chlamydospores from stem infections exhibit mostly a globose (spherical) shape, and their spine numbers seem a little higher when compared with the spores from male flower or kernel infections. The spores of stem infection also show a characteristic folding, when they are placed under high vacuum conditions of the SEM. The spores derived from the male flower infections are mostly spherical, but the chlamydospores from Guerrero teosinte seem to have more spines as compared to the Chalco teosinte smut. The diameter of the spines at the base is slightly greater in the latter species than in the former.

Further, the chlamydospores from kernel infection in a hybrid of Chalco teosinte and maize show variation in spore shape, i.e., ovoid to spherical. Also their spines are slightly elongated and the diameter at the base is narrow compared to the spore spines of other teosinte smuts we have studied. The details of this investigation will be published elsewhere.

We are thankful to Dr. Walton C. Galinat, of the University of Massachusetts, Waltham Field Station, for kindly collecting the spores of teosinte smuts used in this investigation.

Umesh C. Banerjee
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7. The oldest convincing archaeo-palynological evidence for natural introgression between Tripsacum and Zea.

Mangelsdorf and Reeves (1931, 1939) were the first to demonstrate that Zea and Tripsacum can be hybridized artificially. They also postulated that teosinte (Euchlaena mexicana) originated as a result of such natural hybridization between these two genera. Mangelsdorf (1961) further suggested that the South American races of maize may have inherited their tripsacoid characteristics through direct introgression with Tripsacum. But natural introgression between Zea and Tripsacum has not yet been demonstrated conclusively. Our recent palynological investigation with various genetic stocks, which were artificially produced by crossing Zea and Tripsacum, has revealed that such introgression could be predicted precisely using the pollen grain ektexine patterns at the micro-morphological level (Galinat, Barghoorn, and Banerjee, unpublished data). Our palynological data also indicate that teosinte is not a hybrid of Zea and Tripsacum as suggested earlier; we feel perhaps this genus may have evolved parallel with Zea, possibly from a common ancestor. The phenotypic patterns of the pollen grain ektexine of the "pure races" of Zea and Euchlaena are very similar, and are represented by the evenly distributed spinules, although Zea pollen is significantly larger in size both in archaeological and in modern populations. On the other hand, the phenotypic pattern of Tripsacum ektexine in diploid and tetraploid species shows a very distinct "negatively-reticuloid" spinule clumping. When different races of Zea and Euchlaena are hybridized with each other artificially or in the wild, the pollen grains

from hybrid derivatives (including the Type specimen of Zea cannina Wats., in the Gray Herbarium, Harvard University) show a pattern in which a few ektexine spinules are occasionally missing, giving rise to blank areas (Banerjee and Barghoorn, 1972a). This pattern occurs in most of the popcorn races from Mexico which overlap in flowering time with teosinte. Even the popcorn race "Confite Morocho" from Peru was found to exhibit this pattern and hence the presence of teosinte germ-plasm is indicated. However, the phenotypic Tripsacum ektexine pattern is dominant over the Zea and Euchlaena patterns, and the hybrid-derivatives retain some degree of spinule clumping. Our observations also suggest that this criterion could be used conclusively to show introgression of Tripsacum with both maize and teosinte. Recently, we have studied a prehistoric archaeological sample (tassel fragments) of Zea mays L. from the site near Huarmey, Peru, from level #4, dated approximately 2000 to 1600 B.C. (this date is estimated by the archaeologists, personal communication with Professor Mangelsdorf, and Mangelsdorf and Cámara-Hernández, 1967). The pollen grains from this site show a distinct spinule clumping and demonstrate the oldest convincing archaeo-palynological evidence of introgression of Tripsacum with maize. Moreover, we found that a collection of pollen grains of the extant race of Cuzco maize (Zea mays L.) also shows a distinct spinule clumping, and we may assume perhaps that this race of maize has likewise been derived through natural introgression with Tripsacum (Banerjee and Barghoorn, 1972b).

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1. Reaction of germinating maize pollen to Helminthosporium maydis pathotoxins.

Hooker et al. (Plant Disease Reptr. 54: 708-712, 1970) have shown that Helminthosporium maydis race T is virulent for maize plants which carry the Texas (T) male-sterile cytoplasm. Maize with nonsterile cytoplasm, as well as that with C or S male-sterile cytoplasm, is resistant to the race T pathotoxin. When germinating seeds are incubated in solutions of the race T pathotoxin, the elongation of primary roots of seedlings with T cytoplasm is inhibited. Root growth of seedlings with C, S or normal cytoplasm is not inhibited. The race O pathotoxin is not specific as to cytoplasm (Lim and Hooker, Genetics 69: 115-117, 1971). We have conducted studies to determine whether the race T pathotoxin has a similar differential effect on germinating maize pollen grains.

The technique of Cook and Walden (Can. J. Bot. 43: 779-786, 1965) for the in vitro germination of pollen was modified to incorporate toxin into the medium. As sources of race O and race T toxin, we have used both extracts of infected leaves and filtrates of Fries medium in which the fungus has grown. Pollen tubes were both fixed and stained with lactophenol aniline blue.

We have tested many lines and their different cytoplasmic versions, as well as the normal and T cytoplasm versions of some F_1's. Pollen germination of T and P cytoplasm plants is consistently inhibited in the presence of the race T toxin at concentrations which allow growth of

pollen with normal, C and S cytoplasms. The race O toxin has no differential effect on pollen germination.

This procedure can be accomplished in one to two hours and does not require sterile technique. Only small amounts of toxin are required for a test both because the pollen is very sensitive and because only a small volume of test medium is necessary. Plants are tested while they are still flowering and since the cells being assayed are reproductive cells, this procedure may be adapted for use as a selective device, for example, to select for toxin resistance. This procedure may also be of use in the isolation and purification of the race T pathotoxin since it permits the identification of the toxic fraction in a fractionation procedure.

John R. Laughnan
Susan J. Gabay

2. Nuclear restoration of fertility in S male-sterile cytoplasm in maize.

We reported previously (Genetics 60: 226, 1968; MGCNL 45: 30, 1971; Genetics 71: 607, 1972) on numbers of changes of the S cytoplasmic sterile condition to the fertile or semifertile condition. These changes occur at the cytoplasmic rather than nuclear level; they are not pollen transmissible. The fertility was assigned to changes in the cytoplasmic S element with the reservation that transmission of cytoplasm through the male gametophyte may be involved.

The present report deals with studies of four recent independent occurrences of mutations which restore fertility in S sterile cytoplasm and are pollen transmissible. These cases of mutation at restorer loci occurred in the progenies which were being searched for changes in the cytoplasm from male-sterile to male-fertile condition.

Male-sterile and male-fertile (maintainer) versions of five shrunken-2 inbred lines, R839, R851, R853, R853N and M825, were employed in these studies. The male-sterile cytoplasm incorporated into these lines traces to a Vg source which has been shown to be equivalent to the S (USDA) sterile cytoplasm.

Male-sterile plants from the sh_2 inbred lines were crossed by their corresponding maintainer inbred lines. The resulting progeny were searched for male-fertile exceptions, plants with entire tassels fertile or those

with fertile tassel chimeras, among otherwise male-sterile offspring. Over 300 such exceptional plants were identified during the 1971 summer growing season and were tested in the following manner. The exceptional male-fertile plants were either self pollinated or crossed as egg parents by the corresponding maintainer. These plants were also crossed as pollinators with sibling male-sterile individuals, and, in some instances, with male-sterile plants of other $\underline{sh}_2$ inbred lines. A few exceptions were also crossed with an S male-sterile version of WF9. In all but four cases, the progenies of testcrosses with S male-sterile plants indicated that the male-fertile character of the exceptional plants was not transmitted through the pollen. These four cases do not fit the usual pattern of changed cytoplasm described above. In each instance, testcrosses of these exceptional male-fertile individuals with S male-sterile plants produced male-fertile offspring, suggesting a Mendelian, or nuclear, basis for the fertility. The four cases of restorer gene mutation are numbered I through IV and are discussed below.

Case I occurred in the R853N line. The restorer gene mutation occurred in an R853N maintainer strain which was represented in family 71-741. This mutation can not be traced to a single plant. The new gene restores WF9 cms S as well as R853N cms Vg.

Case II occurred in the R853 line and traces to a fertile tassel chimera borne on plant 71-737-16. The chimera involved one side of the main rachis and all florets of twelve lateral branches on that side of the tassel. In addition to R853 cms Vg, this new gene restores M825 cms Vg. Surprisingly, this gene does not restore R839 cms Vg which is ostensibly the same type of male-sterile cytoplasm as is carried by R853 and M825.

Case III also occurred in the R853 line and traces to a single plant, 71-739-37, with an entirely fertile tassel and a tiller that was also entirely fertile. In addition to R853 cms Vg, the case III mutation also restores the M825, R839 and R851 sources of Vg sterile cytoplasm as well as WF9 cms S.

Case IV occurred in the M825 line and traces to a fertile tassel chimera borne on plant 71-727-37. The main rachis and most lateral branches of the tassel of this plant were sterile. Eight contiguous lateral branches on one side of the tassel were fertile. In addition to

M825 cms Vg, this newly-arisen mutation restores WF9 cms S.

The male-fertile exceptions described here can be accounted for formally as mutations at one or more restorer gene loci in the nucleus. So far as we are aware, these are the first reported instances of mutations in restorer genes. That we should have encountered four such male-fertile exceptions seems highly coincidental. We think it may be significant, also, that these changes were encountered in the same strains in which we have identified numerous additional cases of male-fertile exceptions involving cytoplasmic "mutations". We suggest a common basis for the two kinds of events. According to this scheme, given the first appearance, by whatever process, of male-fertile elements in male-sterile cytoplasm, they may become established and continue to propagate either in the cytoplasm or in the nucleus. In the former case, the change registers as cytoplasmic and the new strain has the characteristics of a maintainer which transmits the male-fertile trait through the egg, but not the sperm. In the latter case, the change occurs in the nucleus and the new strain, now behaving as a restorer, transmits male fertility through both egg and sperm.

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1. Monogenic chlorotic-lesion resistance in corn to Helminthosporium maydis.

A source of resistance to race O of Helminthosporium maydis in an East African strain of corn tested in Nigeria (Jeweus Craig and J. M. Fajemisin, Plant Disease Reporter 53:742-743, 1969) was obtained from Dr. Craig. Corn Belt adapted resistant selections (RS) were developed through backcrossing, selfing and selection. Genetic studies in the field and in the greenhouse involving numerous susceptible U. S. inbreds reveal that the resistance in our selections is monogenic recessive in

inheritance. A portion of the seedling data is given in the following table:

Cross	Observed No.		Expected No.		X^2	P value
	R[a]	S[b]	R	S		
W64AxRS	0	20				
(W64AxRS)xRS	262	251	256.5	256.5	0.236	0.50-0.75
(W64AxRS)F_2	186	522	177.0	531.0	0.610	0.25-0.50

[a]Resistant: small chlorotic lesions with limited fungus sporulation

[b]Susceptible: large tan, oval to rectangular lesions with abundant fungus sporulation

The symbol rhm is proposed for the recessive gene conditioning this chlorotic-lesion resistance to H. maydis.

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1. The $Adh_1^{\overline{FC^m}}$ "Operon".

Earlier studies have established that the level of alcohol dehydrogenase activity in the plant is limited by the concentration of a specific factor which is essential for the activity of the Adh gene (Schwartz, 1971). Although various Adh_1 alleles have been shown to differ in their ability to compete for the limited factor, enzyme level in segregating kernels and seedlings is constant and independent of Adh_1 genotype as long as the Adh_2 gene is not active. The Adh_2 gene, which specifies a relatively inactive enzyme, competes with the Adh_1 gene for the limited factor.

The $\underline{Adh_1}^{C^m}$ allele and the $\underline{Adh_1}^{\overline{FC^m}}$ duplication, which consists of two cistrons that specify an F and a C^m polypeptide, were found segregating with an $\underline{Adh_1}^{F}$ allele in a line of maize from Colombia, South America (Schwartz, 1966). The $\underline{Adh_1}^{C^m}$ allele produces a stable but relatively inactive enzyme. $\underline{Adh_1}^{F}/\underline{Adh_1}^{C^m}$ heterozygotes exhibit considerably less enzyme activity than their $\underline{Adh_1}^{F}/\underline{Adh_1}^{F}$ sibs, as expected, since much of the limited factor is used in activation of the $\underline{Adh_1}^{C^m}$ gene which makes inactive enzyme. The two cistrons in the duplication are very tightly linked. No crossovers were detected between the two loci in over 4000 progeny. This analysis involved backcrossing an $\underline{Adh_1}^{\overline{FC^m}}/\underline{Adh_1}^{S}$ heterozygote to $\underline{Adh_1}^{S}/\underline{Adh_1}^{S}$ and individually scoring the progeny by starch gel electrophoresis to determine if in any cases the pseudoalleles were transmitted separately. Recent studies suggest that the two cistrons may in fact be part of a single "operon". They act as a unit in that they contain only a single activation site which competes for the limited factor. The reasoning behind this conclusion is as follows. If the cistrons in the duplication each had an activation site that competed for the limited factor, F_2 $\underline{Adh_1}^{\overline{FC^m}}/\underline{Adh_1}^{\overline{FC^m}}$ progeny should show much less enzyme activity than the $\underline{Adh}^{F}/\underline{Adh_1}^{F}$ sibs since in the former the limited factor would be used in the synthesis of inactive C^m as well as active F polypeptides. This is definitely not the case. Approximately equal enzyme levels are found in both genotypes. Since the C^m subunit stabilizes the F subunit in a heterodimer (Schwartz and Laughner, 1969), the enzyme levels vary slightly in different tissues as a result of differential enzyme turnover.

The operon hypothesis was tested by comparing the relative concentrations of F and C^m protomers produced in $\underline{Adh_1}^{F}/\underline{Adh_1}^{C^m}$ vs $\underline{Adh_1}^{\overline{FC^m}}/\underline{Adh_1}^{\overline{FC^m}}$ genotypes. Use was made of the method of high resolution immunoelectrophoresis (Schwartz, 1972) which permits comparison, at the protein level, of the relative amount of polypeptides produced by two alleles in a heterozygote. $\underline{Adh_1}^{F}$ competes better than $\underline{Adh_1}^{C^m}$ in seedlings, and less C^m than F polypeptides are produced in $\underline{Adh_1}^{F}/\underline{Adh_1}^{C^m}$ genotypes. If the $\underline{Adh_1}^{\overline{FC^m}}$ duplication has a single activation site which binds the limited factor and a bicistronic messenger RNA molecule is produced, one would predict that equal amounts of F and C^m polypeptides would be synthesized in the duplication homozygotes. This is clearly the condition which is observed (unfortunately the

immunoelectrophoretograms cannot be reproduced in the News Letter).

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Drew Schwartz

2. Regulation of alchohol dehydrogenase (ADH) activity in developing maize endosperm.

In immature seeds, high levels of ADH activity are found in both the embryo and the endosperm. During late stages of maturation, the activity decreases strikingly in the endosperm but remains high in the scutellum. The level of activity of an enzyme depends upon the rate at which it is synthesized, as well as the rate at which it is degraded or inactivated. The *Adh*$_1$ gene is probably not active in mature endosperm, but gene repression can not account for the rapid disappearance of ADH activity during the late stages of maturation. The rapid drop in activity must be a result of inactivation of preexisting enzyme. This process has been shown to involve two components, both present in the mature endosperm, which readily inactivate ADH *in vitro*. One component is a protein and the other is dextrin; neither has any effect by itself.

In the tissues which contain active ADH, only one of the components can be found. The embryo contains only the protein. The immature endosperm contains a high level of dextrin, but the protein component can not be detected until the stage of development at which ADH activity rapidly declines.

In order to be effective in the inactivation process the dextrins must be in a certain size range. Large molecules such as starch, glycogen, and even commercially available dextrins are ineffective unless hydrolyzed; prolonged hydrolysis reduces effectiveness. Furthermore, the dextrin must

be branched as hydrolyzed amylose does not have any effect.

The protein component has been characterized and partially purified. It is heat labile and has a molecular weight of approximately 120,000.

Inactivation has been shown to involve only the active site of the enzyme without altering the overall configuration of the molecule, since the inactivated enzyme retains its antigenic specificity. The inactivation is reversible; 70-80% of initial activity can be recovered by 2-3 hours incubation at 55°C. This reactivation may result from destruction of the heat labile protein component in the complex.

This two factor system might be involved not only in the control of ADH activity in the endosperm, but also in inactivation of ADH in other tissues, such as the root and the plumule during germination.

The role which this system plays *in vivo* is being tested by the use of a mutant which does not synthesize the protein factor in the embryo.

Dina Fischer

3. Genetic differences between ADH_1 isozymes revealed by dissociation and reassociation experiments.

Two unlinked alcohol dehydrogenase (ADH) genes, Adh_1 and Adh_2, are found in maize (Schwartz, 1966, Freeling and Schwartz, 1973). ADH_1-FF and ADH_1-SS, the products of two alleles at the Adh_1 locus, differ in their electrophoretic mobilities.

A dissociation and reassociation procedure (freezing in high salt followed by thawing and dialysis) described by Hart (1971) has been adopted recently for dissociation and reassociation studies of ADH in maize. These studies lend further support for the dimeric structure of these isozymes, as concluded from genetic and electrophoretic analysis (Schwartz and Endo, 1966).

If reassociation is a random process, mixtures of crude extracts with equal ADH activities from Adh_1^F/Adh_1^F and Adh_1^S/Adh_1^S kernels should yield dimers in a ratio 1 FF:2 FS:1 SS upon dissociation and reassociation, comparable to *in vivo* subunit assembly in Adh_1^F/Adh_1^S heterozygotes.

In zymograms of such reassociated extracts a deviation from this expected ratio is observed. The isozyme band pattern obtained approximates a ratio of 4 FF: 4 FS: 1 SS, as if only one half of the ADH_1^S

monomers recombined into active enzyme. This result suggests that the ADH_1^S monomers are less stable than ADH_1^F monomers under the conditions of treatment.

The addition of Zn^{++} during dialysis, when the subunits are reassociating, has a striking effect in shifting the ratio back to 1 FF: 2 FS: 1 SS, and increasing the amount of activity which is restored. Without Zn^{++}, the average activity recovered for $\underline{Adh_1}^S/\underline{Adh_1}^S$ extracts was 12% of the undissociated control and 75% for the $\underline{Adh_1}^F/\underline{Adh_1}^F$ extracts. With the addition of Zn^{++}, the activities recovered for $\underline{Adh_1}^S$ extracts increased to 60% while the $\underline{Adh_1}^F$ recovery was unchanged. Our results indicate that Zn^{++} is necessary for the reassociation of ADH_1^S monomers to form active enzyme.

Preliminary results obtained with a modification of this procedure, which yields almost 100% active enzyme upon reassociation, suggest that dissociated F monomers bind Zn^{++} more strongly than do the S monomers.

Experiments are currently underway to determine whether or not zinc plays a role in the dimerization process itself.

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Meir Fischer

4. The products specified by two, unlinked alcohol dehydrogenase genes in maize are immunologically similar.

One way to identify similarities in the primary structures of two different enzyme subunits is to ascertain whether any antibody specified against one subunit will cross-react with the other subunit. The antigen-antibody reaction is extremely specific (see Reichlin, 1972, J. Mol. Biol. 64, 485). There are two unlinked alcohol dehydrogenase (Adh genes; ADH enzyme, EC 1.1.1.1.) genes in maize: $\underline{Adh_1}$ and $\underline{Adh_2}$. Their products dimerize into three electrophoretically separate enzymes of the same molecular size: Set I (ADH1·ADH1), Set II (ADH1·ADH2) and Set III (ADH2·ADH2)

(Schwartz, 1966, Proc. Nat. Acad. Sci. 56, 1431; Freeling and Schwartz, 1973, Biochem. Genet. 8, 27). All three sets of ADH can be induced by anaerobic treatment of primary roots (Freeling and Schwartz, 1973).

It was shown--from data to be reported elsewhere--that some anti-ADH1 antibodies specified against highly purified ADH1 subunits (Set I) also cross-react with ADH2 subunits (Set III). Competitive titrations and a two-dimensional immunoelectrophoretic technique (Schwartz, 1972, J. Chromatogr. 67, 385) were used. ADH1 and ADH2 subunits share some, but not all, antigenic sites. Homogenous ancestry is directly supported.

This result was not expected. The two subunits composing the major lactate dehydrogenases (LDH's) in animals are not immunologically similar although they do have considerable amino-acid sequence homology (see Kaplan, 1964, Brookhaven Symp. Biol. 17, 131). Compared to the animal LDH's, the original Adh duplication event reflected in contemporary maize may be recent. In any case, the Adh gene-system may prove phylogenetically useful. Quantitative immunological comparisons between the ADH's of maize and its relatives would be expected to yield evolutionary relationships. The antigenic similarity of ADH1 and ADH2 in maize, and presumably in maize relatives, may permit the quantitation of rate and extent of divergence of two, unlinked, duplicate genes.

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5. The functioning of the dissimilar sperm of high-loss plants in double fertilization.

Roman (1947, 1948) in his studies with TB-A translocations, where dissimilar sperm are formed by nondisjunction at the second microspore division, reported that the hyperploid sperm with two B^A chromosomes preferentially fertilized the egg while the hypoploid sperm with no B^A chromosome united with the polar nuclei. The fate of the two sperms is dependent on their genetic make-up and not on the segregation of the two B^A chromosomes into a specific nucleus with a preordained function in fertilization (Carlson, 1969). Carlson showed that preferential fertilization of the egg by sperm with two B^9 chromosomes did not occur when several intact B chromosomes were present (the so-called "swamping" effect). Any tendency toward

preferential fertilization was overcome by the predominating influence of the intact B's, which were randomly distributed to both sperm nuclei following nondisjunction. Dissimilar sperm are also formed at the second microspore division in high-loss plants; one sperm is hypoploid, while the other is usually euploid. Since chromosome elimination in high-loss plants occurs only when several B's are present, the potential ability of one of the sperm to undergo selective fertilization should be negated by the swamping effect of the B chromosomes, as suggested by Carlson.

The results of one test of preferential fertilization with a high-loss plant reported in the paper by Rhoades, Dempsey, and Ghidoni (1967) indicated approximately equal frequencies of deficient endosperms and embryos, although the deficient endosperms occurred with a somewhat higher frequency. These rather scant data suggested that the two dissimilar sperm, resulting from chromosome elimination at the second microspore division, were randomly involved in fertilizing the egg and polar nuclei, in agreement with Carlson's prediction. However, much more extensive data acquired subsequent to the 1967 report were at variance with this tentative conclusion. We stated in our 1972 paper in Genetics that "In general, deficient endosperms are found more frequently than are deficient embryos--i.e., selective fertilization does occur. However, the variation found in different crosses is so great that the phenomenon requires further study." This we have done and we now believe that the relative rates of loss in endosperm and embryo, ranging from no difference to frequencies at a much higher rate for the endosperm, reflect the tendency of deficient embryos to abort, producing either germless kernels or those with defective embryos unable to germinate. The triploid endosperm with its diploid genome from the female parent is buffered against the deleterious effects of genic unbalance and is able to develop normally, even though it possesses a deficient chromosome. In contrast, the deficient embryos are not so buffered by polyploidy and with varying frequency, depending upon modifying factors, cease to develop during embryogenesis.

The short arm of chromosome 9 is well suited for the detection of loss events in both endosperm and embryo. High-loss plants with both chromosomes 9 carrying a large terminal knob and the Yg and C alleles in the short arm were used. The consequences of breaks in the short arm of 9 are easily

discernible in the aleurone. Breaks between the C locus and the terminal knob result in a dicentric chromosome which undergoes the bridge-breakage-fusion cycle during endosperm development and produces kernels mosaic for colored and colorless tissue. Breaks proximal to C yield an acentric fragment with the C allele and a dicentric lacking the C locus. The acentric fragment is lost and, although the dicentric undergoes the bridge-breakage-fusion cycle, the aleurone is wholly colorless. The colorless kernels coming from breaks between C and the centromere cannot be distinguished from those arising by nondisjunction, a relatively infrequent phenomenon also occurring in high-loss microspores. In summary, nearly all types of loss affecting the short arm of 9 lead to recognizable aleurone phenotypes. The only exceptions are those rare breaks distal to the C locus in which healing of the broken end occurs or where there is a bridge-breakage-fusion cycle with breaks restricted to one region. On either alternative, a self-colored rather than a mosaic kernel would ensue and the deficiency would not be phenotypically expressed. A sperm with a deficient chromosome 9 coming from breaks anywhere in the short arm, save for the extremely short Yg-knob interval, would yield an F_1 plant with the recessive yg phenotype when it fertilizes the egg nucleus. From the above considerations, it is apparent that the consequences of breaks in the short arm, or loss by nondisjunction, are manifested in both the sporophyte and the endosperm. It follows that the loss of the C allele, measured by endosperm color, should equal the loss of Yg in the F_1 sporophyte if selective fertilization is not operating.

Three plants from the high-loss strain, $\frac{K9\ Yg\ C}{K9\ Yg\ C}$ in constitution, were used as male parents in crosses to yg c testers. The total progeny of 3196 included 2585 kernels with colored aleurone (no loss of C in the sperm uniting with the polar nuclei), 32 with a bridge-breakage-fusion pattern of C-c variegation and 579 colorless kernels. The 32 variegated kernels arose from breaks between C and the large terminal knob and the 579 colorless kernels stem from breaks between C and the centromere or come from nondisjunction. The sum of the variegated and colorless kernels (611 or 19.1% of the total population) represents the fraction of fertilizations in which the deficient sperm united with the polar nuclei.

The three classes of kernels were planted under a favorable environment in a sand bench and the resulting seedlings were scored for the green versus yellow-green trait. The 2585 C kernels produced a seedling population of 2180, consisting of 1823 Yg and 357 yg plants. The germination percentage of the C kernels was 84.3%, a fact which, as we shall see, is of some consequence.

The 611 variegated and colorless kernels gave rise to 546 green seedlings, a germination rate of 89.4%. As expected, none were yellow-green. The embryos all contained a normal chromosome 9 since the sperm with a deficient chromosome had united with the polar nuclei. The frequency of yg seedlings in the total seedling population of 2726 was 13.1%, a value significantly less than the 19.1% of kernels with loss of the C allele in the endosperm. Since breaks in 9S leading to deficient sperm should be as readily detectable in the embryo as in the endosperm, the data, at first glance, indicate that a deficient sperm is more apt to fertilize the polar nuclei than it is the egg nucleus. Two features of the above data are noteworthy. First, all of the yg seedlings came from the C class of kernels and second, the germination was higher in the exceptional colorless kernels (89.4%) than in the colored class (84.3%). It was possible that the decreased germination rate in the C kernels was caused by kernels with yg embryos which aborted, producing germless seeds. The germination rate of the colorless kernels was 89.4%; the 10.6% aborted embryos represent residual abortion most likely caused by deficiencies in other knobbed chromosomes. A similar rate of residual abortion should occur in the class from C kernels. Multiplying .894 X 2585 gives 2311, the number of kernels which should have germinated with no lethality of yg embryos. The difference between the expected number (2311) and the observed number (2180) of seedlings, or 131, is an estimate of the number of colored kernels which failed to germinate because their yg embryo had aborted. Combining the observed yg seedlings (357) and the estimated number of yg zygotes which aborted (131) gives a total of 488 yg zygotes in a seedling population of 2857 (2726 + 131) or 17.1% of yg zygotes. The 10.6% of kernels which failed to germinate (in C, mosaic and c classes) is assumed to include Yg and yg embryos in the same proportion as that determined for the 2857 population. Since the residual abortion is believed to be due either to unknown gm

factors or to loss of chromosomes other than 9, there is no reason to assume that the frequency of yg should differ from the frequency calculated for the estimated population of 2857. The percentage of 17.1 is in good agreement with the 19.1% of endosperms deficient for the C allele and is suggestive of random fertilization.

This conclusion was further tested in studies with chromosome 3. The A marker followed in the endosperm showed a much greater frequency of loss than did the Lg marker in the embryo. The chromosome 3 data are less amenable to a phenotypic analysis of loss events since breaks between Lg and A give rise to endosperm loss of A but not to embryo loss of Lg. However, the percentage of germination was higher among the kernels experiencing endosperm loss than among those with no loss of A. All of the deficient embryos occur in the latter group and presumably a fraction of these failed to germinate. When the data were corrected for the lethal embryos resulting from loss involving chromosome 3, the frequencies of loss in the endosperm and embryo were nearly the same, although the endosperm loss remained somewhat higher.

Dissimilar sperm arise at the second microspore mitosis in both TB-A translocations and high-loss plants, but they differ in their mode of origin. Nondisjunction of the B^A chromosome is unaffected by additional B's; hence, the fate of dissimilar sperm in double fertilization can be studied in plants with and without B's. The selective fertilization of the egg by the hyperploid sperm first found by Roman disappeared when the number of B's was great enough to insure that both sperm possessed them (Carlson, 1969). On the other hand, the dissimilar sperm of high-loss pollen arise only when the plants have several B's. Since dissimilar sperm are not produced in 0 or low B plants of the high-loss strain, it is impossible to determine whether or not selective fertilization would take place in the absence of B chromosomes. Carlson predicted that the dissimilar sperm of high-loss pollen would be randomly involved in double fertilization because of the swamping effect of B's and this appears to be the case when our data are corrected for lethal zygotes. The pattern of fertilization in high-loss plants without B's remains unknown.

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6. A cytogenetic analysis of a terminal deficiency in chromosome 3.

In our 1972 paper in Genetics on the high-loss phenomenon, we reported that 207 of the 208 cases of a modified chromosome 3 involved the deletion of the large heterochromatic knob in the long arm at position 0.6. The one exception, which may be causally unrelated to the high-loss mechanism, had a deficient chromosome 3. Cytological observations at pachynema disclosed that the break in 3L occurred near its distal tip with the terminal deficiency consisting of two or three minute chromomeres. Plants heterozygous for a normal 3 (N3) and the deficient 3 (Df3) produce normal sized pollen having the N3 chromosome and grains markedly reduced in size with the Df3 chromosome. The small pollen grains rarely, if ever, achieve fertilization in competition with normal pollen although they usually contain a considerable amount of starch. In contrast to the failure of the Df3 chromosome to be pollen transmitted is the ability of a varying fraction of Df3 megaspores to form functional embryo sacs, and thus be available for subsequent cytogenetic studies. The testcross data from a number of sib plants heterozygous for the $\underline{Gl}_6$, $\underline{Lg}_2$, and $\underline{A}_1$ loci and carrying a N3 and Df3 are given in Table 1. Individuals heterozygous for the Df3 chromosome have two sizes of pollen grains while homozygous N3 plants have full sized grains only.

The four point linkage data show that the deficiency lies approximately 19 crossover units to the right of the A locus and that the heterozygous deficiency apparently has no inhibitory effect on recombination in the long arm of 3. The terminal nature of the deficiency makes it possible to estimate with some accuracy the total genetic length of 3L. The glossy-6 locus lies within a few crossover units of the centromere, the Gl-Lg interval in the present data has 34 percent recombination, the Lg-A region 36% and the A-Df interval 19% of recombination. Making no allowance for undetected double exchanges in the relatively long Gl-Lg and Lg-A regions, we have a minimum map length of ca. 90 units. The true length is undoubtedly somewhat greater since we have no precise measure of crossing over in the centromere-Gl interval or in the segment comprising the deficiency, but these data provide a fairly good estimate.

Male recombination data were obtained from the reciprocal of the cross given in Table 1. Since no or little Df pollen successfully competes with normal pollen, the percentage of A offspring measures the frequency of

Table 1

Four point testcross data from the cross of $\frac{Gl\ \ Lg\ \ A\ \ Df}{gl\ \ lg\ \ a\ \ N}$ (1) (2) (3) ears by gl lg a N pollen.

(0)	(0)	(1)	(1)	(2)	(2)	(3)	(3)	(1-2)	(1-2)	(1-3)	(1-3)	(2-3)	(2-3)	(1-2-3)	(1-2-3)	
Gl Lg A Df	gl lg a N	Gl lg a N	gl Lg A Df	Gl Lg a N	gl lg A Df	Gl Lg A N	gl lg a Df	Gl lg A Df	gl Lg a N	Gl lg a Df	gl Lg A N	Gl Lg a Df	gl lg A N	Gl lg A N	gl Lg a Df	Σ
63	249	134	41	174	58	59	37	19	75	22	41	12	8	8	4	1004

Transmission frequencies: % Gl = 48.9 % Lg = 46.7 % A = 29.6 % Df = 25.5

Recombination frequencies: Gl-Lg = 34.3% Lg-A = 35.7% A-Df = 19.0%

recombination between A and Df. The testcross data from field grown populations are given below with the crossover regions indicated in parentheses.

(3)	(1-2-3)	(1-3)	(2-3)	(2)	(1)	(1-2)	(0)	
Gl Lg A	Gl lg A	gl Lg A	gl lg A	Gl Lg a	Gl lg a	gl Lg a	gl lg a	Σ
174	34	62	79	461	261	204	538	1813

Transmission frequencies: % Gl = 51.3 % Lg = 49.7 % A = 19.2

Recombination frequencies: Gl-Lg = 30.9% Lg-A = 42.9% A-Df = 19.2%

The recombination between A and Df is the same in the male and female testcross data. The male data give a somewhat lower value for the Gl-Lg and a higher frequency for the Lg-A region than do the female data. The sum of the recombination values for the several regions is approximately the same in male and female meiocytes. Three point data from the testcross of A Et Df/a et N heterozygotes confirmed the location of Df close to the distal end and placed the Et locus nine crossover units proximal to Df.

The following transmission frequencies for the individual ears providing most of the data summarized in Table 1 indicate that modifying genes affect the development of Df megaspores into viable embryo sacs. In some progenies the number of F_1 individuals with the Df chromosome equalled the number with a normal 3, indicating that Df megaspores were as viable as those with N3, while other sib families had much lower frequencies of functioning Df ovules. Because of the linkage of A and Df, there is a strong correlation between the percentage of A kernels on the testcrossed ears and the percentage of functioning Df embryo sacs.

Plant	% A kernels on ear	% N/Df plants	Field population
30910-3	30.6	36.1	72
" 5	38.5	28.0	143
" 15	26.1	25.5	184
" a	46.8	45.8	203
" b	17.8	14.5	202
" c	46.3	51.2	123
" d	18.4	10.6	179
" e	22.0	17.1	205

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7. Absence of chromatin loss during embryo sac development in high-loss plants.

In our earlier papers, we stated that loss of knobbed A chromatin was restricted to the second microspore division and that it did not occur during development of the megaspore into the embryo sac. This statement was based on the failure to find kernels with colorless aleurone when plants from the high-loss strain with knobbed chromosomes 3 homozygous for the A_1 allele were used as the female parent in crosses with recessive a_1 pollen parents. The reciprocal cross gave as much as 20% colorless kernels in some cases. However, the lack of colorless kernels in the female cross cannot be taken as conclusive evidence that chromatin loss did not take place. The embryo sac has a more complicated life history than does the male gametophyte. Three instead of two mitoses occur and the mature female gametophyte is an 8-nucleated structure. One of the polar nuclei and the egg nucleus, which are sisters, come from the micropylar half of the embryo sac while the other polar nucleus is derived from the chalazal portion. The triploid endosperm arises from the fusion of a sperm with the two polar nuclei, one coming from the micropylar and the other from the chalazal end of the embryo sac. The frequency with which embryos and endosperms deficient for the A locus are expected following chromatin loss of the A allele at the first, second, and third megaspore mitoses is given below. It is assumed that the mechanism of loss, if loss does occur, is the same as in the second microspore division, where only one of the two chromatids is deficient.

Time of postulated loss in embryo sac development	Frequency of embryos deficient for A (%)	Frequency of endosperms deficient for A (%)
1st megaspore mitosis	50	0
2nd megaspore mitosis		
Loss in micropylar nucleus	50	0
Loss in chalazal nucleus	0	0
Coincident loss in both	50	25
3rd megaspore mitosis		
Loss in any one of the four nuclei	12.5	0
Loss in any two	25.0	4.2
Loss in any three	37.5	12.5
Loss in all four	50.0	25.0

In the above tabulation it is evident that chromatin elimination during embryo sac formation will lead to deficient endosperms much less frequently than to deficient embryos. In order to more accurately assess the occurrence of loss in the megaspore mitoses, crosses were made using plants of the high-loss line with knobbed chromosomes 3 carrying the dominant $\underline{Gl}_6$, $\underline{Lg}_2$, and $\underline{A}_1$ alleles as the female parent in crosses with gl lg a pollen. The recessive gl and lg alleles produce glossy and ligule-less seedlings, respectively, when homozygous or hemizygous. These high-loss plants gave from 10-12 percent of A loss when used as the pollen parent but produced no kernels with colorless endosperm when used as the egg parent. In a population of over 4000 from crosses with high-loss plants as the female parent, all of the F_1 kernels were colored and no F_1 sporophytes were found exhibiting the recessive gl, lg, or a phenotypes expected following elimination of part or all of chromosome 3--i.e., there were no deficient embryos. Our conclusion that B-chromosome induced loss of knobbed A chromosomes is restricted to the second microspore division and does not take place during embryo sac development is confirmed by these more exacting tests.

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1. Intraspecies variation of ribosomal gene redundancy in Zea mays.

Ribosomal genes in eukaryotes are highly redundant. Considerable variation in the level of rDNA cistron redundancy among species has been reported but it seems to be generally accepted that intraspecies variability in redundancy level is small. Ribosomal DNA variation as a result of natural variation, mutation, deletions, or duplications has been reported for a few species (3, 6, 7, 9, 10).

While examining the question of rDNA arrangement at the nucleolar organizer region (NOR) and differential activity and competition of the

NOR or NOR portions in translocated stocks (translocation through the NOR) in maize, it was necessary to establish the base level of rDNA redundancy. In these studies we noted that a variation in the levels of rDNA redundancy existed in the different maize stocks. The first report on the number of rRNA genes and the localization of rDNA cistrons at the NOR in maize and higher plants was published by Phillips et al. (7). Ingle et al. (4) later reported a different level of DNA-rRNA hybridization for maize. The saturation hybridization levels for maize reported by the two laboratories are 0.37%, 0.26% (7) and 0.27% (4).

The differences in the percent of rDNA in maize reported by Phillips and Ingle may be attributed to differences in hybridization techniques or to differences in DNA values used to determine the genome size. However, our experimental data supports the hypothesis that the number of rDNA cistrons is not constant in maize but varies from one strain to another. Since our results (Table 1) coincide with results reported by the two different laboratories plus additional values, we feel that such variations are not due to technique differences or other factors, but are real variations in genetically controlled gene redundancy.

Table 1

Number of rRNA genes of different inbred strains of maize (Zea mays)

Strain	% DNA hybridized at saturation	rRNA genes/2C[1]
FS (hybrid)	0.391	1.82×10^4
432	0.360	1.68×10^4
Black Mexican, no B's	0.358	1.67×10^4
Black Mexican + 4 B's[2]	0.314	
Black Mexican corrected	0.358	1.67×10^4
KYS	0.339	1.57×10^4
W22	0.254	1.18×10^4

1. 15.5×10^{-12} g/2C cell (McLeish and Sunderland, 1961).
2. B chromosome 3.8% (Ayonoadu and Rees, 1971).

We examined the variation in redundancy levels of rDNA cistrons in several inbred lines of maize by molecular DNA-rRNA hybridization. The assay consists of saturation hybridization of ^{32}P-labeled, MAK purified maize rRNA with MAK purified DNA from nuclei (8). Hybridization was carried out on millipore filters; the amount of DNA per filter was determined by HCl extraction after counting (2). The results shown on Table 1 are based on the mean value of at least four separate extractions and four separate hybridization experiments. All hybridization results are reported as percent of nuclear DNA which hybridizes with rRNA.

The amount of nuclear DNA which hybridizes with rRNA in the different inbred lines examined, varied from 0.254% to 0.391%; the stock with the higher level having approximately 54% more rDNA cistrons than the line with the lower level. The number of rDNA cistrons per diploid genome thus ranges from 1.18×10^4 to 1.82×10^4.

Since our study utilizes strains with B chromosomes, it is necessary to correct for the additional DNA contributed by the B chromosome which is considered genetically inactive and not part of the normal genome. The Black Mexican strain with and without B chromosomes was used as control. Table 1 shows that the saturation level of Black Mexican strain without B chromosomes was 0.358% while saturation level of Black Mexican strain with four B chromosomes was 0.314%. Gene redundancy would appear to be different between the two strains. Each B chromosome contains approximately 3.8% of the DNA of the total genome (1). By making the correction for the four B chromosomes found in this strain, the saturation level changes from 0.314% to 0.358% per normal diploid genome. This shows that correction is possible and necessary in order to determine the normal diploid rDNA redundancy of strains with and without B chromosomes and that the level of redundancy may be genetically controlled since these strains have been maintained separate for some time.

In light of the range in rDNA cistron variation that exists in maize, it is important that the background level of redundancy be established for each inbred line that is used. In experiments using lines with B chromosomes or any form of aneuploidy, the base level of rDNA cistrons must be known, otherwise any variation from the normal would definitely

be altered if the background was not known or a different background used for its base level, e.g. 0.25% or 0.39%.

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2. Isolation and purification of maize nuclear DNA.

A large number of methods have been published for extraction of nucleic acids from plant material. The variety of approaches used attests to the difficulties of plant material. Our own experience and discussions with people working with maize suggest that DNA extraction from maize is particularly difficult.

A method has been devised in our laboratory for preparing highly purified DNA from maize nuclei in high yield. The method borrows primarily from reports of Marmur (2) and Kirby (1) and incorporates modifications and/or suggestions from several other laboratories (3, 5).

Materials: Nuclei were prepared from 5-6 day old corn seedlings and mature leaf material with good results. Seedlings were obtained by germinating seeds on paper toweling moistened with 0.1 mM $CaCl_2$ after surface sterilization with 10% sodium hypochlorite. Leaf material from mature plants was harvested from field or greenhouse grown plants, washed and deribbed.

Buffers: Grinding Sucrose-Tris Buffer - 0.5 M sucrose; 0.01 M $MgCl_2$; 0.05 M Tris; 0.025 M KCl; 0.005 M mercaptoethanol; pH 8.2 (3).

Saline-EDTA Buffer - 0.15 M NaCl; 0.1 M EDTA; pH 8.0.

Standard Saline Citrate (SSC) - 0.15 M NaCl; 0.015 M Na citrate.

Nuclear isolation:

1. Wash and soak seedlings and deribbed leaves in 0.001 M NH_4OH for 1 to 2 hours.

2. Drain, blot, chop and infiltrate the tissue with grinding sucrose-tris buffer under full vacuum for at least 30 minutes and store at 2-4°C overnight.

3. Drain and grind the tissue in an equal-weight of fresh sucrose-tris buffer with ice-cold acid-washed sand, mortar and pestle.

4. Filter the homogenate through four layers of cheesecloth and four layers of Miracloth. Centrifuge at 1000 x g in an SS-34 head (Sorvall RC-2) for 10 minutes at 2°C.

5. Resuspend, wash and centrifuge the pellet, containing nuclei and chloroplast, as above for three successive times with half grinding volume of sucrose-tris buffer containing 3.5% Triton X-100 (Rohm and Haas) to remove the chloroplasts.

DNA extraction: 6. Resuspend the nuclear pellet in 1 ml NaCl-EDTA per 2.5 g of fresh tissue.

7. Lyse the nuclei by adding solid sodium dodecylsulfate (SDS) to make 2% SDS and shake gently for 0.5-1 hour at room temperature.

8. Add solid $NaClO_4$ to make the solution 1 M to dissociate proteins (solid NaCl to make 2.5 M also gives good results).

9. Extract and deproteinize the DNA three times with chloroform-n, octanol (19:1 by volume), and centrifuge at 10,000 rpm for 10 minutes.

10. Layer the aqueous portion (nucleic acids) with two volumes of 95% cold ethanol. The DNA is either spooled out with a glass rod or centrifuged at 5,000 rpm for 5 minutes. Suspend the pelleted DNA in 0.1 X SSC.

11. Further purify the DNA by digesting for 0.5-1 hour at 37°C with 50 ug/ml of pancreatic RNase (dissolved in 0.1 X SSC, pH 5, and predigested at 80°C for 10 minutes) and 50 units of T_1 RNase. Add self-digested pronase to a concentration of 50 ug/ml and incubate for an additional 30 minutes at 37°C. Adjust the NaCl concentration to 2.5 M by adding solid NaCl, 1.46 g per 10 ml of solution and reextract the solution twice with an equal volume of chloroform-n, octanol. Precipitate the DNA with two volumes of 95% cold ethanol and suspend in 0.1 X SSC.

12. Chromatograph the DNA suspension on a methylated-albumin-kiselguhr (MAK) column (4) and elute with a 0.2 to 1.2 M NaCl (0.05 M PO_4, pH 6.8) buffer gradient.

Rationale: This procedure involves isolation of nuclei. This is necessary for our purpose because it eliminates chloroplast or mitochondrial DNA contamination. It has the additional advantage of eliminating chloroplast pigment contamination. Due to heavy cell walls, high nuclease content and heavy shearing forces required for disrupting the cells, vacuoles are disrupted and active nucleases released. In a number of plants the

vacuolar pH is also very low and this plus the nucleases quickly denature and degrade DNA. Vacuolar membranes are likely to be disrupted before cells are broken and Tris and/or other buffers may not penetrate rapidly enough to offset acidity or nucleases. Stern (5) suggested that carrying out the entire isolation at high pH would effectively neutralize nucleases. Thus it is possible that by removing the nuclei from the presence of cytoplasmic degradative enzymes (especially hydrolytic enzymes released from broken vacuoles) prior to lysis, additional protection is afforded the DNA. These enzymes are probably the major cause of poor results in DNA extraction from plants.

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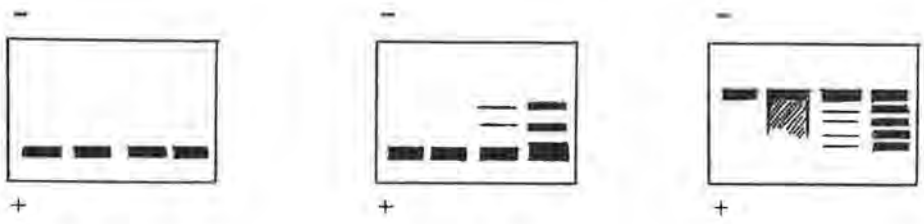
1. Isozyme pattern of catalase in the developing maize endosperm.

It is known that in maize endosperm the enzyme catalase is controlled by the $\underline{Ct}_1$ locus (Scandalios, J.G. 1969). Furthermore, a second locus, $\underline{Ct}_2$, has been detected in the scutella acting to control catalase synthesis. The products of the $\underline{Ct}_2$ and $\underline{Ct}_1$ loci tend to aggregate and, as a result, at a certain stage of seed germination a catalase pattern emerges consisting of 5 isozymes. In this paper we report the isozyme pattern of catalase in the developing endosperm. The study was carried out on 32 inbred lines of maize and 22 interline hybrids. The endosperm was fixed in solid carbonic acid at 13, 16, 19 and 25 days after pollination.

Electrophoretic analysis of endosperm isozymes was carried out according to standard procedures (Poulik, M.D., 1957 and Scandalios, J.G., 1969).

The 32 lines studied were assigned to 3 groups which we have designated: 1-catalase, 3-catalase and 5-catalase. 1-catalase (12 lines) were those in which only one electrophoretic catalase variant was revealed from days 13 to 25 after pollination. 3-catalase (15 lines) were those in which one electrophoretic catalase variant was detected on day 13 and two additional isozymes were established in the developing endosperm. They stain less intensely than the first isozyme. 5-catalase (5 lines) were those in which one electrophoretic catalase variant was found in the endosperm on day 13, and on day 16 a new band was observed which on day 19 displayed five distinct isozymes (Fig. 1).

Fig. 1



Based on these findings, it is considered that in some lines there functions a second locus in the endosperm controlling catalase synthesis. Just as in scutella, there seems to be no hindrance to the aggregation of the subunits of the two loci so that some lines produce 5 isozymes on the zymogram. We cannot attribute this observation to heterozygosity for the Ct_1 locus, since, in this case, the zymogram shows 4 isozymes with a characteristic distribution of staining intensity of each zone which reflects the double dosage effect of the maternal allele (Scandalios, J.G., 1969); moreover, in the segregating corn-cob half of the seeds would have a single zone of catalase activity. In 5-catalase lines all the seeds exhibited a specific pattern with the electrophoretically slowest isozyme staining most intensely. The other 4 isozymes stained equally well. The

existence of 5-catalase and 3-catalase lines may be explained, on the one hand, by the different activity of the second locus in various lines (i.e., the different amounts of gene product per time unit). It may be suggested, on the other hand, that subunits and catalase molecules differ in the rates of their synthesis or degradation. For this reason, we found no products of the second locus in the developing endosperm in 12 lines studied, in 15 lines the relative contributions of the products of the two loci were such that only three isozymes were distinguished on the zymogram and in 5 lines the proportions of the two types of subunits were more favorable permitting resolution of all 5 isozymes. Scandalios has described a maize line containing 3 catalase isozymes and believes that they are controlled by a sixth allele of the $\underline{Ct}_1$ locus referred to as V'. In the light of our observation, it may be assumed that in this line a second catalase locus is active.

1-catalase, 3-catalase and 5-catalase lines were used in crosses and backcrosses. In these hybrids, the pattern of isozyme catalase was studied in the developing endosperm. Hybrids between lines with the same catalase pattern (same number of isozymes and similar electrophoretic mobility) gave a pattern identical with that observed in these lines. When the crosses involved lines differing in the electrophoretic variant of catalase, a hybrid pattern occurred consisting of 4 isozymes with the distribution frequency of isozyme staining reflecting the effect of double dosage of the maternal allele. In crosses between the 1-catalase line with the 3-catalase and 5-catalase lines, the results depended on the type of cross. When 3-catalase or 5-catalase lines were used as paternal lines, the hybrid pattern of catalase was unaffected. In backcrosses, where the 3-catalase or 5-catalase lines were used as the maternal plants, the pattern deviated from the one usually observed: the bounding lines of the isozymes were blurred and some bands frequently merged into one spot. This is indirect support of the idea that additional isozymes altering this pattern are the product of a second functioning locus.

Thus, evidence has been obtained corroborating a two-loci system which controls catalase synthesis in the developing endosperm of maize.

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1. Effect of segment relocation on intragenic recombination (continued).

In two previous notes (MNL 44 and 45), experiments were described and results presented on the effect of segment relocation on intragenic recombination. The assay of intragenic recombination was that of the wx locus utilizing 4 alleles and 5 proximal relocations (see figure 1), i.e., five relocations of the wx locus away from the centromere and 5 distal relocations, i.e., 5 different sized segments distal to the wx locus. Each of the heteroalleles (ex. wx^x/wx^y) was analyzed as a homotranslocation. The results of these experiments can be summarized as follows:

(1) In most instances, the frequencies of wx intragenic recombination in the rearranged chromosomes, both in proximal and in distal series, were lower than those in the controls.

(2) Among the relocated segments, there is a linear correlation between the wx-centromere distances and the wx recombination frequencies (see figure 2). The longer distance between the wx locus and the centromere results in higher recombination values. This supports the concept of a continuing distribution of exchange events proportional to distance and subject to limitations imposed by the centromere effect on linked exchange and in this instance on intragenic recombination.

(3) Each of the heteroallelic combinations responded differently with respect to differences from the control in percentage change in recombination value at the same proximal relocated position. This is influenced principally by the closeness of the wx-breakage point distance. A greater diversity in degree of change from control is associated with the shorter distance between the wx locus and the breakage point.

(4) On homoalleles:

(a) The frequency of occurrence of Wx pollen grains from homoalleles was lower among inbreds than from outcross sources indicating background effect on this change.

FIGURE 1.

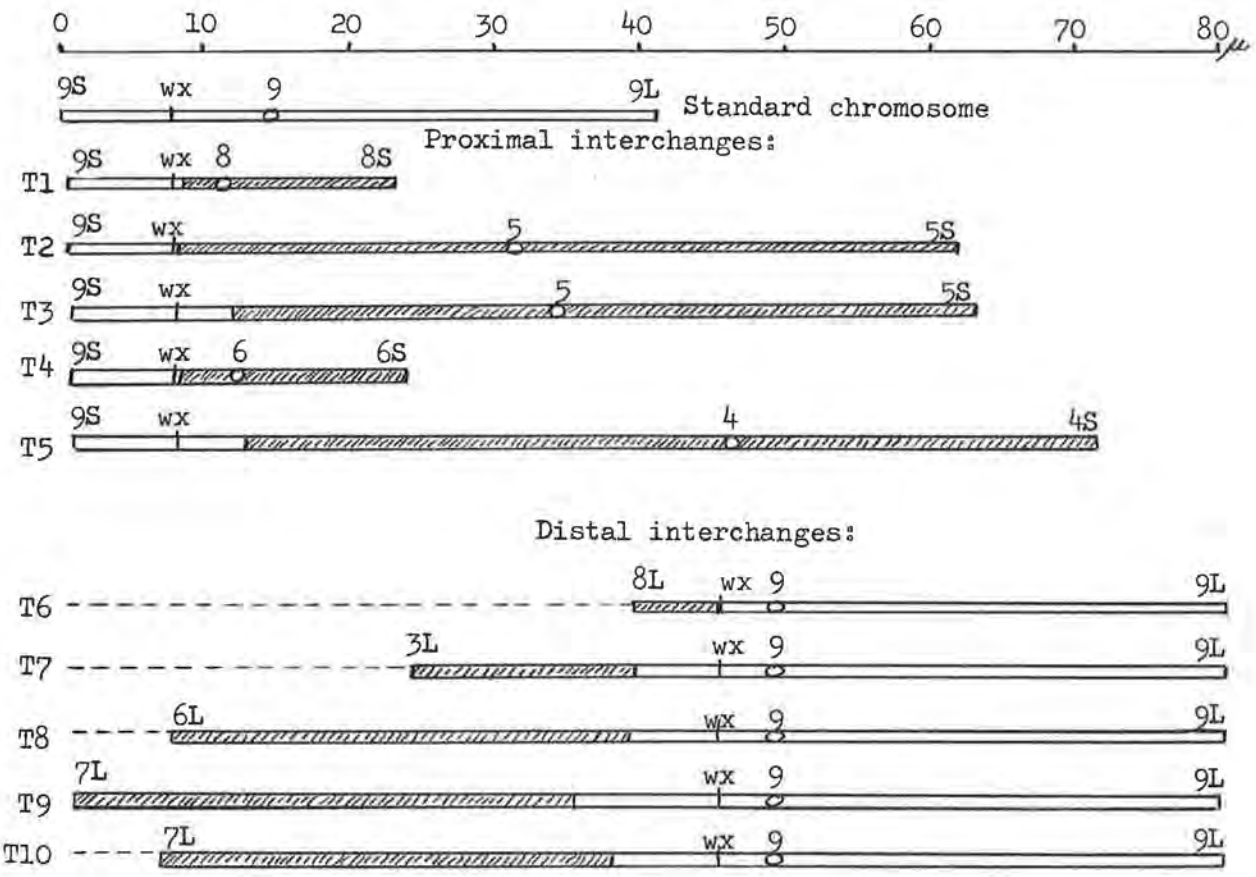
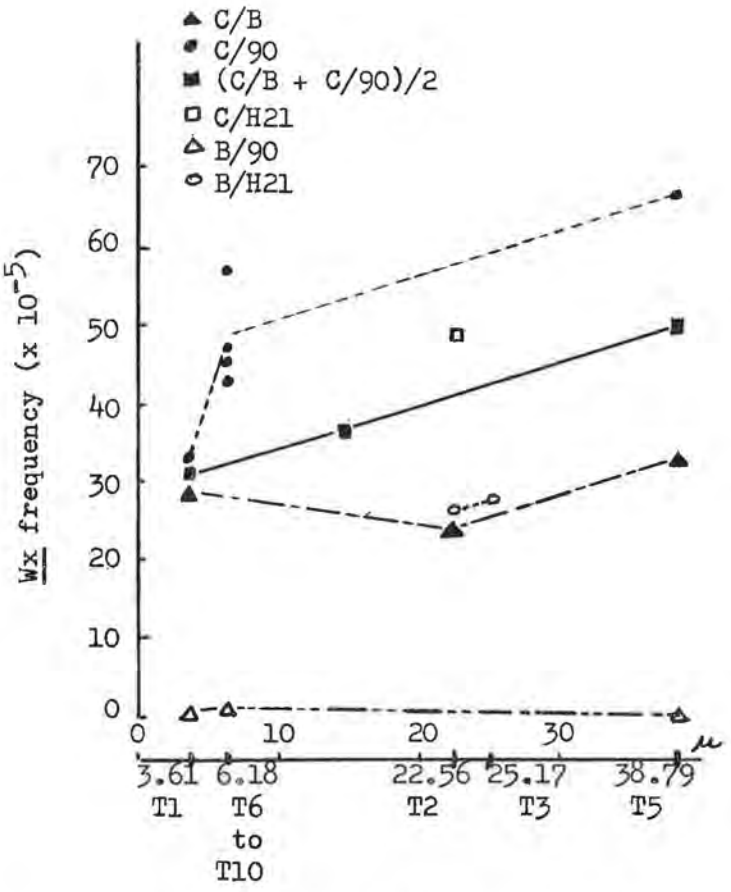

0
10
20
30
40
50
60
70
80
9S
wx
9
9L
Standard chromosome
Proximal interchanges:
T1
9S
wx
8
8S
T2
9S
wx
5
5S
T3
9S
wx
5
5S
T4
9S
wx
6
6S
T5
9S
wx
4
4S
Distal interchanges:
T6
8L
wx
9
9L
T7
3L
wx
9
9L
T8
6L
wx
9
9L
T9
7L
wx
9
9L
T10
7L
wx
9
9L

FIGURE 2.


C/B
C/90
(C/B + C/90)/2
C/H21
B/90
B/H21
Wx frequency (x 10⁻⁵)
70
60
50
40
30
20
10
0
0
10
20
30
u
3.61
6.18
22.56
25.17
38.79
T1
T6 to T10
T2
T3
T5
wx-centromere distance (u)

(b) When proportionate changes from the controls are compared, significantly different Wx frequencies are found for the same homoallelic combinations at the same position. These differences are not assignable to the influence of the wx-centromere or wx-breakage point distances.

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2. The $a_2^{m(r\text{-}pa\text{-}pu)}$ allele: phase changes.

The $a_2^{m(r\text{-}pa\text{-}pu)}$ allele is a derivative of $a_2^{m\ 1\ 1511}$ (Peterson 1968, Genetics 59: 391) of the En system. In the absence of En this allele shows a uniform pale pigmentation and in the presence of En it shows purple, pale and colorless sectors in a colorless background. Changes in the mutability pattern from higher to lower levels have been observed among kernels and some of these have been ascribed to changes in En. Thus, En undergoes phase variation changing from periods of high activity to various levels of lower activity that is expressed in a reduced ability to suppress the $a_2^{m(r\text{-}pa\text{-}pu)}$ allele and the responsive $a_2^{m(r)}$ allele. This altered activity, designated En^v (En variable) originally exhibiting a low level of activity expressed a higher level of activity in kernels of ears from tillers than in kernels of ears from main stalks of the same plant. These different levels of En^v expression were inherited in the next generation in main stalk ears indicating that En^v itself had been altered. Thus, En^v is in a labile or unstable condition and susceptible to environmental alterations that influence its level of activity.

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3. Hydrolytic enzymes during development of SCLB.

Resistant (N-Normal) and susceptible (T-Texas male sterile) varieties of maize, infected with Helminthosporium maydis race T (SCLB -

Southern corn leaf blight disease), show differences in hydrolytic enzyme activity. At various stages during the development of lesions, leaves were assayed for β-amylase, ribonuclease, acid phosphatase and β-glucosidase and compared with uninfected leaves. The greatest responses approach two-fold increases in the amount of activity. Each enzyme shows a distinctive pattern of activity changes with time. β-amylase activity in N leaves rises sharply after infection and drops again after 4 days, but in T leaves, only a small change in activity occurs with the maximum at 7 days after inoculation. β-glucosidase activity rises sharply in both N and T leaves, but the maximum activity is reached later in T than in N leaves. The response of ribonuclease in N and T leaves is very similar during the first 4 days after inoculation. After this period, the activity declines in N leaves, but continues to increase in T. Acid phosphatase activity does not decline in the later stages of the experiment as did the other activities; a larger response to infection is seen in T rather than in N leaves.

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4. β-amylase activity between cytoplasms and among inbreds.

In a comparison of the cytoplasms of Normal (N) and Texas (T-Texas-cytoplasmic male sterile), consistently higher total and specific β-amylase activity was found in 10-day-old leaves of N plants. This was consistent in 9 inbred lines tested. The inbred lines differed markedly in total amylase activity ranging from a low of 2.7 to a high of 112.5 (mg maltose/g/hr). Differences in β-amylase activity between cytoplasms and between inbred lines were statistically significant. There was no interaction between cytoplasms and inbred lines indicating a constancy of effect due to the cytoplasm. For β-amylase activity T-restored lines were not consistent when compared to N though, in 4 out of 5 cases, the values of T-restored were closer to N than to T-sterile.

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1. Location of the modifier gene of the cl_1 locus confirmed.

In last year's News Letter (M.G.C.N.L. 46:93-95, 1972) I reported results that indicated the Cl_M locus is located on chromosome 8. The Cl_M locus has a series of dominant alleles (Cl_M^2, Cl_M^3, Cl_M^4, Cl_M^5, Cl_M^{M14g}, and Cl_M^{W22}) that modify the albino seedling phenotype in plants homozygous for one of the recessive alleles (cl_1, cl_p or w_{7716}) at the cl_1 locus (white-albino mutant) on chromosome three. In the presence of the modifiers, the albino phenotype can range from pale green to green depending on the modifier present and whether it is homozygous or heterozygous. The endosperm phenotype remains unaltered (i.e., white or pale yellow).

The tests reported last year involved an F_2 generation in the cross between a w_{7716} line without a Cl_M allele and the $wxT8\text{-}9_{6673}$ translocation in an M14 background which carried Cl_M^{M14p} (a pale green modifier). When the white or pale yellow seeds from this F_2 generation were separated for starchy and waxy and planted, there was a definite surplus of albino seedlings in the starchy class (219 pale green : 102 albino) and a deficiency of albinos in the waxy class (68 pale green : 6 albino). These results indicated that Cl_M^{M14p} was located on chromosome 8. However, the Cl_M^{M14p} gene has not as yet been tested for allelism with the other modifiers, so the possibility remained that we were dealing with a second modifier locus. This seemed unlikely since it had been shown previously that six independently occurring modifiers were all allelic.

Tests completed this year have confirmed that the Cl_M locus is on chromosome 8. In these tests, a $wxT8\text{-}9_{6673}$ line that had been crossed two times to the inbred OH43 was used. This inbred is known not to contain any dominant alleles at the Cl_M locus. The translocation line was crossed to a $cl_1\ cl_1\ Cl_M^3\ Cl_M^3$ stock (white or pale yellow endosperm; green seedlings and plants). The F_2 segregated for yellow and pale yellow seeds and the waxy alleles. The pale yellow seeds were separated into starchy and waxy classes and planted. If the modifier is carried on chromosome 8, the starchy seeds should show a deficiency for the albino seedling and the

waxy seeds should produce a surplus of albino seedlings. The results shown in Table 1 agree with these expectations and confirm the location of $\underline{Cl}_M$ on chromosome 8. As was the case with last year's linkage data with $\underline{wx}T8\text{-}9_{6673}$, there is a deficiency in the waxy class in the data reported in Table 1. The reason for this deficiency of waxy seeds is not known. However, the deficiency does not in any way obscure the linkage between $\underline{wx}$ and $\underline{Cl}_M^3$ since there are marked deviations in the expected directions from a 3:1 ratio in both the starchy and waxy classes. Chi square tests of the deviations from a 3:1 ratio in both classes give chi square values so large that their expected probabilities are considerably less than 0.01.

Table 1

Types of seedlings arising from pale yellow seeds in the F_2 from a cross between $\underline{wx}T8\text{-}9_{6673}$ (OH43) and $\underline{cl}_1\ \underline{cl}_1\ \underline{Cl}_M^3\ \underline{Cl}_M^3$.

Plant	Wx		wx	
	green	albino	green	albino
71-5185-1	20	1	4	5
-2	20	3	3	6
-3	22	3	6	3
-4	15	2	3	4
-5	23	6	4	5
-6	22	4	5	1
-7	31	5	2	7
-8	24	3	0	4
-9	14	1	4	3
-10	18	5	4	3
-11	34	4	2	1
-12	23	3	0	3
-13	25	4	3	8
-14	19	2	2	1
-15	26	6	5	5
-18	14	1	4	2
-19	28	5	2	8
-20	14	2	1	1
Total	392	60	54	70

That this linkage with waxy is due to the presence of the translocation is shown by the absence of any indication of linkage when the translocation is not present. Although not reported last year, an F_2 population from a cross between waxy-M14 and $\underline{w}_{7716}$ did not give any indication of linkage between the waxy locus and $\underline{Cl}_M^{M14p}$. An F_2 involving waxy-OH43 and $\underline{cl}_1$ $\underline{cl}_1$ $\underline{Cl}_M^3$ $\underline{Cl}_M^3$ also did not give any indication of linkage between waxy and $\underline{Cl}_M^3$ in tests completed this year. Since linkage with waxy only occurs in the presence of the 8-9 translocation and does not occur when the translocation is absent or in the presence of other wx-9 translocations involving chromosomes other than 8, the $\underline{Cl}_M$ locus must be located on chromosome 8.

Since $\underline{Cl}_M^3$ and $\underline{Cl}_M^{M14p}$ are both located on chromosome 8, it is very likely that $\underline{Cl}_M^{M14p}$ is indeed an allele at the $\underline{Cl}_M$ locus. Allele tests confirming this should be completed this year.

Donald S. Robertson

2. The ordering of $\underline{y}_9$ and $\underline{bf}_2$ on the linkage map of chromosome 10.

The $\underline{y}_9$ mutant has a pale yellow endosperm and seedlings that vary from yellow green to green. In the M.G.C.N.L. 44:81-83, 1970, I reported linkage of $\underline{y}_9$ with $\underline{g}_1$ and $\underline{bf}_2$ but the genes could not be ordered at that time. Tests with TB-10a (breakpoint 10L.35) (M.G.C.N.L. 44:84-91, 1970) showed that $\underline{y}_9$ was not uncovered by this translocation. Hypoploid tests indicated that $\underline{y}_9$ was about 22.8 or 15.2 units from the TB-10a breakpoint depending upon whether the hypoploid plant was used as a male or female, respectively, in the testcrosses.

Crosses were made in which plants carrying $\underline{y}_9$ and $\underline{bf}_2$ in coupling were pollinated by TB-10a plants. No $\underline{bf}_2$ seedlings were seen in the progeny of this cross, indicating that this locus is also proximal to the TB-10a breakpoint. Hypoploid plants from the above tests were in turn crossed to homozygous $\underline{y}_9$ $\underline{bf}_2$ plants. The results of these crosses are summarized in Table 1.

Table 1

Data from the crosses of $\underline{y}_9\ \underline{bf}_2/++$ hypoploid TB-10a plants with $\underline{y}_9\ \underline{y}_9\ \underline{bf}_2\ \underline{bf}_2$.

$+\ +$	$+\ \underline{bf}_2$	$\underline{y}_9\ +$	$\underline{y}_9\ \underline{bf}_2$	Total	% $+\ +$	% $+\ \underline{bf}_2$
80	68	0	323	471	17.0%	14.4%

The data from Table 1 indicate that $\underline{y}_9$ is distal to $\underline{bf}_2$ with respect to the TB-10a breakpoint. Crossing over between $\underline{bf}_2$ and the breakpoint results in the $+\ +$ class while crossovers between $\underline{y}_9$ and $\underline{bf}_2$ result in the $+\ \underline{bf}_2$ class. In order to get $\underline{y}_9\ +$ a double crossover would have to occur--one between $\underline{y}_9$ and $\underline{bf}_2$ and the other between $\underline{bf}_2$ and the breakpoint. Such double crossovers were not observed. These data indicate the order of $\underline{y}_9$-$\underline{bf}_2$-$\underline{g}_1$ in chromosome 10. This order was confirmed by a three-point test involving $\underline{y}_9$, $\underline{bf}_2$ and $\underline{g}_1$. The results of this testcross are given in Table 2; they indicate a linkage map of $\underline{y}_9$-3.6-$\underline{bf}_2$-18.2-$\underline{g}_1$.

Table 2

Testcross results from the trihybrid $+\ +\ \underline{g}_1/\underline{y}_9\ \underline{bf}_2\ +$.

	$+\ +\ \underline{g}_1$	$\underline{y}_9\underline{bf}_2+$	$+\ \underline{bf}_2\ +$	$\underline{y}_9\ +\ \underline{g}_1$	$+\ +\ +$	$\underline{y}_9\underline{bf}_2\underline{g}_1$	$+\ \underline{bf}_2\underline{g}_1$	$\underline{y}_9++$	Total
No.	229	257	14	5	52	57	1	2	617
%			3.1		17.7		0.5		

Donald S. Robertson

3. Linkage relationships of chlorophyll defective mutants on chromosome 6.

As part of a study of luteus seedling mutants, six ($\underline{w}_{8896}$, $\underline{l}_{Brawn\ \#1}$, $\underline{l}_{Blandy\ \#3}$, $\underline{l}_{4120}$, $\underline{l}_{10}$ and $\underline{l}_{4920}$) were found which showed close linkage with $\underline{y}_1$ on chromosome 6. Allele tests of the mutants demonstrated allelism only between $\underline{l}_{Brawn\ \#1}$ and $\underline{l}_{Blandy\ \#3}$. The linkage relationships of the

mutants were investigated to determine if there might be a cluster of closely linked luteus mutants on chromosome 6.

Phenotype

The basic phenotype of all these mutants was yellow (luteus). However, considerable variation of the basic luteus phenotype was observed in some of the mutants. Some of this variation is undoubtedly due to environmental differences, such as temperature, and differences in the genotypic background of the plants. The following is a brief phenotypic description of each mutant:

(1) $\underline{w}_{8896}$: In the original stock obtained from the Maize Genetics Cooperation, this was a dark yellow mutant. However, on crossing into our stocks the mutant was found to vary in its expression from a strong, to light, to very pale luteus, to an off-white. No obvious chlorophyll is present in this mutant.

(2) $\underline{l}_{4120}$-A: Dark yellow mutant that sometimes shows some greening of the leaf tips.

(3) $\underline{l}_{10}$: The darkest yellow of the five mutants with no obvious chlorophyll present.

(4) $\underline{l}_{\text{Brawn \#1}}$ and $\underline{l}_{\text{Blandy \#3}}$: Moderately dark yellow mutants that frequently but not always show considerable greening.

(5) $\underline{l}_{4920}$: This mutant shows variation in the amount of yellow, from a moderate degree of yellowing, to pale yellow, to off-white. Frequently the tips of the leaves will be green and occasionally chlorophyll is observed elsewhere on the leaf.

Table 1 gives the chlorophyll, carotene, and xanthophyll content of these mutants. For these analyses, seedlings were grown under conditions of 27°C and 2,000 foot candles of light. The phenotype indicated in the table is that observed for the seedlings before they were harvested for extraction.

Table 1

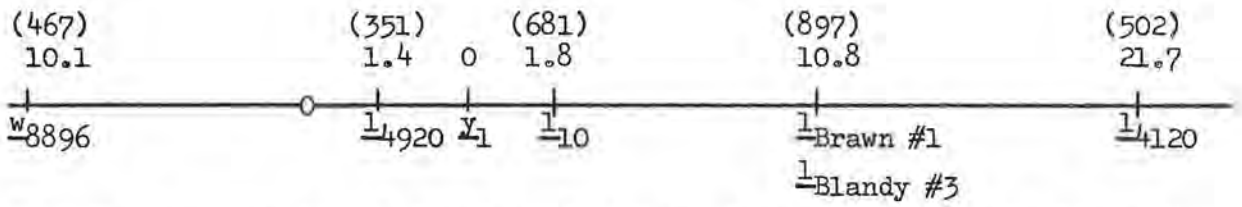
Chlorophyll, carotene, and xanthophyll determinations for a series of luteus mutants on chromosome 6.
(From M.G.C.N.L. 42:85-88, 1968.)

Mutant	Phenotype	chlorophyll mg/gm	carotene mg/gm	xanthophyll mg/gm
$\underline{w}_{8896}$	pale yellow	Trace	.006	.0090
$\underline{l}_{4120}$	yellow, green leaf tips	.0935	.0021	.0175
$\underline{l}_{10}$	good yellow	Trace	.0029	.0140
$\underline{l}_{\text{Brawn \#1}}$	yellow-green	.5939	.0222	.0800
$\underline{l}_{\text{Blandy \#3}}$	yellow with some green	.3115	.0176	.0432
$\underline{l}_{4920}$	pale yellow, green leaf tips	.0486	.0021	.0117
Normal	green	1.674	.0980	.1140

Linkage

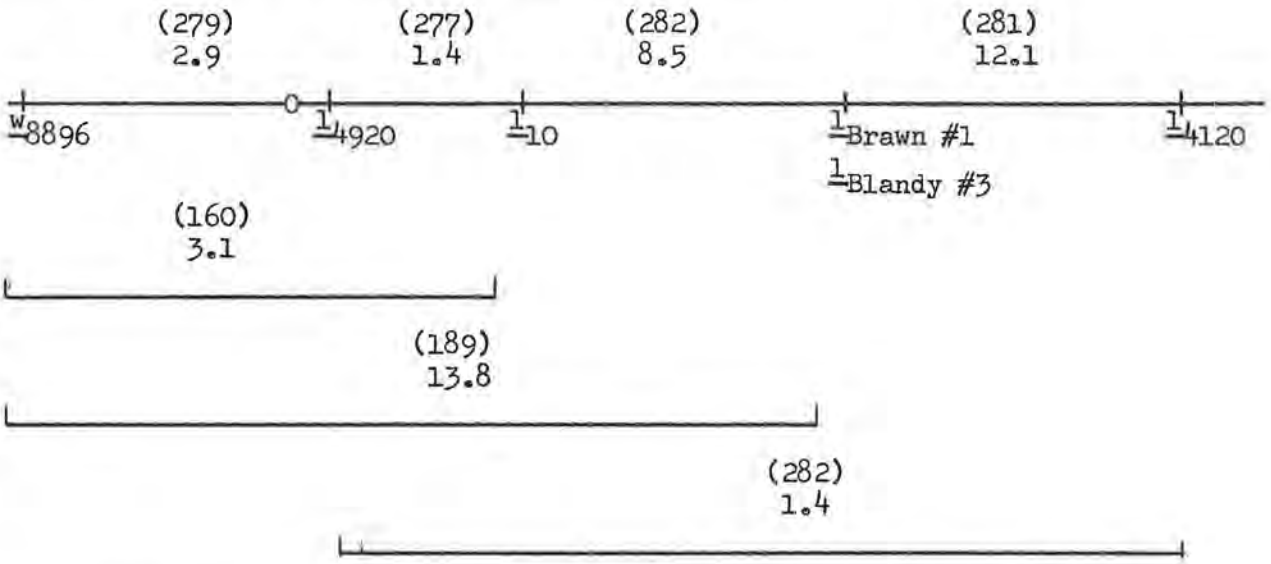
Each of the mutants was involved in three-point tests with $\underline{y}_1$ and one or more translocations. These tests permitted the placement of the genes on the linkage map with respect to $\underline{y}_1$. Tests of $\underline{w}_{8896}$ with T 1-6$_{4456}$ (6L.30) and T 1-6a (6L.54) indicated a map of $\underline{w}_{8896}$ - $\underline{y}_1$ - T. Crosses of $\underline{l}_{4120}$ with T 1-6$_{4456}$ (6L.30) and T 1-6a (6L.54) indicated an order of $\underline{y}_1$ - T - $\underline{l}_{4120}$ for T 1-6$_{4456}$ and an order of $\underline{y}_1$ - $\underline{l}_{4120}$ - T for T 1-6a. When $\underline{l}_{10}$ was tested with the same two translocations the order was determined to be $\underline{y}_1$ - $\underline{l}_{10}$ - T for both. Tests of $\underline{l}_{\text{Blandy \#3}}$ with T 1-6a (6L.54) gave a map of $\underline{y}_1$ - $\underline{l}_{\text{Blandy \#3}}$ - T, while crosses of T 6-9$_{4778}$ (6S.80) with $\underline{l}_{\text{Brawn \#1}}$ indicated a map of T - $\underline{y}_1$ - $\underline{l}_{\text{Brawn \#1}}$. The map for $\underline{l}_{4920}$ was determined to be $\underline{l}_{4920}$ - $\underline{y}_1$ - T from crosses with T 1-6a (6L.54) and T 1-6$_{4456}$ (6L.30). These tests placed $\underline{w}_{8896}$ and $\underline{l}_{4920}$ to the left and $\underline{l}_{10}$, $\underline{l}_{\text{Brawn \#1}}$ ($\underline{l}_{\text{Blandy \#3}}$) and $\underline{l}_{4120}$ to the right of $\underline{y}_1$.

Two-point tests of these genes with $\underline{y}_1$ established the following map. (Figures in parentheses indicate the total number of individuals in the testcross populations):



This map does not reveal a tight clustering of the five loci but rather a wide distribution extending over 30 map units.

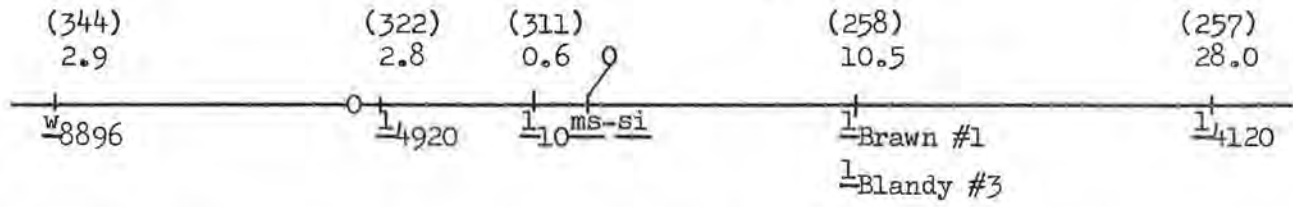
Seven intercrosses were made between these five loci and the crossover values determined for each cross. The following map summarizes the results of these crosses. (The genes are placed on this map in the positions determined by the y_1 linkage tests above):



Two discrepancies are obvious when this map is compared with the y_1 map. First, all linkage data of w_{8896} with other mutants would suggest that this gene should be closer to l_{4920} than the y_1 data indicated. The linkage values with l_{4920}, l_{10}, and $l_{Brawn\ \#1}$ are consistent in indicating this. Other linkage data to be presented below are in agreement with the location of w_{8896} closer to l_{4920}. Second, the l_{4920} - l_{4120} distance is much too short and is not in agreement with the y_1 data or the l_{10} - $l_{Brawn\ \#1}$ and $l_{Brawn\ \#1}$ - l_{4120} distances. The latter two agree with the y_1 data. The distance indicated by the l_{4920} - l_{4120} test probably is in error.

Linkage data were obtained for these five genes with ms-si (2 units to the right of y_1). These are presented on the following map. (The

genes are again placed in accordance with the $\underline{y}_1$ data.)



All linkage data are in agreement with the $\underline{y}_1$ data except for $\underline{w}_{8896}$. Again the data indicate $\underline{w}_{8896}$ should be closer to $\underline{l}_{4920}$. In this regard, the data agree with the intercross values.

Linkage tests were made between Pl and the five luteus genes. The linkage values obtained are indicated on the following map (gene position as determined by the $\underline{y}_1$ data):

Except for $\underline{l}_{4120}$, the data are consistent with the previous linkage tests. It is possible that a new mutant had occurred in the stocks used for this test and the new mutant was confused with $\underline{l}_{4120}$. Again notice that the data suggest $\underline{w}_{8896}$ should be closer to $\underline{l}_{4920}$; in fact, the data, in this instance, would place $\underline{w}_{8896}$ to the right of $\underline{l}_{4920}$.

Linkage data with $\underline{ms}_1$ were obtained for $\underline{w}_{8896}$ and $\underline{l}_{4120}$. We have obtained values that indicate $\underline{ms}_1$ is 2.8 units from $\underline{y}_1$, direction unknown. The $\underline{ms}_1$ - $\underline{l}_{4120}$ distance was 23.2 (177 plants tested), which is in close agreement with other linkage data for this locus. The $\underline{w}_{8896}$ - $\underline{ms}_1$ distance was 3.7 (294 plants tested). Again the linkage data indicate that $\underline{w}_{8896}$ should be closer to $\underline{l}_{4920}$ than the $\underline{y}_1$ data had indicated.

Also linkage of $\underline{su}_2$ with $\underline{l}_{4920}$ and $\underline{l}_{Blandy\ \#3}$ was determined. The crossover values were 28.1 for $\underline{l}_{4920}$ (235 plants tested) and 21.4 for $\underline{l}_{Blandy\ \#3}$ (214 plants tested). Although the reliability of these data is in doubt, because of the difficulty in classifying $\underline{su}_2$ in our stocks, they are in general agreement with other linkage values for these loci.

As was mentioned above, these luteus genes were crossed with several translocations involving chromosome 6. In addition to the translocations previously mentioned, tests were made involving 6-9c (6L.15) and 6-9e (6L.18). In general, the crossover values obtained with translocations were lower than those with genes. This is as expected, since translocations are known to frequently interfere with crossing over. However, in most instances where more than one gene was tested against a given translocation, the comparative magnitudes of the crossover values were as expected for the placement of genes from the $\underline{y}_1$ linkage data. As was indicated earlier, $\underline{l}_{4120}$ mapped to the right of T 1-6_{4456} (6L.30) and to the left of T 1-6a (6L.54). The crossing over was 1.9% (322 plants tested) with T 1-6_{4456} and 26.5% (298 plants tested) with T 1-6a. This places $\underline{l}_{4120}$ between 6L.30 and 6L.54, and probably close to 6L.30.

Table 2 presents a summary of the distances between these five genes as determined by the four two-point tests that were made.

Table 2

A summary of the distances determined between the five luteus genes on chromosome 6 as determined by four two-point tests.

Two-point test with:	$\underline{w}_{8896}$-$\underline{l}_{4920}$	$\underline{l}_{4920}$-$\underline{l}_{10}$	$\underline{l}_{10}$-$\underline{l}_{\text{Brawn \#1}}$	$\underline{l}_{\text{Brawn \#1}}$-$\underline{l}_{4120}$
$\underline{y}_1$	8.7	3.2	9.0	10.9
Intercrosses	2.9	1.4	8.5	12.1
ms-si	0.1	2.2	11.1	17.5
Pl	-1.5	1.5	6.7	----

The data are fairly consistent for each interval except $\underline{w}_{8896}$ - $\underline{l}_{4920}$, which shows considerable variation between tests and even reversal in order in the case of the Pl crosses. The weight of the evidence would suggest that $\underline{w}_{8896}$ is closer to $\underline{l}_{4920}$ than indicated by the $\underline{y}_1$ data.

Donald S. Robertson

4. Linkage relationships of w_1 and w_{8657} with chromosome 6 markers.

The albino mutant w_1 is an off-white mutant that interacts with l_1 to produce yellow seedlings. The w_{8657} mutant is a paper-white albino similar in seedling phenotype to the white endosperm albino mutants but with yellow seed color. Previous studies have indicated that both of these mutants are located on chromosome 6.

The results of two-point linkage studies of w_1 with ms_1, ms-si, Pl and su_2 are shown in Table 1. The loose linkage with ms_1 and ms-si, which are located within three units of y_1, indicate that w_1 is some distance from these loci. The linkage with Pl and su_2 would indicate that w_1 is located distal to y_1 in the long arm of chromosome 6 probably proximal to Pl by about 19 units.

Table 1

Linkage data from testcrosses involving w_1 and ms_1, ms-si, Pl and su_2.

Marker gene	Parental classes	Recombination classes	Total	% recombination
ms_1	80	66	146	45.2
ms-si	143	77	220	35.0
Pl	196	45	241	18.7
su_2	212	59	271	21.8

Linkage tests of w_{8657} with several chromosome 6 markers are given in Table 2 and testcross data involving several translocations are given in Table 3. The data from Table 2 indicate that w_{8657} is located in the long arm of chromosome 6, probably about 20 units distal to su_2. If this location is substantiated by further studies, it would make w_{8657} the most distal marker on chromosome 6. The translocation linkage data from Table 3 are consistent with the placement of w_{8657} well out in the long arm of chromosome 6, probably in the vicinity of the T1-6a breakpoint (L.54).

Table 2

Testcross data involving $\underline{w}_{8657}$ and chromosome 6 marker genes.

Marker gene	Parental classes	Recombination classes	Total	% recombination
y_1	158	86	244	35.2
ms_1	132	102	234	43.6
ms-si	168	115	283	40.6
Pl	157	49	206	23.8
su_2	93	23	116	19.8

Table 3

Testcross results involving $\underline{w}_{8657}$ and chromosome 6 translocations.

Trans-location	Chrom. 6 breakpoint	Parental classes	Recombi-nation classes	Total	% recombi-nation
6-9a	S.79	101	88	189	46.6
6-9c	L.15	174	44	218	20.2
6-9e	L.18	192	46	238	19.3
$1\text{-}6_{4456}$	L.30	200	67	267	25.1
1-6a	L.54	217	2	219	0.9

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1. The rate of alcohol dehydrogenase in vivo decay in endosperm of maize.

In studies relating to genetic control of enzyme activity, it is most important to know the rate of in vivo decay of the enzyme. In an earlier study (Efron and Schwartz, Proc. Natl. Acad. Sci. 61: 586-591, 1968) we have described a two factor system for the in vivo inactivation of maize alcohol dehydrogenase. In this study we have found that the ADH enzyme from embryo extracts is stable over prolonged periods of incubation at room temperature. However, we did not have information on the in vivo stability of the enzyme. In later studies (e.g. Efron, MNL 45: 25-27) we have described three inbred lines (AD-1, AD-7 and AD-19) having different activities of ADH. Until now we have not ruled out the possibility that the variation in ADH activity is due to differences in enzyme stability.

Endosperm from the developing kernel has been used in this study. We have followed ADH activity in the developing endosperm of the inbred lines AD-1, AD-7 and AD-19. Plants of the three lines were self pollinated in the field, harvested at different days after pollination and stored at -20°C. ADH activity was tested by following the rate of NAD reduction at 340 mμ. The results were calculated as ADH activity units/single kernel/ μg protein.

The three lines showed clear differences in their ADH activity in the endosperm (Table 1). AD-19 and AD-7 showed the highest and lowest activities, respectively. However, the relative change in activity with time was similar in all three lines. The relative activity (percent of the highest activity) was increased from eight to twenty-one days after pollination in about the same rate. From 21 days on, a slow decrease in activity was observed. ADH activity was not tested daily. Therefore, it is possible that higher activities could be found before or after 21 days. Nevertheless, these results suggest that ADH is synthesized in the endosperm during the first three weeks after pollination. It is assumed that the Adh_1 structural gene is "turned off" at this time and therefore the decrease in ADH activity may reflect its in vivo decay. Since it is

Table 1

ADH activity (Activity units/kernel/ug protein) in the developing endosperm of the inbred lines AD-1, AD-7 and AD-19.

Days after pollination	AD-7			AD-1			AD-19		
	Activity units	Relative rate of synthesis (%)	Relative rate of decay (%)	Activity units	Relative rate of synthesis (%)	Relative rate of decay (%)	Activity units	Relative rate of synthesis (%)	Relative rate of decay (%)
8	1.6	5.0		2.0	4.1		2.2	3.7	
10	5.0	15.6		8.4	17.4		10.2	17.2	
13	11.2	35.0		18.3	38.0		32.4	54.8	
17	28.4	88.7		40.5	84.0		53.7	90.8	
21	32.0	100.0		48.2	100.0		59.1	100.0	
26	30.1		100.0	44.0		100.0	54.0		100.0
30	26.2		87.0	37.2		84.5	48.2		89.2
35	20.6		68.4	32.3		73.4	44.3		82.0
40	15.8		52.4	26.1		59.3	36.1		66.8

possible that higher activities could be found between 21 and 26 days, the activities found at 26 days after pollination were used as a reference (100%) for the rate of decay.

The rate of the average daily decay was relatively very low (Table 2). The average decrease in activity units was similar in the three lines. But, since the levels of activity were different, the relative loss of activity was somewhat different too.

Table 2

Mean daily loss of ADH activity in the endosperm of AD-1, AD-7 and AD-19.

Days after pollination	Mean daily loss of ADH activity					
	Activity units			Percent of activity		
	AD-7	AD-1	AD-19	AD-7	AD-1	AD-19
26-30	.98	1.71	1.45	3.25	3.88	2.70
30-35	1.12	.98	.78	4.28	2.64	1.62
35-40	.96	1.24	1.64	4.68	3.96	3.72
26-40	1.02	1.28	1.28	3.40	2.90	2.40

Thus, it might be concluded that ADH is a stable enzyme unless it is actively inhibited (Efron and Schwartz, 1968), and that the differences in ADH activities of the three lines are not due to differences in enzyme stability.

Y. Efron

2. Extent of variation in ADH activity and Adh_1 genotypes among inbred lines of maize.

The subject of variation in enzyme activity and its genetic control is under investigation in our laboratory for the last few years. We have shown variation in acid phosphatase activity in maize (Efron, Biochem. Genet. 5: 33-44, 1971) and alcohol dehydrogenase activities in maize

Table 1

Distribution of ADH activity and *Adh*$_1$ genotypes among 213 different lines of maize.

ADH activity unit/min/mg dry seeds	No. of inbred lines	Adh_1 genotype			Both Adh_1^S and Adh_1^F are present
		Adh_1^S/Adh_1^S	Adh_1^F/Adh_1^F	$Adh_1^{F/C(m)}/Adh_1^{F/C(m)}$	
3.1 - 6.0	4	-	2	2	-
6.1 - 9.0	2	-	2	-	-
9.1 - 12.0	20	1	19	-	-
12.1 - 15.0	58	8	48	1	1
15.1 - 18.0	64	1	62	-	1
18.1 - 21.0	36	4	30	-	2
21.1 - 24.0	19	3	14	-	2
24.1 - 27.0	7	3	4	-	-
27.1 - 30.0	3	1	2	-	-

Table 2

Relative frequencies of $\underline{Adh_1}$ genotypes in three ADH activity levels.

Range of activity	No. of inbred lines	Adh_1 genotype			Both Adh_1^S and Adh_1^F are present
		Adh_1^S/Adh_1^S	Adh_1^F/Adh_1^F	$Adh_1^{F/C(m)}/Adh_1^{F/C(m)}$	
3.1 - 12.0	26 (100%)	1 (3.8%)	23 (88.5%)	2 (7.7%)	-
12.1 - 21.0	158 (100%)	13 (8.2%)	140 (88.6%)	1 (0.6%)	4 (2.5%)
21.1 - 30.0	29 (100%)	7 (24.1%)	20 (69.0%)	-	2 (6.9%)

(Efron MNL 45: 25-27, 1971) and in the genus Carthamus (Efron, Ashri and Peleg, Biochem. Genet., in press). We have concluded that such variation may be common and could be an important factor in evolution.

In the present study we have initiated a large scale investigation on the extent of variation in ADH activity and $\underline{Adh_1}$ genotypes among different inbred lines of maize. $\underline{Adh_1}$ genotype was tested by starch gel electrophoresis and ADH activity by following the rate of NAD reduction at 340 mμ. The activity was calculated in activity units/min/mg ground dry kernels.

Two hundred and thirteen different inbred lines have been tested so far. The results are summarized in Table 1.

Three $\underline{Adh_1}$ alleles, $\underline{Adh_1}^S$, $\underline{Adh_1}^F$, and $\underline{Adh_1}^{C(m)}$, have been described by Schwartz and Endo (Genetics 53:709-715, 1966) in maize. Two other alleles ($\underline{Adh_1}^u$ and $\underline{Adh_1}^w$) were induced artificially by EMS (Schwartz, personal communication). All three naturally occurring alleles have been found among the inbred lines tested. However, their frequencies were completely different (Table 1). About 90 percent of the inbreds were homozygous $\underline{Adh_1}^F/\underline{Adh_1}^F$ and only 10 percent $\underline{Adh_1}^S/\underline{Adh_1}^S$. The $\underline{Adh_1}^{C(m)}$, whose products do not show ADH activity, was found in only three lines and only as a duplication together with the $\underline{Adh_1}^F$ allele.

About tenfold differences in ADH activity have been found between the highest and lowest lines. Only the four lines with the lowest activity could be classified as a distinct group with very low activity. The other lines showed a continuous variation (Table 1). A study of this type is subjected to experimental variation despite all efforts to unify the experimental technique. Therefore, it is also possible that there were a number of distinct activity groups masked by the experimental variation. Most of the lines (about 75 percent) showed intermediate levels of activity (12-21 units), which may suggest that lower or higher activity levels are not desirable.

The comparison between the relative frequencies of the $\underline{Adh_1}^F/\underline{Adh_1}^F$ and $\underline{Adh_1}^S/\underline{Adh_1}^S$ homozygous lines among the activity groups is of most interest (Table 2). The relative proportion of the $\underline{Adh_1}^S/\underline{Adh_1}^S$ lines among the lines with the highest activity was significantly greater than among the lines with lower activity levels. This suggests again that electrophoretic mobility is not the only difference between allelic isozymes.

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1. Variation among inbreds for black spot maturity and filling period.

Recent interest in physiological maturity of maize grain determined by formation of a black layer at the base of the kernel prompted our investigation of the variation among inbreds for black spot maturity (planting to harvest) and filling period (pollination to harvest). Twenty maize inbred lines were examined over a three year period.

Appearance of the black spot always coincided with maximum dry weight accumulation. Moisture content at black spot maturity was significantly different among inbreds but the year means did not differ. There was a significant year x inbred interaction.

The growing degree days required to reach black spot maturity were significantly different among the inbreds and among years. However, interaction of inbreds with years was found to be minor. The variability among inbreds was always much greater than among years. Similar conclusions were made for the growing degree days required during the filling period.

The growing degree days required for the filling period had positive phenotypic and genotypic correlations with the growing degree days required for pollination, but the correlations were small enough to suggest possible selection for types with long filling periods and short time to pollination. The rate of kernel dry weight accumulation during the filling period was significantly different among inbreds and years but had a significant inbred X year interaction. The rate of kernel dry weight accumulation was not correlated with any character other than dry weight at black spot maturity.

A brief summarization of the three year averages is presented in Table 1.

Table 1

Three year summary of black spot maturity (BSM) and filling period variation among 20 maize inbreds.

	High inbred	Low inbred	Average of twenty inbreds
GDD from planting to BSM	1808	1337	1648
% moisture at BSM	35.0	15.4	25.2
Dry wt. at BSM (mg/k)	322	192	237
GDD from planting to pollination	1060	818	940
GDD in the filling period	821	512	708
Rate of dry wt. accumulation (mg/k/day)	9.7	6.2	7.7

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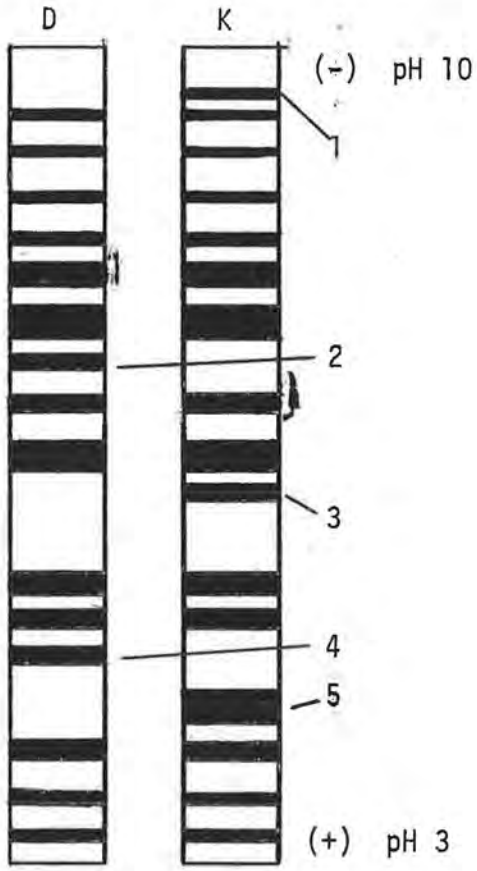
1. Electrophoretic separation of peroxidases of lines carrying two different male-sterile cytoplasms.

The two male-sterile cytoplasms, D and K, both arose in Turkey (Beckett, 1971) and respond alike to restoration alleles (Beckett, 1971). Moreover, maize lines carrying either of the two cytoplasms are resistant to attack by the fungus Helminthosporium maydis (Gracen, et al., 1971). Hence, by three phenotypic criteria, i.e., male-sterility, response to restoration alleles, and fungal resistance, the D and K mutant cytoplasms appear to be identical. However, the three phenotypic manifestations

Figure 1

Polyacrylamide Gels Stained For Peroxidase Activity Following Isoelectricfocusing of Cellular Proteins of Plants With D or K Male-Sterile Cytoplasm



mentioned above are gross expressions of an unknown number of interacting biochemical pathways and may not reveal differences between the two cytoplasms at the protein level.

I have subjected the cellular proteins of fourteen to sixteen day old corn seedlings, carrying D or K cytoplasm, to isoelectric focusing on polyacrylamide gels and stained the gels for peroxidase, esterase and acid phosphatase activities. Only the gels stained for peroxidase activity show any differences between the two cytoplasms. As shown in figure 1, the gel from K proteins has bands of peroxidase activity at points 1, 3, and 5 that are lacking on the D gel. At points 2 and 4 the K gel lacks a band that is present on the D gel.

The possible explanations of the multiple band differences are many. The easiest, and perhaps most naive, explanation is one of differential synthesis of protein species in the cells of the two cytoplasm-sterile lines. That is, the synthesis of the species of peroxidase at points 1, 3, and 5 is repressed in cells carrying D cytoplasm while the species of peroxidase at points 2 and 4 is repressed in cells carrying K cytoplasm. However, other explanations, e.g., aggregation of molecules and repressed activity, are possible and cannot be ruled out at this time.

A major question is one of isogenicity of the two lines. The nuclear background in both lines is CO192, the original lines having been backcrossed to CO192 as the recurrent parent for six generations. While it is possible that the five differential peroxidase bands actually represent five segregating genes, it seems unlikely in that esterase patterns (13 bands) and acid phosphatase patterns (4 bands) of the D and K gels are identical.

It is also impossible to correlate the five peroxidases with either a plasmagene or nuclear gene code. It would appear that the data are demonstrating different cytoplasmic-nuclear interactions in the peroxidase activities of several proteins.

References:

Beckett, J. B. 1971. Classification of Male-Sterile Cytoplasms in Maize (Zea mays L.). Crop Sci. 11: 724-727.

Gracen, V. E., M. J. Forster, and C. O. Grogan. 1971. Reactions of Corn (Zea mays) Genotypes and Cytoplasms to Helminthosporium maydis Toxin. Pl. Dis. Rptr. 55: 938-941.

Clyde F. Smith

2. Effects of the toxin of Helminthosporium maydis on cell membrane permeability.

Corn hybrids having the Texas male-sterile (Tcms) cytoplasm are more susceptible to southern corn leaf blight than are those having the normal male-fertile cytoplasm (N-cmf). Since diseased plants often display increased membrane permeability, the following experiment was undertaken in an effort to assess the effects of the toxin of the causal organism of the blight, Helminthosporium maydis Nisikado and Miyake, race T, on the cell membranes of susceptible plants.

An isolate of H. maydis, race T, was obtained from Dr. O. D. Morgan, Department of Botany, University of Maryland, College Park, Md. After inoculation into 500 ml lots of modified Fries' medium, the fungus was allowed to grow at room temperature for two weeks. The culture was harvested by filtration through Miracloth, after which the pH was raised to 7.0. Boiling for 5 minutes to kill spores was followed by freezing, and the culture filtrate was stored in the freezer until used.

Since earlier work in our laboratory had shown that effects of the crude culture filtrate were similar to those of partially purified toxin, the preparation of which requires many additional hours, the crude culture filtrate was used in the investigations.

Cut stem ends of the shoots of 13-day-old corn plants carrying either T-cms cytoplasm or N-cmf cytoplasm were placed in crude culture filtrate at 0° for 2 hours. Controls were placed in glass-distilled water. After toxin uptake, 1-gram samples of leaf tissue, cut into pieces 1-2 cm in length, were washed 5 times in glass-distilled water and transferred to 100 ml Ehrlenmeyer flasks which were then shaken at the rate of 110 strokes per minute at 25° C. Ten ml aliquots of the ambient solution were withdrawn at the end of 4, 8, 12, and 16 hours.

These samples were then analyzed, using a Perkin-Elmer 303 Atomic Absorption Spectrophotometer, for K^+, Na^+, Ca^{++}, and Mg^{++}.

The results, shown in Tables 1, 2, 3 and 4, indicate increased loss of all 4 cations from susceptible tissue as compared with loss from the controls and from the toxin-treated resistant tissue. We conclude from these preliminary data that one of the specific effects of the fungal toxin is the damage of cell membranes of lines carrying T-cms cytoplasm but not

N-cmf lines. Current investigation is directed at 1) defining the molecular damage and 2) characterization of the cell membranes from T-cms and N-cmf lines for differences in protein and/or lipid quality and/or quantity.

Table 1

Cytoplasm-Treatment	Leaching Time (hrs) 4	8	12	16
T-toxin	1.4*	1.6	1.7	2.8
T-water	1.2	1.2**	1.1	1.1
N-toxin	1.1	1.2	1.2	1.2
N-water	.9	1.2	1.2	1.2

*Numbers are ppm K^+ in leachate; mean of five determinations

**Mean of four determinations

Table 2

Cytoplasm-Treatment	Leaching Time (hrs) 4	8	12	16
T-toxin	25*	25	26**	29
T-water	26	24**	23	23
N-toxin	29	26**	20	21
N-water	26	29	26	20

*Numbers are ppm Na^+ in leachate; mean of five determinations

**Mean of four determinations

Table 3

Cytoplasm-Treatment	Leaching Time (hrs) 4	8	12	16
T-toxin	1.7*	2.4	3.3**	3.9
T-water	1.8	2.0**	2.5	2.5
N-toxin	2.4	3.6	2.5	2.3
N-water	1.9	2.6	2.5	2.3

*Numbers are ppm Ca^{++} in leachate; mean of five determinations

**Mean of four determinations

Table 4

Cytoplasm-Treatment	Leaching Time (hrs) 4	8	12	16
T-toxin	.24*	.23	.51**	.66
T-water	.16	.14**	.32	.33
N-toxin	.28	.34	.33	.29
N-water	.19	.35	.33	.35

*Numbers are ppm Mg^{++} in leachate; mean of five determinations

**Mean of four determinations

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1. Two systems that transform a two-ranked spike into a four-ranked spike.

The eight-rowed ear of maize (four-ranked with paired spikelets) can be derived from the two-ranked spike (distichous) of teosinte and certain variants in maize by two different systems as follows:

1. Condensation alone in which a lack of internode elongation in the rachis forces a primordial slippage or twisting in order to spatially

accommodate the expanding and differentiating cupules and their spikelets. The result is more than two vertical ranks. The degree of condensation varies greatly and appears to be polygenic in inheritance.

2. A direct induction of the decussate spike independent of condensation although frequently combined or superimposed upon it. The inheritance of the directly induced type of decussate spike appears to be simple.

Some northern flints have the directly induced decussate ear with some condensation superimposed upon it, especially at the butt. This decussate trait may be transferred to teosinte at a low-level of condensation in which yoked fruit cases alternate at 90°.

When a mutant gene (tr) for a two-ranked ear found in northern flint was transferred to A158 maize (normally 12 to 16 rowed), the phenotype was eight-rowed, sometimes changing to four-rowed (two-ranked with paired spikelets) near an elongate tip. That these eight-rowed ears are more than two-ranked due to condensation alone is shown in their hybrids with northern flint eight-rowed maize. The hybrids between these two types of eight-rowed maize are 10 to 12 rowed, apparently due to a combination of condensation derived from the tr A158 parent superimposed upon the induced decussate condition from the northern flint parent. Although a sufficiently large F_2 from such hybrids has not been grown to yield the two-ranked condition, it was recovered in an F_3 family from this cross. Emerson and Smith (1950) reported a slight increase in kernel row number from crosses of different eight-rowed inbreds which they described as a "plus increment of hybrid vigor". The increase in row number from 8-rowed X 8-rowed which they obtained was not as great as that in our experiments.

The several two-ranked cobs among the oldest Tehuacán specimens are mostly four-ranked at the base, suggesting that condensation is the basis for the many-ranked condition in these oldest-known specimens of archaeological cobs.

Walton C. Galinat

2. The location and penetrance of a gene (tr) for two-ranked (distichous) spike from Tripsacum.

One of the phenotypic effects of Tripsacum chromosome Tr9 when transferred to maize that was observed first in an interchange derivation of this chromosome by Maguire (1961) is that of reducing the number of kernel rows on the ear. When we recently altered the background of our chromosome 2 tester gene stock to that of 8-rowed or 4 ranked ear, the effect of the extra alien chromosome Tr9 in reducing kernel row number was manifested as a change to the four-ranked or two-ranked spike, a taxonomic trait of both Tripsacum and teosinte which distinguishes, in part, these relatives from maize. This evidence suggests a gene for two-ranks in Tr9 which is only able to express itself when the maize background is fixed at a low degree of floral compaction (condensation). The basic change controlled by this gene on Tr9 appears to be distichous vs decussate spike.

Because the long arm of Tr9 is known to carry at least 6 loci in common with the short arm of maize M2, a linkage test for a tr mutant gene out of northern flint was made with the M2 marker genes lg_1 gl_2 v_4. In each case the F_2 repulsion phase data indicated independent assortment with the tr gene. Because Tr9 is also known to have pairing affinity with M10, a linkage analysis of tr with chromosome 10 markers is being made.

Walton C. Galinat

3. A formula for giant ears in maize.

Ears up to 22 inches long when wet and grown at wide spacing in Waltham, Massachusetts have been developed from the following combination of characteristics.

1. Heterozygosity for teosinte chromosome 9. A factor on this chromosome elongates the rachis internodes in the upper half of the ear and, thereby, eliminates a fasciated tip by allowing interlocking of cupules and spikelets. In highly condensed ears of certain North American corn, the surface area necessary for development of many rows near the ear tip comes from a flattening (fasciation) of the rachis.
2. Homozygosity for fasciation (high condensation) of the ear. This causes the cob to be highly vascularized at its base.

3. A single main ear borne lower than half-way down the stalk.

4. A tall (9 to 10') late flowering plant with tillers.

5. A long central-spike in the tassel.

Wide row spacing in the field is essential for the development of maximum ear size by this formula. Its plants may even be barren at high population densities. Because the current fad requires that U.S. corn be adapted for close row spacing, this giant eared corn may be better suited to tropical and sub-tropical areas where the greater food demands are met by intercropping such as the ancient corn-beans-squash eco-system or possibly this in combination with multiple cropping or with crops of different maturities. For example an early small sweet corn in alternate rows might be harvested before it competed with the giant eared corn for solar radiation.

Walton C. Galinat

4. Pollen size and the origin of maize.

Because the size of the oldest known Zea pollen is larger than that of present-day Mexican teosinte (Table 1), one can only conclude that the oldest known pollen could not be that represented by any of the present-day Mexican teosintes. Because this oldest known pollen compares more favorably in size with that of present day corn, it has also been concluded that "the ancestor of corn is corn and not (Mexican) teosinte," (Mangelsdorf).

If we consider Mexican teosinte and maize to be products of co-evolution resulting from disruptive selection between man and nature for features involved in their different adaptations for seed dispersal and survival, the above pollen size data could also be interpreted as the result of an alternate possibility involving a large-pollen teosinte such as certain types from Guatemala. The smaller pollen (and fruit case) in present day Mexican teosinte may be, like the block inheritance distinguishing these species, part of a system evolved to cope with some aspects of gene flow from maize. Increased condensation together with increased kernel size due to maize introgression into teosinte produces an incongruous combination of parts for survival in the wild because an expanded kernel that protrudes outside a condensed fruit case is left

Table 1

A comparison of pollen diameter frequencies (%) of 200 pollen grains each from two Guatemalan teosintes (Jutiapa 40-202A and Lake Retana 40-203) to a teosinte growing in the Valley of Mexico and maize from the lowest level of Bat Cave. All pollen treated with lactic acid.

Pollen Diameter Microns	Valley of Mexico Teosinte*	Jutiapa Teosinte**	Lake Retana Teosinte**	Bat Cave Level V Maize*
60	0.5			
62	0.5			
64	1.5	1.0		
66	3.5	1.5		
68	2.5	1.5		
70	6.5	3.0	1.0	
72	11.5	6.5	1.0	
74	14.0	2.5	1.5	
76	12.5 Av.	5.5	4.0	
78	17.0	12.0	6.0	1.5
80	13.5	7.5	15.5	1.5
		Av.		
82	9.0	7.0	13.0	1.5
			Av.	
84	3.0	11.5	18.0	5.5
86	2.0	9.5	7.5	6.5
88	2.0	9.0	14.0	11.5
90	0.5	6.0	11.0	13.5
92		7.5	5.0	12.0
				Av.
94		3.0	1.5	14.5
96		2.5	1.0	10.0
98		1.5		7.0
100		1.0		8.5
102		0		3.5
104		0		2.0
106		0.5		0
108				1.0
110				

*Data from P. C. Mangelsdorf (unpub.).

**Stunted plants from 32 year old seed (from cold storage) grown in the greenhouse, Waltham, Mass. winter 1972-73.

unprotected. The evolution of kernel-size suppressors would develop in the teosinte genotype in order to allow a freedom to absorb certain amounts of maize germplasm (i.e., mimetic and heterotic traits). That such suppressors may have a pleiotropic effect in reducing pollen size as well is suggested by the following:- The primitive Guatemalan teosintes have larger pollen approaching the size of Bat Cave pollen (Table 1) together with large fruit cases and large staminate spikelets (long glumes suggesting a Chapalote-like tunicate allele and large anthers). The slight shift to the right for pollen size distribution for Bat Cave maize over that of Jutiapa and Lake Retana teosintes from Guatemala is exactly the type of change one might get during domestication. The few grains of the much older fossil pollen could as well be a part of the Jutiapa size distribution as the Bat Cave and Chapalote size distribution.

The Guatemalan teosintes are sometimes considered as more tripsacoid than the Mexican ones. However, the much smaller size of Tripsacum pollen makes this suggestion inconsistent with the pollen-size data. Thus, on a basis of the pollen evidence available so far, the Guatemalan teosinte could represent a relic from a common stem stock that once produced by disruptive selection the sympatric partners, maize and Mexican teosinte. Work continues on a possible pleiotropic effect of certain Mexican teosinte germplasm (especially chromosomes 1 and/or 7) in reducing both pollen and kernel size in maize.

The waxy locus controls the quality of both pollen and endosperm. Other loci controlling the quantity of both pollen and endosperm storage which are not in the category of heterozygous translocations nor in that of defective seed are probable. Thus, pleiotropic suppressors for size of both pollen and kernel such as hypothesized here for present day Mexican teosinte seem possible. The popcorns with small kernels and small pollen may be secondary products of introgression by present-day Mexican teosinte. Maize with large kernels and pollen may have long ago escaped the bonds of this sympatric coexistence in Mexico. In contrast to such pleiotropy, the correlation of pollen size to ear (style) length is thought to be an evolved assemblage. The combination of large pollen and small kernels in certain Guatemalan teosintes and possibly certain primitive maize, not

being intensively subjected to disruptive selection and coevolution, would not be expected to fit such a relationship.

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5. An interchange between two different Tripsacum chromosomes that are partially homoeologous to maize chromosome 4.

We have reported previously that several different Tripsacum chromosomes carry individual loci assembled on maize chromosome 4 (M4). Tripsacum chromosome 7 (Tr7) bears the Su_1 locus in common with the short arm of M4 while Tr13 bears the Gl_3 locus in common with the long arm of M4. Neither Tr7 nor Tr13 carries certain other M4 loci tested (ra_3, bm_3, j_2). We have reported the pachytene morphology of Tr7 and Tr13 in the 20+2 condition (MNL44:126-128, 1970 and Ann. Rev. Gen. 5:470, 1971).

Tr7 and Tr13 were combined in the 20+1+1 condition on *su* gl_3 maize for pairing studies by selecting for the Su^d and Gl_3^f combinations. After four generations of inbreeding, we collected cytological material from 38 of the double dominant plants in 1971. In six plants carrying the standard knobless Tr7 (Su^d) and standard knobbed Tr13 (Gl_3^f), we did not observe any pairing between them although they were occasionally observed in close proximity at pachytene. However, one family of the Tr7-Tr13 combination carried two knobbed but different Tripsacum chromosomes. Thus, we suspected that the originally knobless Tr7 had acquired a knob from Tr13 by an interchange during an earlier generation.

To determine if the Tr7 chromosome had acquired this knob from Tr13, we made cytological collections from the Su^d gl_3 phenotypes segregating in this family which would be 20+1 carrying the Su^d marked Tr7 but not the Gl_3^f marked Tr13. These 20+1 individuals did indeed carry a knob on their extra Tr7 chromosome.

The reciprocal event producing a knobless Tr13 was recovered in some of the Gl_3^f 20+1 segregates from an earlier generation of this line in which Gl_3^f showed an abrupt increase in transmission frequency, as described last year (MNL 46:114-115, 1972). Furthermore, we have now recovered all four possible combinations of the knobbed and knobless forms of Tr7 and Tr13 from these 20M + 1Tr7 + 1Tr13 families. When both Tripsacum chromosomes were knobbed or both were knobless, one was longer than the other as in

the original Tr7 and Tr13. When only one of the two Tripsacum chromosomes was knobbed, it could terminate either the longer or the shorter one, according to independent assortment of these reciprocal alterations with their standard counterparts.

It is not known how much, if any, chromatin interchange took place accompanying the knob transfer nor into which arm of Tr7 the knob was transferred. It might be possible to obtain information on this in the heteromorphic bivalent condition now being developed by crossing the old standard $\underline{Su}^{d}$ (20+2) line of Tr7 with its altered Tr7+K as well as the old standard $\underline{Gl_3}^{f}$ (20+2) line of Tr13 with its altered (knobless) Tr13-K.

Studies of interchanges (translocations) between different Tripsacum chromosomes that are partial homoeologs to the definitively important fourth chromosome of maize and teosinte are important in uncovering the course of evolutionary differentiation in the chromosomes of the American Maydeae.

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6. Results of crossing annual and perennial teosinte with ig/Ig maize.

Pollinations were made on Kermicle's stock of ig/Ig W-23 maize with three varieties of annual and perennial teosinte in an attempt to recover androgenetic monoploids of both annual teosinte and "diploids" (polyhaploids) from tetraploid perennial teosinte.

Studies of meiosis in haploid teosinte have not been reported. Perennial teosinte is generally considered as an autotetraploid, although it is questionable if it originated by chromosome doubling from one of the present day annual teosintes. If a "diploid" can be obtained from the perennial teosinte, one can observe if it will still remain perennial and if its cytological behavior reflects any genome differentiation.

The kernels from these pollinations were classified as follows: (1) those showing aleurone color at the crown of the kernel or at the scutellum and embryo axis; (2) those that are shrivelled (defective) and (3) those with no obvious color (Table).

Variety of Teosinte	No. of Kernels with Colored Scutellum	No. of Shrivelled (Defective) Kernels	No. of Kernels with No Scutellum Color	Total No. of Kernels	% Kernels with No Scutellum Color
Guatemala 51764	907	40	16	963	1.66
Nobogame	1,011	31	23	1,065	2.16
Chalco 62-394	371	30	9	410	2.19
Perennial	690	62	30	782	3.84

The anthocyanin pigmentation is produced by the $\underline{R}^{nj}$ factor in the maize parent. All of the kernels showing aleurone color and/or scutellum color probably represent maize-teosinte hybrids. The defective kernels might be due to abnormalities in endosperm development associated with the ig gene. At least some of the colorless kernels probably contain androgenetic teosinte embryos. Chromosome numbers from root tips will be checked before transplanting to the field in June.

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7. Preliminary studies on the inheritance of abscission layer development in Zea.

A disarticulation of the maize cob requires the transfer of genetic control of abscission in two areas. (1) Abscission through the rind which extends along the interface between the beak (roof) of the cupule with the glume cushion from above, angling upward to the pith. (2) Abscission through the pith at a level adjacent to the deepest indentation of the cupule. This second area of pith abscission is variable in expression, developing sometimes as a tear at these morphologically predetermined points of weakness. The tear may quickly heal leaving only a trace-like band extending across the pith or it may open into a gap usually bound by abscission layers.

Preliminary analysis of the linkage data for chromosome four indicates control of pith abscission ($\underline{ab}^{p}$) on the short arm and control of rind abscission ($\underline{ab}^{r}$) on the long arm. Although the genes controlling these two areas of abscission may be 50 crossover units or more apart, they tend to be inherited together in hybrids of corn four with Nobogame teosinte four because of a reduction in crossing over in the $\underline{Su}$-$\underline{Gl}_3$ region. Crossing over is normal in similar hybrids with the fourth chromosome from Florida teosinte, a variety of the primitive Guatemalan teosinte, Jutiapa. Final analysis of the linkage data awaits completion of the tedious sawing of longitudinal sections through some 1000 highly lignified corn cobs.

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1. Chemical mutagenesis following treatments at different developmental stages.

The study of the effectiveness of chemical mutagens, administered at different stages of ontogeny, upon induction of mutations might bear useful information of both theoretical and practical value. We chose for this analysis an alkylating agent, ethyl methane sulphonate (EMS), whose mutagenic power has been established in many organisms.

The following treatments were performed:

1. Seeds: soaking for 24 hr. in the mutagen solution at 23±2° C
2. Seedlings: immersion of the primary root in the mutagen solution after removal of its distal portion to insure better uptake
3. Plants at the time of male meiosis: injection of 10 cc of EMS solution into a Pasteur pipette with its tip inserted into the plant stem
4. Pollen grains: as in previous stage

For each treatment a freshly prepared 2% EMS solution adjusted to pH7 with phosphate buffer was used.

The following mating scheme was adopted: Control and treated sibs, homozygous for R_2^{nc} Pr Sh and Y, were reciprocally crossed with a corresponding multiple recessive stock (r^g pr sh y). The resulting ears were then scored for production of sectors of seeds with nonparental phenotype. If the dominant markers, Y, Sh and Pr, are lost or mutate the recessive character may appear, while any change leading to resumption of R function is registered by production of pigment in the aleurone layer of the endosperm. The effect of EMS upon R expression will be considered elsewhere; results from studies with the other three genes are presented in Table 1.

Table 1

Frequency of mutations induced by EMS at specific loci (y, pr and sh).

Plant stage treated	n(a)	Kernels scored	Mutants	Fr. (x 10^{-3})
Control	102	14653	0	--
Seeds	61	12021	2(b)	32.78
Seedlings	83	8431	1(c)	12.04
Premeiotic	37	5032	0	--
Pollen grains	28	7144	0	--

(a) total number of ears and/or tassels

(b) 1 sh and 1 pr

(c) y

From each M1 ear, three or four kernels were taken and planted to measure the frequency of mutation in M2 and M3 generations. Kernels were removed from different areas of each M1 ear to avoid a duplication of any mutation, on the assumption that induced mutations transmitted through the female gametophyte tend to occur in clusters. The M2 ears were then scored for segregation of "endosperm mutants". This category includes aborted and small seeds, defective endosperm and viable mutants with abnormal endosperm morphology. From each ear a sample of 50 seeds was germinated in the sand-bench. The resulting seedlings were scored for the appearance of mutants

Table 2

Frequency (%) of endosperm and seedling mutation as measured in M2 and M3 progenies.

Plant stage treated	n	Mutations affecting:									Total
		Endosperm			Chlorophyll synthesis			Seedling morphology			
		(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	
control	148	.00	---	---	.00	---	---	1.35	1.35	---	1.35
seed	109	26.60	13.76	12.84	11.92	11.00	.92	17.43	6.42	11.01	55.96
seedling	99	15.15	3.03	12.12	8.08	8.08	---	2.02	1.01	1.01	25.25
premeiotic	46	.00	---	---	.00	---	---	2.17	---	2.17	2.17
pollen grain	104	.00	---	---	.96	.96	---	.00	---	---	.96

(a) total; (b) 3 : 1 segregation; (c) segregation other than 3 : 1

affecting chlorophyll synthesis or seedling development. The results obtained are presented in Table 2.

In the table each group of mutants has been subdivided, by means of a chi-square test, into two subgroups, i.e., those fitting a 3:1 ratio, presumably point mutants, and those with a significant shortage of segregating mutants, presumably small intercalary deletions. This subdivision shows that, while both endosperm and seedling mutants are variously distributed among the two subgroups, the majority of chlorophyll mutants fit the 3:1 segregation. Furthermore, as in the case of the mutagenesis at specific loci (Table 1), the stage most sensitive to EMS treatment appears to be the seed, followed by seedling, while later treatments seem to be ineffective.

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G. Gavazzi

2. A test of the response of some chlorophyll mutants to different temperature and nutrients.

Mutants with identified blocks in the synthesis of an essential metabolite are very rare in higher plants (cf. Nelson, 1967 and Redei, 1970 for a review). Maize offers a large series of mutants well characterized genetically but not yet investigated from the point of view of their metabolic effect. These mutants, being involved in the control of essential functions like chlorophyll and chloroplast synthesis, plant development, and morphogenesis, represent good material for the analysis of the chain of events linking the gene to its phenotypic effect.

Among all those available, we chose the "chlorophyll mutants", i.e., mutants characterized by a more or less strong reduction in chlorophyll content or by its complete absence. We made a test of the response of these mutants to exogenous sources of nutrients and to different temperature. The test consisted in growing excised mutant embryos under sterile conditions in testtubes containing either mineral or supplemented media at two temperature levels (20 and 30° C) in a growth chamber under continuous light (approximately 300 foot candles). The composition of the mineral medium (M.M.) in mg/l bidistilled water is as follows: NH_4NO_3 600; $MgSO_4 \cdot 7H_2O$ 400; $CaH_4(PO_4)_2 \cdot H_2O$ 400; KH_2PO_4 400; K_2HPO_4 160; $FeC_6H_5O_7 \cdot 3H_2O$ 6.

The "complete medium" (C.M.) was obtained by adding casein hydrolysate, yeast extract, yeast hydrolysate (100, 20, 20 mg/l, respectively) and 4% coconut milk to the mineral medium. Both media were supplemented with 2% saccharose to provide better growth and solidified with 8% agar.

For a quantitative estimate of the response of the mutants to the different growth conditions, their pigment concentration at a given developmental stage (i.e., first two leaves fully extended, the third not yet unfolded) was calculated. Pigments were extracted by grinding leaf material in aqueous 80% (V/V) acetone with a homogenizer. Following centrigugation, pigment concentration was determined spectrophotometrically using the formulas given by Arnon (1949) for chlorophylls and by Wettstein (1957) for carotenoids.

The mutants so far analyzed have the following origin: Albino and luteus were kindly provided by Dr. Robertson, Sienna was from Dr. Anderson, those with a subscript letter have been isolated in our laboratory following E.M.S. treatment of a W22 inbred line and all the others were furnished by Dr. Lambert, Maize Genetics Cooperative. They can be grouped in the two categories of lethals and nonlethals. Results referring to each of these two groups will be presented separately.

Lethal mutants

The following mutants were tested:

$\underline{w}_1$, $\underline{w}_2$, $\underline{w}_3$, $\underline{w}_{7752}$, $\underline{w}_{7748}$, $\underline{w}_{8657}$, $\underline{w}_{8896}$, $\underline{w}_{wisc.2}$, $\underline{w}_{mumml}$, $\underline{w}_{it.1}$, $\underline{w}_{turk}$, $\underline{cl}_1$, $\underline{lw}_1$, $\underline{l}_3$, $\underline{l}_4$, $\underline{l}_{10}$, $\underline{l}_{4120}$, $\underline{l}_{4932}$, $\underline{l}_{42\text{-}4106}$, $\underline{l}_{60\text{-}1106}$, $\underline{l}_{62\text{-}4117}$, $\underline{l}_{blandy\ 1}$, $\underline{l}_{blandy\ 2}$, $\underline{l}_{blandy\ 4}$, $\underline{l}_{brown\ 1}$, $\underline{yd}$, $\underline{l}_a$, $\underline{l}_b$, $\underline{py}_a$, $\underline{py}_d$, $\underline{Sienna}_{7748}$.

They were grown as whole seeds in soil or as excised embryos in testtubes on both mineral and complete medium, in a greenhouse (t=25$\pm$ 4° C). No difference in their pigment content was visible after growth in the two media or in soil except for Sienna. Homozygous Sienna seedlings grown in soil are pale green while those grown in the testtube turn green quickly. In either case, necrotic areas are formed on the leaf tissues as soon as the third leaf unfolds.

Nonlethal mutants

They were grown on both mineral and complete medium at 20 and 30° C. Their pigment content values are reported in Table 1. Each value is the average of three determinations based on the pigment extract of six seedlings.

Table 1

Pigment concentration in mg per gram fresh weight of normal and mutant seedlings grown from excised embryo on mineral and complete medium.

Mutant	30°C			
	C.M.		M.M.	
	chlorophylls	carotenoids	chlorophylls	carotenoids
wild type (v_8^+)	1.476 ± .099	.228 ± .005	1.389 ± .086	.223 ± .004
wild type (W22)	1.335 ± .061	.236 ± .007	1.461 ± .107	.245 ± .012
v_1	.806 ± .014	.111 ± .007	.819 ± .096	.108 ± .015
v_2	.928 ± .024	.215 ± .014	1.048 ± .117	.249 ± .017
v_4	1.042 ± .069	.153 ± .010	1.019 ± .073	.149 ± .010
v_8	.712 ± .111	.133 ± .017	1.029 ± .025	.182 ± .003
v_{12}	1.095 ± .057	.222 ± .007	.920 ± .079	.193 ± .015
v_{16}	1.156 ± .094	.220 ± .007	1.116 ± .096	.282 ± .079
v_{18}	.957 ± .087	.174 ± .011	1.037 ± .054	.188 ± .016
wt	1.046 ± .068	.188 ± .000	.883 ± .038	.162 ± .000
ws_3	.545 ± .023	.104 ± .005	.532 ± .048	.093 ± .008
yg_1	.584 ± .032	.158 ± .007	.627 ± .049	.177 ± .005
py_c (W22)	.627 ± .040	.140 ± .000	.512 ± .014	.139 ± .000
g_1 (W22)	.672 ± .016	.172 ± .008	.635 ± .031	.140 ± .004
et	.622 ± .037	.147 ± .006	.708 ± .004	.163 ± .004
$pg_{11}pg_{12}$	.662 ± .002	.129 ± .005	.612 ± .000	.125 ± .005

Table 1 (continued)

Mutant	20°C			
	C.M.		M.M.	
	chlorophylls	carotenoids	chlorophylls	carotenoids
wild type (v_8^+)	1.745 ± .040	.302 ± .010	2.005 ± .020	.327 ± .033
wild type (W22)	1.636 ± .160	.270 ± .018	1.987 ± .209	.304 ± .027
v_1	.725 ± .011	.157 ± .000	.777 ± .043	.157 ± .002
v_2	.517 ± .005	.160 ± .007	.704 ± .046	.188 ± .004
v_4	.672 ± .051	.148 ± .006	.638 ± .051	.135 ± .011
v_8	.095 ± .027	.038 ± .010	.037 ± .013	.016 ± .003
v_{12}	.551 ± .040	.189 ± .006	.536 ± .005	.184 ± .002
v_{16}	.318 ± .014	.107 ± .003	.243 ± .050	.097 ± .017
v_{18}	.781 ± .020	.181 ± .005	.893 ± .081	.187 ± .014
wt	.963 ± .141	.226 ± .017	.895 ± .069	.222 ± .010
ws_3	.491 ± .044	.110 ± .008	.581 ± .032	.124 ± .001
yg_1	.579 ± .048	.151 ± .007	.660 ± .034	.145 ± .006
py_c (W22)	.445 ± .026	.133 ± .000	.250 ± .040	.093 ± .010
g_1 (W22)	.964 ± .125	.199 ± .008	.996 ± .072	.208 ± .011
et	1.156 ± .074	.258 ± .013	1.026 ± .050	.246 ± .003
$pg_{11}pg_{12}$	.670 ± .034	.209 ± .010	.743 ± .062	.191 ± .004

In Table 2 the pigment content of the mutant analyzed is expressed as mutant/normal ratio while in Table 3 the chlorophyll a/b ratio is reported.

Table 2

Chlorophyll and carotenoid content of mutant seedlings expressed as mutant to normal ratio.

Mutant	30°C				20°C			
	C.M.		M.M.		C.M.		M.M.	
	chlor.	carot.	chlor.	carot.	chlor.	carot.	chlor.	carot.
$\underline{v}_1$	0.54	0.48	0.58	0.48	0.41	0.51	0.38	0.48
$\underline{v}_2$	0.62	0.94	0.75	1.11	0.29	0.52	0.35	0.57
$\underline{v}_4$	0.70	0.67	0.73	0.66	0.38	0.49	0.31	0.41
$\underline{v}_8$	0.48	0.58	0.74	0.81	0.05	0.12	0.01	0.04
$\underline{v}_{12}$	0.74	0.97	0.66	0.86	0.31	0.62	0.26	0.56
$\underline{v}_{16}$	0.78	0.96	0.80	1.26	0.18	0.35	0.12	0.29
$\underline{v}_{18}$	0.64	0.76	0.74	0.84	0.44	0.59	0.44	0.57
$\underline{wt}$	0.70	0.82	0.63	0.72	0.55	0.74	0.44	0.67
$\underline{ws}_3$	0.36	0.45	0.38	0.41	0.28	0.36	0.28	0.37
$\underline{yg}_1$	0.39	0.69	0.45	0.79	0.33	0.50	0.29	0.44
$\underline{py}_c$	0.46	0.59	0.35	0.56	0.27	0.49	0.12	0.30
$\underline{g}_1$	0.50	0.72	0.43	0.57	0.58	0.73	0.50	0.68
$\underline{et}$	0.42	0.64	0.50	0.73	0.66	0.85	0.51	0.75
$\underline{pg}_{11}\underline{pg}_{12}$	0.44	0.56	0.44	0.56	0.38	0.69	0.37	0.58

Table 3

Chlorophyll a/b ratio in normal and mutant seedlings grown from excised embryos.

Mutant	30°C		20°C	
	C.M.	M.M.	C.M.	M.M.
wild type (v_8^+)	3.82	3.82	3.62	3.69
wild type (W22)	4.62	4.81	4.41	4.17
$\underline{v}_1$	4.48	4.46	5.61	5.77
$\underline{v}_2$	3.76	3.64	3.69	3.48
$\underline{v}_4$	3.89	3.97	3.59	3.46
$\underline{v}_8$	4.03	4.27	2.59	1.53
$\underline{v}_{12}$	2.92	2.87	3.93	3.53
$\underline{v}_{16}$	4.40	4.44	3.88	3.95
$\underline{v}_{18}$	4.46	4.34	4.76	4.48
$\underline{wt}$	4.68	4.75	5.22	4.44
$\underline{ws}_3$	3.95	3.68	3.78	3.71
$\underline{yg}_1$	3.49	3.67	3.59	3.13
$\underline{py}_c$	3.90	3.57	3.66	3.75
$\underline{g}_1$	4.86	3.87	4.14	4.39
$\underline{et}$	5.37	4.88	4.95	4.71
$\underline{pg}_{11}\underline{pg}_{12}$	3.59	3.56	5.44	4.71

Non mutant (wild type) values are those of green seedlings in the progeny of a selfed $\underline{+/v}_8$ ear or in the W22 inbred line. The latter are used as reference values for mutants in the W22 genetic background, while the former furnish reference values for the other mutants tested.

The results indicate that the majority of the mutants tested are thermosensitive in the sense that they show an increase in chlorophyll and carotenoid concentration when grown at a high temperature (30°C). This increased pigment concentration at 30°C is observed among the $\underline{v}$ series as well as in other mutants ($\underline{yg}_1$, $\underline{wt}_1$ and $\underline{ws}_3$). Even though all these mutants respond to temperature treatment in the same direction, the mutations do

not affect the pigment levels equally. The capacity of recovering at high temperature in fact ranges from a barely visible effect (see $\underline{v}_1$) to a 28-fold increase (see $\underline{v}_8$).

Three mutants are worth considering in more detail. They are:

1. golden ($\underline{g}_1$). Previous studies (Smith et al., 1956) had shown that the $\underline{g}_1$ chlorophyll deficiency is not the result of insufficient production of chlorophyll precursor, but of an increased chlorophyll destruction. The temperature effect here reported seems to indicate that the rate of chlorophyll destruction is significantly reduced at low temperature. Thin layer chromotography of acetone extracts of golden leaves show that they differ from wild type tissues by the absence of a yellow spot. This mutant is presently under investigation.
2. etched (et). Like $\underline{g}_1$, this mutant shows an increased pigment concentration when grown at low temperature. Contrary to $\underline{g}_1$, however, this effect is observable only if et is grown on complete medium. These are the results expected from a thermosensitive auxotrophic mutant. Further experiments are necessary to confirm this interpretation and to establish the nutritional requirements of et.
3. pale yellow ($\underline{py}_c$). At emergence, this mutant has a green coleoptile and the tip of the first leaf is green, while the rest of the leaf tissues remain white. This pattern is repeated in succeeding leaves. Most plants die before reaching maturity. The same phenotype is observed when embryos are grown at 20°C. In this condition, however, the mutant grows slowly till emergence of the third leaf, while at 30°C it grows normally and appears pale green in phenotype.

The low temperature inhibition is released as soon as the mutant is transferred to high temperature. $\underline{py}_c$ is thus a conditional lethal. It is also clear from Table 1 that the growth medium has an effect on the pigment concentration; this will be further investigated. It might be of interest to recall that of the five EMS induced mutants tested, $\underline{py}_c$ is the only thermosensitive one. This suggests that this mutant is probably a

point mutant as opposed to the more common chromosomal mutations induced by chemical mutagens in maize.

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3. Genetic properties of an atypical chromosome 10.

In previous notes (MNL 45: 115-119 and 46: 120-122) the nonrandom transmission of a chromosome 10 was described. From the crosses made with the trisomic condition, it appeared as though the lower frequency of transmission of that chromosome was the result of an abnormal pairing at meiosis and of male gametophyte competition. It was suggested that an unfavorable chromosomal condition, linked to $\underline{R}^{st}$, the marker used to follow this chromosome, was the cause of the low recovery of two classes ($\underline{R}^{st}$ and $\underline{R}^{nj}\underline{R}^{st}$) in reciprocal crosses involving putative trisomic parents ($\underline{R}^{nj}\underline{R}^{st}\underline{r}$) and a tester ($\underline{rr}$).

The linkage mentioned was confirmed since such crosses produced 41 progenies in 40 of which the situation remained unchanged, while in one, two doses of this abnormal chromosome were apparently present, respectively marked by $\underline{R}^{st}$ and by $\underline{R}^{nj}$. The individual found with two such chromosomes is believed to be the result of recombination between the marker and the chromosomal condition, involving an abnormal and a normal chromosome, followed by the recovery of two abnormal chromosomes in the same spore. The low recombination frequency is probably the result of both physical linkage and nonrandom pairing, similar to that observed by Dr. Rhoades in K10, k10, k10 individuals (Preferential Segregation in Maize, in "Heterosis," 1954). Out of the 41 putative trisomics tested, four gave no transmission of the marker $\underline{R}^{st}$, while two gave no transmission of the marker $\underline{R}^{nj}$. In the first four cases $\underline{R}^{nj}$ was transmitted in typical disomic ratios, while in the other two cases $\underline{R}^{st}$ was transmitted at a much lower

frequency than the allele r. These six cases were regarded as the result of nondisjunction of one of these chromosomes in the second microspore division, which could explain the noncorrespondence between the endosperm and the embryo, although other mechanisms may be the cause of loss of one of the markers, such as somatic nondisjunction, chromosome breakage eliminating only the marker, etc.

The abnormal chromosome, marked by $\underline{R}^{st}$, was removed from the trisomic condition and its transmission was observed in the disomic condition, i.e., in plants where this chromosome was present together with a normal chromosome marked by r.* A few individuals regarded as disomic $\underline{R}^{st}$ r were reciprocally testcrossed to an rr tester, and the results are given in Table 1 and in Table 2. From the data reported here and in the earlier notes, it appears that the chromosome marked by $\underline{R}^{st}$ is constantly transmitted at a lower frequency regardless of the chromosomal condition, whether trisomic or disomic.

Table 1

Progeny of the cross $\underline{R}^{st}\underline{r}$ x rr

$\underline{R}^{st}$	r	Total	% $\underline{R}^{st}$	s.e.
58	175	233	25.0	2.84
48	151	199	24.1	3.03
94	277	371	25.3	2.26
164	130	294	55.8	2.89
115	334	449	25.6	2.06
80	214	294	27.2	2.59
72	168	240	30.0	2.96
174	155	329	52.9	2.75
220	120	340	64.7	2.59
70	218	288	24.2	2.52
37	101	138	26.8	3.77
1,132	2,043	3,175	35.6	0.85

*Since the disomic condition was not ascertained cytologically in all plants, the possibility exists that some of these $\underline{R}^{st}$ individuals have the genotype $\underline{R}^{st}\underline{r}\ \underline{r}$ (trisomic).

Table 2

Progeny of the cross $\underline{rr} \times \underline{R}^{st}\underline{r}$

$\underline{R}^{st}$	$\underline{r}$	Total	% $\underline{R}^{st}$	s.e.
44	260	304	14.5	2.02
31	374	405	7.6	1.32
47	373	420	11.9	1.58
94	236	330	28.5	2.48
56	347	403	13.9	1.72
3	24	27	11.1	6.04
34	455	489	6.0	1.07
76	332	408	18.6	1.93
227	285	512	44.3	2.19
36	481	517	7.0	1.12
44	316	360	12.2	1.72
692	3,483	4,175	16.6	0.57

Cytological observations were made on root tip metaphases. The abnormal chromosome appears sharply different from a normal chromosome 10, in that 1) it shows a long arm exceeding by more than 30% the length of the long arm of the normal chromosome and 2) the long arm is club-shaped with a constriction at .35-.40. This chromosome looks quite similar to the well known K10. The similarity with K10 is supported by the frequent exclusion from pairing and by the lower recombination frequency in the region distal to R. However, a remarkable difference between the two is apparent in the genetic behavior of this chromosome, namely its low transmission and the male gametophyte effect. Sporocytes have not been studied yet.

A. Ghidoni

4. Nondisjunction and preferential fertilization in balanced and hyperploid structural heterozygotes for the translocation TB-9b.

Nondisjunction of the B^9 chromosome in the presence of the 9^B chromosome occurs at highly variable rates in the second pollen mitosis, while preferential fertilization of the egg by hyperploid sperm occurs at more constant rates. The data of various investigators were briefly reviewed by

D. S. Robertson in Maize News Letter 46:88-93.

In the translocation TB-9b two markers with easily classifiable phenotypes are useful in estimating the rates of nondisjunction and of preferential fertilization. The Wx allele is a good marker of the 9^B chromosome because of its close linkage with the translocation breakpoint. The C^I allele is useful because of its location in the B^9 chromosome and also because of the dosage effect observed in endosperms heterozygous for I and C. A single dose of C^I is unable to completely inhibit pigment formation in the aleurone, which appears more or less tinged; moreover, sharp spots of deep pigment are frequently observed following losses of the inhibitor factor. On the other hand, two doses of the C^I allele are able to completely inhibit pigment formation in the aleurone, which appears colorless with infrequent and smaller spots of color; these are the consequence of coincidental loss of both inhibitor factors in the same clone of cells.

A stock of TB-9b, homozygous for the translocation and marked with Wx and C, was crossed to a wx C^I (normal) stock in 1967 in order to obtain a Wx C^I marked translocation. The F_1 was crossed as male parent to a C wx (normal) stock. Among the progeny, two types of crossovers were then selected: those with C^IWx endosperm, colorless scutellum (balanced translocation 9^BWx, B^{9C^I}, resulting from regular disjunction of B^{9C^I}), and those with C Wx endosperm, colorless scutellum (hypoploid endosperm, hyperploid embryo, resulting from nondisjunction of B^{9C^I} at the second microspore division). The selected individuals were then crossed as male parents to a C wx (normal) stock with colored scutellum. Four classes of offspring were observed with regard to presence and distribution of pigment in the endosperm and scutellum. The Wx and wx types were separated within each class, and the data are reported in Table 1. The main characteristics of each class are briefly described:

Class I (purple aleurone, purple scutellum): The large wx group comes from fertilization by sperm carrying nontranslocated chromosomes; the few Wx types are the result of crossing over bringing C back to the B^9 chromosome. The fraction of such B^{9C} chromosomes undergoing nondisjunction cannot be detected by seed classification.

Table 1

Progeny of crosses involving the TB-9b translocation as the male parent.
The classification of the progeny was made for the markers C,C^I and Wx,wx.

Type of cross	Class		I		II		III		IV	
	Aleurone		purple		purple		colorless		tinged, purple spots	
	Scutellum		purple		colorless		purple		colorless	
	No. of ears		Wx	wx	Wx	wx	Wx	wx	Wx	wx
9Cwx, 9Cwx x 9Cwx, 9^BWx, B^{9C^I} (balanced translocation)	2		20	241	143	0	89	0	92	110
9Cwx, 9Cwx x 9Cwx, 9^BWx, B^{9C^I}, B^{9C^I} (hyperploid translocation)	31		26	268	3,412	13	2,250	11	454	1,610

Class II and Class III (respectively: purple aleurone, colorless scutellum, and colorless aleurone, purple scutellum): Nearly all individuals belonging to these classes are Wx because of the presence of 9^B; the exceptional wx individuals are the result of heterofertilization or of crossing over between Wx and the translocation point. Class II has hyperploid embryos with two B^{9C^I} chromosomes and hypoploid endosperms with no B^9. The reverse situation is found in Class III, with hypoploid embryos and hyperploid endosperms.

Class IV (tinged aleurone with spots of deep pigment, colorless scutellum): The classification of Wx and wx individuals allows an estimate of the rate of regular disjunction of B^{9C^I} in the presence of 9^B or with a normal chromosome 9. Some wx individuals inherited the C^I allele with chromosome 9; their frequency can be estimated from the frequency of reciprocal crossovers (Wx individuals of class I).

The rate of nondisjunction of B^9 in the presence of 9^B is estimated as follows: the total number of individuals in class II and class III, plus a fraction of the crossovers in the left column of class I (see Table 1), is divided by the total of the Wx types. The rate of preferential fertilization is obtained by dividing the number of individuals of class II by the total number of individuals in class II and class III. The values found are reported below, with standard errors:

Pollen parent	Rate of nondisjunction of B^9 in the presence of 9^B	Rate of preferential fertilization
Balanced translocation ($9,9^B,B^9$)	71.0% (2.5)	61.5% (3.1)
Hyperploid translocation ($9,9^B,B^9,B^9$)	92.3% (.4)	60.7% (.6)

Despite the inconstancy of nondisjunction rates frequently found for the B^9 chromosome, the present data indicate that there may be a significant difference, in this regard, between the two conditions of the translocation (i.e., balanced and hyperploid). W. Carlson reported comparable results (Ph.D. Thesis, Indiana University, 1968) for nondisjunction rates of B^9: 75% (balanced translocation) and 84% (hyperploid translocation). If

this difference is confirmed by further tests, an explanation should be sought. It is not obvious why the nuclear conditions existing prior to the meiotic separation of homologues should influence the nondisjunction of B^9 during the second microspore division.

Another peculiarity of the two genotypes (balanced and hyperploid) was observed after comparing the total ratio of Wx : wx.

	Total Wx	Total wx
Balanced translocation	344	351
Hyperploid translocation	6,142	2,002

In the case of balanced translocation, the ratio found is close to the expectation since the loss of all deficient spores (9^{B}Wx) is compensated by the loss of a considerable number of hyperploid spores (9wx, B^9) in the male gametophyte. In the case of the hyperploid translocation, an enormous excess of Wx was found. Since the two B^9's are expected to undergo a fairly regular meiotic segregation, most of the microspores will be either 9^{B}Wx, B^9 (balanced) or 9wx, B^9 (hyperploid). The latter type is frequently lost by gametophyte competition to the extent indicated by the observed ratio of Wx : wx.

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1. Test for cytoplasmic mutants induced by E.M.S. seed treatment.

The tests for induced mutations with which I am familiar were not planned to test for possible cytoplasmic mutants. This experiment was planned to test only for that type.

The first experiment was set up in 1970, but the treatment was too heavy. The main growing point tissue in many plants was killed or so heavily damaged that the plants were highly deformed. Many of them developed tillers but only a few had ears or tassels. The next experiment was begun in 1971, using a less severe treatment. In both experiments, the

plants grown from the treated seed were remarkably uniform. The treatment procedures were those recommended by our colleague, Dr. Robert A. Heiner, for obtaining uniform and repeatable results with E.M.S. seed treatment. The plants grown from treated seed in 1971 were uniformly shorter than those from the check from late seedling stage to maturity. At the time of harvest of plants from the treated lot, I noted that some had only 3 nodes below the ear node, none with more than 4 (avg. 3.6), whereas for the untreated check only an occasional one had 4 and most of them had 5 (avg. 5.0) below the ear node.

Pollen from untreated A619 was used on the plants from treated seed of inbred A619. Each ear was tested in a 75-seed row in the field in 1972. Isolation was such that open pollination was relied on for increase.

The 242 rows descended from treated and 53 from untreated seed were checked for off-type characters in the seedling and later stages. At pollen shedding, a check was made for male sterility. Had cytoplasmically inherited mutants occurred, at least in sectors which included portions of the ears, they should have appeared (probably in variable numbers) among the progeny in the rows that descended from E.M.S.-treated seed and not from the untreated seed. None were observed. In 36 of the rows from treated seed and in 15 from untreated seed, there was an occasional plant, usually only one in the row, which was thinner-stalked and shorter than normal. Many of these did not extrude their anthers. Also, all but one of the 22 such plants with seed had only a few kernels, but these were all plump and normal in appearance. Had the off-types been triploids, seed size should have varied.

The conclusion is that E.M.S. is not a very effective agent for inducing cytoplasmic mutants. Acknowledgements: Mr. Tom French for making the pollinations in 1971 and checking in 1972 for male sterility and other adult characters. Also the gratuitous help of Dr. Helmy Ghobrial in planting the 1972 field test. Dr. Richard V. Kowles set up the treatments in 1970 and 1971.

Charles R. Burnham

2. Chromosome aberrations from polymitotic.

In 1958, progeny were grown from 21 polymitotic plants crossed as ♀ with inbred A188. The number of progeny varied from 1 to 51, but 13 had 10 or more. For the seven cultures that had one or more semisterile or low sterile plants, the numbers were: 1 in a total of 9, 1 in 12, 2 in 33, 2 in 44, 3 in 28, 4 in 51, and 5 in 29; a total of 18 partially sterile plants. Eleven of the partially sterile (P.S.) plants were crossed on inbred A188. All but two segregated for P.S. in the next generation. Fertile plants from selfs of P.S. plants were testcrossed on normal to establish lines homozygous for the change. Studies were resumed after several years. Only seven of the lines that continued to segregate for P.S. plants were continued. Two lines that had plants with about 25% sterility were tested extensively without obtaining the homozygotes. Three homozygous lines were established. Two that traced back to the same original po plant had interchanges. One is T4-7, the other a T7-9. The third line is probably an inversion, since crosses with the chromosome identification set of interchanges give only a ring of 4. The results indicate that in polymitotic plants interchanges and other chromosome changes that can be recognized by partial sterility in progeny from crosses with normal do occur, probably at meiosis. They also suggest that different P.S. plants from the same po plants may be separate events.

The results reported here are only from tests in which partial sterility was transmitted through the pollen in generations subsequent to the first one.

If the experiment is repeated, tests for ♂ and ♀ transmission should be made to answer the question: do pollen abortion (pa) type changes also occur in polymitotic plants? As pointed out in M.N.L. 45: 133 (1971), certain of these might have a practical value in the use of male-sterility in the production of hybrid corn.

Chas. R. Burnham

3. Chromosomal interchanges from colchicine treatment.

In 1968, Neubauer and Thomas (Crop Sci. 6:209-210) reported that, when solutions were made with different commercial lots of colchicine,

the pH value varied. In order to obtain repeatable results, it was important to adjust the pH level. In alfalfa, pH4 gave the highest frequency of chromosome doubling in root tip cells of treated seeds.

In 1963, one of us (Neubauer) injected a colchicine solution at pH6 into the developing ears and tassels of corn plants growing in the field. Progeny were grown from two treated plants, 90 plants from one and 107 from the other. He found partially sterile plants in both. Three of the 8 plants in the first group examined cytologically had a ring of 4 not associated with the nucleolus. Four of the 5 plants in the second group had a ring of 4, two of them associated with the nucleolus (?-T6+?), two of them not. Homozygous lines were established from 6 different semi-sterile plants in the first group and 9 different ones in the second group. Since a change that occurred in a sector might produce several seeds with the same interchange, one line (now identified as a T2-6) was crossed with 6 of the others in the second group. One of the crosses had a ring of 4 and sometimes 10II, showing that the two lines involve the same two chromosomes, but have different breakpoints. The crosses with the other 5 all had 2 rings of 4, indicating that they had a different interchange. Intercrosses between the five show that three of them have an identical aberration, later identified as T4-7; the other two have interchanges involving either 4 or 7 plus some other chromosome. Hence, there are at least 4 different interchanges among this group of 9 homozygous lines.

One of the lines from the other group of 6 has been identified as a T1-5 interchange. No intercrosses have been made with the others in that group.

Until pachytene analyses are completed, we cannot rule out contamination as the source of the T1-5 and T2-6 interchanges since we were growing the entire series of these Coop stocks for our chromosome pairing studies. Since we were not growing any T4-7 interchanges, we conclude that at least this one and the other one that is T4 or 7 + ? were produced following colchicine treatment.

Interchanges from colchicine treatment may be of interest, since Garber and Dhillon (Genetics 47:461-467, 1961) have shown in Collinsia

that, with respect to chromosome segregation and fertility, they differ from those produced by x-ray treatment.

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4. Effects of colchicine, using multiple interchange heterozygotes.

In M.N.L. 42:120 (1968), Ghobrial reported that, when seedlings heterozygous for two rings of 10, e.g., T1-5-6-7-8 x T3-2-4-9-10, were treated with colchicine, a few of the plants produced sectors that extruded their anthers and shed pollen. The anthers had normal-appearing pollen that was much larger than normal. We concluded that tetraploidy had restored fertility. In M.N.L. 44:146-147 (1970), we reported that three plants were obtained from selfing, but the one that matured had a well-filled ear and kernels that varied somewhat in size. Plants from both classes of seed were diploid.

The crosses of those plants with the standard normal mentioned in that report have been grown. All F_1 plants were fertile, and hence the plants tested carried no interchanges. Barring an error, the 2n fertile plant from selfs using pollen from the fertile sector must have come from a ♂ and a ♀ gamete that carried only the normal chromosomes from the heterozygote with two rings, each ring having 5 normal and 5 interchanged chromosomes. If they arose by a haploidization process followed by chromosome doubling, only a combination which had all normal chromosomes, all the interchanged chromosomes plus the other 5 normal ones from either parent that contributed the ring of 10, or all the interchanged chromosomes from both parents would be able to produce viable diploid tissue. Certain aneuploids might also be viable. This still would not account for the abnormally large size of the pollen. Except for that point, the results appear to be similar to those reported by Franzke and Ross (1952, Jour. Hered. 43:107-115) in which true-breeding new types arose in C_2 progeny from colchicine-treated seed. Our experiment with corn should be repeated. The multiple interchange stocks are available from the Coop. If haploidization followed by chromosome doubling does occur,

this might be a method of obtaining a line homozygous for the interchanges present in both rings of 10 chromosomes. This I have been unable to do by conventional breeding methods (see following note). I hope to repeat this phase of the experiment but if anyone wishes to try it also, they will have my best wishes for success.

Charles R. Burnham

5. Progress report on establishing a line with all chromosomes interchanged.

As reported earlier, we have the following stocks which will produce large rings when crossed with normal: (1) T1-5-6-7-8, (2) T3-2-4-9-10, (3) T3-2-4-6-8, (4) T5-7-1-9-10, and (5) T5-7-1-9-10-8. Crosses of #1, 2, 3, and 4 with normal give a ring of 10 + 5II. The cross of #5 with normal gives a ring of 12. These F_1's are highly sterile but will set seed from self-pollination. The cross of (5) x (3) gives a ring of 20. The crosses of (1) x (2) and (3) x (4) produce plants with 2 rings of 10 which do not shed pollen although the anthers have a few normal appearing pollen grains. Plants obtained by backcrossing these F_1 plants to either parent should include some that are homozygous for the interchanges from the recurrent parent and heterozygous for the interchanges from the other parent. Their progeny from self-pollination should include plants homozygous for both groups of interchanges. These could then be x-rayed to combine them in a single line that would produce a ring of 20 when crossed with normal stocks.

Plants with 2 rings of 10 do not shed pollen, but, when backcrossed as $\frac{\circ}{+}$ to either parental ring of 10 homozygote, they have 0 to 7 or 8 seeds.

In 1972, we grew 800 seeds from those backcrosses and in the last two years have checked testcrosses of 66 fertile second generation descendants from those produced in previous years. No plant has been found that had all the interchanges from both multiple interchange parents.

This past summer, certain of the testcrosses did not shed pollen, but cytological examination shows that ones with the highest number of interchanges had only a ring of 10 + 2 rings of 4 + 1 II. What apparently happens is that crossovers occur between homologous differential segments in the chromosomes in each ring of 10 in the F_1 with 2 rings of 10,

producing combinations with fewer interchanges. Ghobrial (Ph.D. thesis, 1968) reported for the 5-7-1-9-10 ring, that 24% of the progeny from N x ring of 10 crosses had smaller rings ranging from a ring of 4 to a ring of 8. It would be helpful in planning the backcrosses if similar information were available for the other rings of 10.

Thus far, not enough fertile descendants from the backcrosses have been tested to insure including one that has all the interchanges that are present in the 2 rings of 10. Tests of another group of normals will be made this coming summer.

Another approach, that of adding a T8-10 interchange to T6-3-2-4-8, has not been completed. When and if it can be completed, the cross of T6-3-2-4-8-10 with 5-7-1-9-10-8 (the T8-10 here is the same one I am trying to add to T6-3-2-4-8) should produce an F_1 with 2 rings of 10, in which random segregation should include a viable combination that has all the interchanges. Again, there is the problem of undesired crossovers as well as the problem of increasing the spore with the desired combination when it does occur.

Charles R. Burnham

6. Interchanges not in the ARS, 1961 list, and changes needed in certain of the information in that list.

Translocation	Symbol	Listed (Longley 1961) breakage points ratio	ratio	New information, breakage points, etc.	
*1-2	a			1L.5	2L.4
1-5	8347	1S.84	5L.51	1	2
1-5	018-5	1S.53	5L.52	1	2
1-5	6899	1S.32	5S.20	1S.37	5L.11
1-5	055-4	1S.32	5L.31	1	8
1-5	040-3	1S.17	5L.61	1	2
1-5	024-5	1S.09	5L.98	1	2
1-5	8782	1 ctr.	5 ctr.	1S.02	5L.01
1-5	4331	1L.03	5S.02	7	10
1-5	e	1L.03	5L.09	1S.08	5S.16
1-5	6178	1L.04	5L.05	1	2
1-5	7219	1L.15	5S.19	1S.15	5L.33
1-5	48-34-2	1L.19	5L.76	1	4
1-5	8388	1L.30	5S.25	1	2
1-5	a	1L.52	5S.42	1L.58	5L.45
1-5	8041	1L.80	5L.15	1L.80	5S.10
1-6	e	1S.37	6L.21		6S ring of 6 only
1-6 (with 1-2)	5537	1S.31	6L.22		

*Stocks only at Minnesota, not in Longley, 1961 list.

Translocation	Symbol	Listed (Longley (1961) breakage points ratio	ratio	New information, breakage points, etc.	
1-6	6189	1S.23	6L.17		6S
*1-6	b			1L.25	6 sat
1-7	b	1L.53	7S.12		7L (Gopinath and B.)
*2-3	a			2S.9	3L.6
2-4	b	2L.59	4S.40		
There are two listed as 2-4b. This one should be 2-4L, see 1958 report.					
2-6	4394	2S.91	6L.12	4	6
2-6	2786	2S.90	6S.77		6S Org.
2-6	001-15	2S.72	6S.87		6S sat.
2-6	6671	2S.22	6L.22	5S.49	6L.35
2-6	e	2L.18	6L.20	2S	6S
2-6	5648	2L.25	6L.19	1	6
2-6	a	2L.28	6L.20	2L.4	6S.5
2-6	c	2L.37	6L.25	2(S?)	
2-6	9002	2L.57	6L.50	1	6
2-6	f	2L.79	6L.87	1	6
2-6	014-11	2L.81	6L.20	inseparable from an version in 6	
2-6	5419	2L.82	6S.79		6S Org.
2-6	8441	2L.94	6S.79		6S Org.
*2-6	Burnham #6049			2S.15	6L.06
*2-6	" #6052			2S.6	6L.6
3-6	b	3S.73	6S.82		6S sat.
3-6	6566	3L.41	6L.35		6S (Ibrahim)
*3-6	Roberts (Conn.)	3	6	2	3
3-9	c	3L.09	9L.12	3S.15	9S.20(E. Clark)
*4-5				4L.7	5L.7
4-6	025-12	4S.44	6L.34		6S
4-6	011-16	4S.31	6L.33		6S
4-6	8591	4L.17	6L.24		6S
4-6	Li	4S.7	6S.2	1	6S (org.tip)
5-6	5622			5S.87	6L.47 Phillips
5-6	8818			5L.91	6L.93 "
5-6	d	5S.64	6S.89	5S.58	6 sat "
5-6	8590	5S.29	6L.25	5S.25	6L.61 "
5-6	5685	5L.27	6L.20	5S.24	6L.23 "
5-6	8665	5L.58	6L.25	(independent of chrom. 5 genes)	"
5-6	8219	5L.76	6S.84	5L.69	6 sat. "
5-6	c	5L.81	6L.08	5L.89	6S.00 Burnham
Others:					
*5-6	b (McClintock)**	5L.72	6L.21	5S.1	6S sat. "
5-6	Burnham (5786)			5L or S.2	6L.2 "
*5-9	a	5L.69	9S.17	5L.86	9S.38 "
6-7	5181	6S.79	7L.86	6S Org.	7L.86 Phillips

**this is the one published in Genetics, 1950.
another one is listed as 5-6b.

Translocation	Symbol	Listed (Longley 1961) breakage points ratio	ratio	New information, breakage points, etc.	
6-7	4964	6S.76	7L.72	6S Org.	7L.63 Phillips
6-7	6498	6L.16	7S.48	6L.23	7S near cent.
6-7	4545	6L.25	7S.73	6L.07	7S near cent.
6-7	013-8	6L.31	7L.22	6L.27	7L.63
6-7	8143	6L.35	7L.36	6L.18	7L.16
6-9	a	6S.79	9L.40	6S Org.	
*6-10	McClintock			6S.5	10L.58
5-10	6061	5S.60	10L.57	2	10
*2-3	Clark (r-tester)			2	3

General Notes

1. The 1-5 interchanges: 058-2, 004-14, 4832, 5537, 5512, and 5813 were not received from the Coop or from Ames according to my records. All the others not in the list of changes have been tested enough to know they are 1-5 interchanges. The breakpoints of most have been verified cytologically, many genetically as to arm (Burnham et al. 1972, Genetics 71:111-126).
2. The 1-6 interchanges: For the following, the breakpoints are in the long arm of 6 as listed: 5013, c, and h.
3. The 4-6 interchanges: For the following, the breakpoints are in the long arm of 6 as listed: b, 6623, 8428 and 8927.
4. The 5-6 interchanges (Phillips, 1969, Genetics 61:107-116): For the following, the breakpoints are as listed: 6522, 4933, and only slightly different for 5906.
5. The 6-7 interchanges (Phillips, 1969): The breakpoints are only slightly different for: 4573, 7380, 6885, 4337.

Chas. R. Burnham
(assisted by many over the past years)

7. Progress report on the all-arms single interchange marker series after at least 8 backcrosses.

T2-9c	is shown to be T1-6
T4-7(7108)	is shown to be T3-4
T5-8a	is shown to be T3-4
T6-9(5454)	which gives low sterility: probably an inversion.

This means that certain chromosome arms may be marked with only one interchange. For the list, see M.N.L. 42:122-123 (1968).

Charles R. Burnham

8. "Discussions in Cytogenetics" reprinted.

A private reprinting of my book "Discussions in Cytogenetics" is available. Copies may be obtained for $9.80 plus mailing costs. Anyone wishing to order a copy should write to my home address: 1539 Branston St., St. Paul, Minnesota, 55108; the bill will be enclosed.

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1. An epistatic phenomenon resulting in aleurone color inhibition in maize.

In reciprocal crosses between individuals having the genotypes RR and rr, respectively, the expectations are: (1) self-colored kernels when the former is used as the female parent, and (2) mottled kernels when the latter is used as the female parent. We report here an exceptional case in which an interaction between a specific modifier, Ma, and a modifier-sensitive R allele, R*, results in the absence or near absence of anthocyanin in aleurone cells having the genetic constitution rrR*.

The epistatic phenomenon resulting in the absence or near absence of anthocyanin in rrR* cells was first observed in the cross: Inbred line KYS (BzBz, cc, rr) X "Bronze" (bzbz, CC, RR). The reciprocal of this cross produced only self-colored kernels. On the other hand, when our K^L-9 knob stock (BzBz, CC, RR) was reciprocally crossed to KYS, only self-colored or mottled kernels were obtained.

In order to determine the genetic difference between "Bronze" tester and K^L-9 stock plants, the F_1 and F_2 individuals of the cross, K^L-9 X "Bronze," were used as pollen parents in crosses to KYS. The results of the tests are shown in Table 1.

Table 1

cc, rr (Female) X CC, RR (Male)		Ear Phenotype: Per cent of kernels self-colored or mottled		
		100%	50%	0%
1) KYS	"Bronze"			x
2) KYS	K^L-9	x		
3) KYS	F_1		x	
4) KYS	F_2	x (19 ears)	x (49 ears)	x (17 ears)

The 1:1 colored to "colorless" kernel ratio obtained from crosses in Entry No. 3, and the 1:2:1 ear ratio obtained from those in Entry No. 4 of Table 1, indicate that the difference between the two RR stocks is due to a single gene pair. That this difference does not involve the bz_1 locus is clearly indicated by the fact that each of the bzbz, Bzbz, and BzBz F_2 individuals when crossed to KYS produced an ear which was either 100% colored kernels, 50% colored kernels, or 0% colored kernels. If the bz_1 allele was involved in the production of "colorless" kernels, then, only the bzbz plants in the F_2 generation when crossed to KYS should have produced "colorless" kernels.

That the c_1 locus is also not involved is deduced from the following line of evidence. The C-bearing chromosome 9 in the "Bronze" tester was marked with the sh_1 allele, while the C allele in the K^L-9 plants and the c allele in KYS were associated with the Sh_1 allele. Inasmuch as the c and sh loci are tightly linked, the two C alleles are traceable through the employment of the alleles found at the sh locus. On an ear obtained from a cross in Entry No. 3, 78 colored and 72 "colorless" kernels were obtained. Approximately 50% of the colored kernels (37) were found to be ShSh in genotype; similarly, about 50% of the "colorless" kernels (37) were found to be ShSh in genotype. Half of the "colorless" kernels, then, received the C allele from the "Bronze" tester, the other half receiving the C allele from the K^L-9 stock. Thus, it is obvious that the genetic difference between "Bronze" and K^L-9 does not involve the c locus.

Beckett's 1610A-2-30 (R allele from Acc. No. 749) and 1608-1 (R allele from Tama Flint) both give results identical to those of our "Bronze" tester when crossed to KYS. On the other hand, Coe Stock No. 3, Neuffer Stock No. 1, and our Abnormal Chromosome-10 (K10) stock all give results identical to those of K^L-9 when crossed to KYS. All of these five stocks are BzBz, CC, RR in genotype. Our conclusion that the genetic difference between "Bronze" and K^L-9 involves the r locus is based on the aforementioned observations. Specifically, our data indicate that the R allele found in the "Bronze" tester is not identical to the R allele found in the K^L-9 stock.

In order to determine whether or not the inhibition of aleurone color was strictly the function of a specific R allele (R^*), several other inbreds were examined. These inbreds were: W23, N6, Wf9, Oh43, Oh51A, K55, C103, M14, Hy2, L317 (all cc, rr), and Ky27, 38-11, and our tester g r sr (all CC, rr). Only the Inbred Line L317 gave results identical to those of KYS when crossed to either the "Bronze" or the K^L-9 stocks. The unavoidable conclusion is that the inhibition of aleurone color is either (a) an epistatic phenomenon, or (b) the result of allelic interaction between a specific r allele and a specific R allele.

To test the Allelic Interaction Hypothesis, KYS X W23 hybrids were produced and used as female parents in crosses to the "Bronze" tester. The r allele contributed to the hybrid by KYS was tagged with the plant color component "r" (r^r), while that contributed by W23 was labelled with the "g" component (r^g). The R allele in the "Bronze" tester was tagged with the "g" component (R^g). From the cross KYS, r^r / W23, r^g X R^g/R^g (Bronze), 136 colored and 125 "colorless" kernels were obtained. The 1:1 colored seedling (r^r/R^g) to colorless seedling (r^g/R^g) ratio realized in both the colored kernel class (70:66) and the "colorless" kernel class (64:61) negates the hypothesis that the inhibition of aleurone color results from a specific allelic interaction. What the data clearly indicate is that the R^* allele of "Bronze" is being influenced by a genetic factor other than specific r alleles in rrR^* aleurone cells.

The data in hand permit us to rule out the involvement of two modifiers, namely, M^{st}, the modifier of R^{st} which is tightly linked to the r locus (6 units), and M_r, the mutator of R^m which shows linkage to the c

locus (14 units). If $\underline{M}^{st}$ were involved in the inhibition of aleurone color and were the modifier in KYS and L317 interacting with the R* allele, then the "colorless" kernels obtained from the cross KYS, $\underline{r}^{r}$ / W23, $\underline{r}^{g}$ X $\underline{R}^{g}/\underline{R}^{g}$ (Bronze) should have given rise to only colored plants, save for rare recombinants. The 1:1 seedling color ratio observed in both the colored and "colorless" kernel classes is contrary to the expectation of the hypothesis which invokes the $\underline{M}^{st}$ gene. If, on the other hand, the mutator $\underline{M}_{r}$ were involved, then the $\underline{M}_{r}$-sensitive R (in this situation, the R*) allele in both of the reciprocal crosses: KYS X "Bronze" and "Bronze" X KYS, should have responded to the presence of $\underline{M}_{r}$. And the response should have resulted in kernels having colorless aleurone patches in an otherwise colored background aleurone. We observed, it will be recalled, inhibition of aleurone color in only one of the reciprocal crosses and the kernel phenotype to be either colorless or near-colorless, not mosaic.

We, therefore, propose the existence of an aleurone color modifier, Ma, which interacts specifically with a modifier-sensitive R allele, R*, the result being the absence or near-absence of color in aleurone cells having the genotype rrR*.

That the dosage relationship between the Ma and the R* genes is also critical in aleurone color inhibition can be gleaned from the following observations:

a) rrR* , MaMama : Colorless or near-colorless Aleurone

b) R*R*r , mamaMa : Self-colored Aleurone

It would be interesting to learn what are the necessary conditions that lead to complete absence of anthocyanin in rrR* aleurone cells containing the Ma gene. Future experimentation should lead to a more precise characterization of both the R* and Ma genes as well as of the epistatic phenomenon involved.

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1. Conditional colored and colorless alleles at the C_1 locus.

Besides the dominant colored allele, C_1, and inhibitor, C_1^I, recessive colorless, c_1, can be differentiated into two allelic forms: c^p (p for positive), the conditional colored which becomes colored in the light during germination (MGCNL 44:153, 1970), and c^n (n for negative), the colorless which fails to give color at any time. Their dominance relationship is $C^I > C > c^p > c^n$. It has been found that *c* testers derived from W22 and K55 contain conditional colored c^p alleles while most other *c* tester stocks and KYS contain colorless c^n alleles. Four point linkage data have been obtained to support the differentiation of *c* alleles into two forms (Table 1).

Some properties of this conditional colored c^p allele have been observed. Light is necessary for pigment formation in c^p tissue. Peeling off the husk to expose c^p tissue to the light does not induce pigment formation during normal seed development. However, c^p vp_1 kernels on the cob can synthesize pigments (although pigments are reduced) if the husk is removed. Thus, light and germination are two critical conditions for anthocyanin formation in c^p tissue. The c^p allele has many properties in common with *C*. It is inhibited by C^I and shows mottled expression with *R r r*. It also requires A_1, A_2, C_2, Bz_1, and Bz_2 to be present in the dominant form for pigment formation. Unlike *C*, c^p seeds from c^p/c^n F_2 segregating ears sometimes show a wide range of color variation, from very light to very intense. The variation is not heritable. It is possible that the variation is due to dosage effect at the *C* locus, to background factors, to varying physiology and vigor of germinating seeds, or to environmental factors.

The relative concentration of cyanidin to pelargonidin (obtained by acid hydrolysis of pigments) in c^p germinated seeds is much lower than that in normally pigmented *C* seeds (Kirby & Styles, Can. J. Genet. Cytol. 12:934, 1970). We observed similar results (lower cy/pg ratio) in c^p

Table 1

Four point linkage data for $\frac{+\ c^p\ +\ +}{yg\ c^n\ sh\ wx}$ x yg c^n sh wx

Test cross	Parental	R1	R2	R3	R1 & R2	R1 & R3	R2 & R3	R1, R2 & R3	Total
c^p (W22)	1731 1580 3311	584 510 1094 19.59%	63 105 168 3.01%	492 462 954 17.08%	14 5 19 0.34%	25 6 31 0.56%	2 0 2 0.04%	4 1 5 0.09%	5584
	Recombination percentages = yg c 20.58, c sh 3.48, sh wx 17.77								
c^p (K55)	963 892 1855	233 279 512 16.20%	68 79 147 4.65%	339 272 611 19.33%	1 5 6 0.19%	5 8 13 0.41%	4 5 9 0.28%	4 4 8 0.25%	3161
	Recombination percentages = yg c 17.05, c sh 5.37, sh wx 20.27								

tissue in K55 do not show a decrease in cy/pg ratio, compared with that of $\underline{C}$ tissue (Table 2). Within W22 background, F_1 ($\underline{C}\ \underline{C}$ x $\underline{c^p}\underline{c^p}$) and F_2 $\underline{C}$ --

Table 2

Relative concentration of anthocyanidins from $\underline{C}$ and $\underline{c^p}$ tissues in different backgrounds (average O.D. of 4 replicates).

Background	W22			K55		
Anthocyanidin	cy	pg	cy/pg	cy	pg	cy/pg
Genotype						
A C R	2.64	0.17	15.53	1.60	0.39	4.10
A c^p R	1.69	0.92	1.84	0.64	0.14	4.57
F_1(C C x c^pc^p)	1.54	0.10	15.40			
F_2 C --	1.64	0.14	11.71			
F_2 c^pc^p	0.59	0.32	1.84*			

*two replicates
cy = cyanidin, pg = pelargonidin

seeds have a cy/pg ratio similar to that of the $\underline{C}\ \underline{C}$ parent, while F_2 conditional colored $\underline{c^p}\underline{c^p}$ seeds have a ratio similar to that of the $\underline{c^p}\underline{c^p}$ parent. The data favor the hypothesis that physiological conditions of germination in W22 result in the increase in pelargonidin. Anthocyanidin constitutions of F_1 and F_2 between K55 $\underline{c^p}$ and W22 $\underline{c^p}$ have also been studied (Table 3).

Table 3

Relative concentration of anthocyanidins of F_1 $\underline{c^p}$ (W22) x $\underline{c^p}$ (K55), F_2, and backcrosses (average O.D. of 4 replicates)

Anthocyanidin family	Relative O.D.		
	cy	pg	cy/pg
F_1	1.64	0.70	2.35
F_2	0.71	0.38	1.87
F_1 x $\underline{c^p}$ (W22)	1.50	0.74	2.03
F_1 x $\underline{c^p}$ (K55)	1.12	0.44	2.54

The data indicate that the two $\underline{c}^p$ alleles present in W22 and K55 $\underline{c}$ testers have essentially the same effects. The materials used for K55 $\underline{c}^p$ were from very weak inbred ears, and this may be the reason for the higher cy/pg ratio observed in K55 $\underline{c}^p$ tissue. There seems to be a general trend such that the more vigorous the germinating seed, the lower the cy/pg ratio.

It is clear that there are two allelic forms present in $\underline{c}$ testers: $\underline{c}^p$, the conditional colored, and $\underline{c}^n$, the colorless. Light and germination are two required conditions for anthocyanin formation in the $\underline{c}^p$ tissue. The pigments formed in $\underline{c}^p$ tissue have a lower cyanidin/pelargonidin ratio than that in normally pigmented $\underline{C}$ tissue.

Shu-mei Chen

2. An unsuccessful search for mutations affecting anthocyanin distribution.

Large populations of one inbred line, Ky 27, grown in isolation, have been observed closely for mutations of factors controlling anthocyanin distribution. This inbred is $\underline{A}\ \underline{C}\ \underline{r}^r$ and has purple plumule; a search was conducted in the plants and in the seeds produced on them for pigment formation in new locations--for example, in culm, husk, glume bar, and aleurone tissue. Among more than 10,000 plants studied, no distinctive plants or sectors were found; among 3.62×10^6 kernels, several colored contaminations were identified, but no valid mutations were found, either as whole-kernel exceptions or as sectors down to the limit of naked-eye resolution. Considering that any single mutation-competent locus (for example, $\underline{r}^r$) is present in the aleurone in 3 doses (i.e., 10.86×10^6 chromosomes entering into the triple fusion), and that the twofold observation protocol used here should identify events through at least the first ten divisions (i.e., 10^3 sites for minimum detectable mitotic events; 10^6 mitoses per kernel), no mutation to anthocyanin synthetic capacity was found in around 10^{13} mitotic replications. This observing load was lightened by the help of Paul Bolen, Shu-mei Chen, John Cousins, Kenneth Hall, Henry Lee, Marion Murray, Donald Smith, Jean Spengel, and Charles Williamson.

E. H. Coe, Jr.

3. Organic solvents as pollen suspending media.

Among solvents tested and reported previously, paraffin oil has been found very satisfactory for protracted suspension of pollen before pollination, an aqueous sucrose-$CaCl_2$ medium suitable for short-term suspension, and glycerol unsuitable. Tests of carbon tetrachloride, cyclohexane, and p-dioxane were conducted in 1972. All three solvents are injurious to silks, and few or no kernels result following application of fluid suspensions. However, the volatility of these solvents permits recovery of dry pollen grains after suspension and drying. Pollen suspended in carbon tetrachloride for one minute, allowed to dry for two minutes and applied conventionally to silks yielded nearly normal sets (300 or more kernels); pollen suspended for two minutes before drying yielded reduced sets (50 or so); five or ten minutes yielded few or no kernels. Pollen suspended in cyclohexane for 1, 2, 5, or 10 minutes before drying was powdery and free-flowing in conventional pollinations, and yielded fully set ears indisguishable from the usual. In dioxane, one minute or longer exposure before drying destroyed functioning of the pollen.

Although cyclohexane is not a very broad-spectrum solvent, its promise as a suspending agent is considerable: It is a low-density solvent in which pollen grains distribute easily yet settle quickly; it volatilizes rapidly, leaving dry, loose pollen grains that are easily applied in conventional fashion; it appears to be harmless to pollen for exposures as long as 10 minutes, and possibly for much longer exposures. Any agent that can be solubilized in this solvent could be applied (and washed free) with facility before pollination.

E. H. Coe, Jr.
Paul L. Bolen

4. Selection for resistance of pollen to ultraviolet light.

Studies reported in 1971 (Newsletter 45:140) examined whether two generations of selection by exposure of pollen to ultraviolet light (UV) resulted in changes in types or frequencies of mutational events induced by UV upon a third exposure; the data were suggestive but required retests. Tests have been completed, partly in parallel (two generations of selection)

and partly in extension (three generations of selection). The populations were similar in size to those reported in 1971. The results were negative unequivocally--i.e., no differences in types or frequencies of events were found among unselected, twice-selected, and thrice-selected lineages when the pollen was once more treated with high doses of UV.

The tests for increase in transmission frequency of mutants under UV selection, also reported in 1971, have been expanded with negative results also. Those mutants that had significantly higher transmission in the initial tests were tested in numbers averaging three to four times greater, and none was found to show an increase in transmission under UV selection pressure.

E. H. Coe, Jr.

5. Tentative map positions of genes and A-B translocations on chromosome 10.

Following are the results of tests of chromosome 10 genes with TB-10a, TB-10b, and TB-10c (yes = gene uncovered [distal to translocation], no = gene not uncovered):

Gene	TB-10c	TB-10b	TB-10a
oy	yes	no	no
tn_2	yes	no	no
y_9	yes	no	
sr_3	yes	no	no
nl	yes	no	no
zn		no	no
du_1	no	no	no
li		yes	no
bf_2	no	yes	no
ms_{10}		yes	no
g_1	no	yes	yes
r	no	yes	yes
w_2	no	yes	yes
sr_2	no	yes	yes

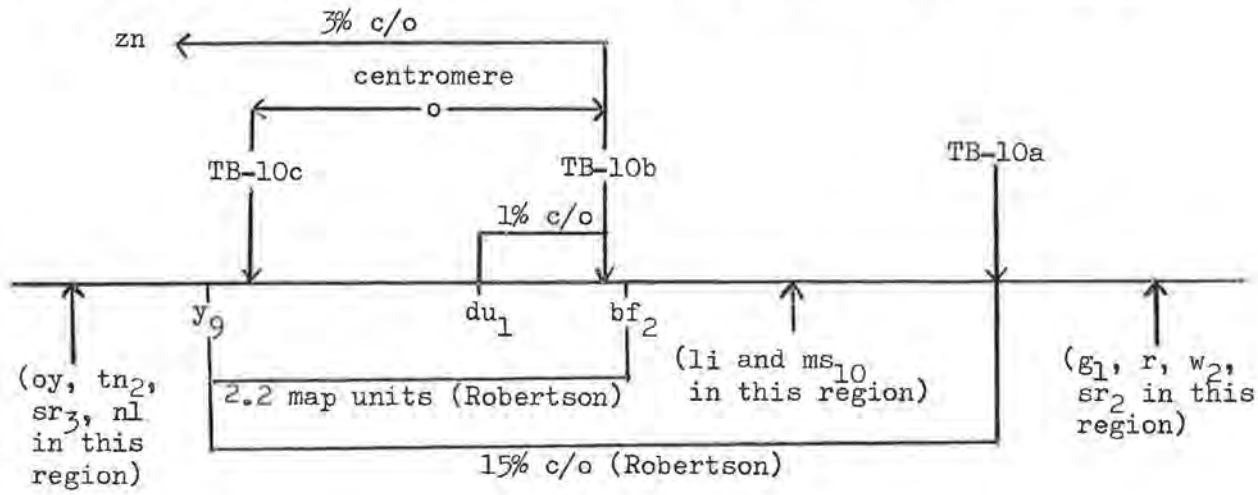
Additional data have been gathered on zn. Two hypoploid plants from a cross of zn by TB-10b were self-pollinated and a total progeny of 5

normal and 70 zn plants was obtained. Since the 10^B chromosome is not transmitted through either egg or pollen, all offspring will be zebra necrotic unless a crossover has occurred. If possible contamination and misclassification are ignored, the crossover percentage between the breakpoint of TB-10b and zn can be calculated. Because the 10^B strands fail to function, the binomial distribution $p^2 + 2p(1-p) + (1-p)^2$ applies, so $(1-p)^2 = 70/75$ or 0.9333 and $p = 0.0339$ or 3.4% crossing over.

Similar data on the position of du have been obtained. Although du alone is difficult to classify, wx du gives a brittle phenotype that is easier to work with. To generate suitable material, a +/wx TB-10b stock was crossed onto wx du. No "bt" grains appeared on the resulting ears, so du must be proximal to TB-10b. Waxy kernels from this cross were planted and seven hypoploid plants were self-pollinated and outcrossed to wx du females. A total of 8 normal and 401 "bt" kernels were obtained from the selfed hypoploids. Use of the binomial method gives a crossover frequency of 0.98%. From 8 outcrosses of the same 7 hypoploids, 5 normal and 1004 "bt" grains were obtained. Using straightforward calculation in this case, 5/1009 = .0050 = 0.50% crossovers. Reduced pairing near the breakpoint is likely, but the actual map distance is surely not large.

The crossover distance from du to TB-10a was calculated in the same way as for TB-10b. Ninety-six normal and 305 "bt" kernels were obtained from selfing 4 hypoploids, giving 12.8% crossing over. Five outcrosses to wx du gave 76 normal and 439 "bt" kernels, so 14.76% crossing over was obtained.

From the above data, plus Robertson's data (MGCNL 44:81-91), a tentative map of a portion of chromosome 10 can be devised, as shown below:



(Note that crossover data from hypoploids are shown as such and not equated to conventional map distances.)

J. B. Beckett

6. Two virescent mutants on the long arm of chromosome 8.

In 1964, a virescent trait segregated in a stock of Chapalote derived from Beckett Accession 552. In both field and sandbench, virescent seedlings typically greened first at leaf edge and tip, with the color grading smoothly from yellow or light green in the middle to green at the edge. In 1970, +/v-A552 plants were crossed by the A-B translocation set; virescent hypoploids segregated in the progeny of TB-8a.

Recently, among the mutants produced by Neuffer by EMS treatment, a virescent was uncovered by TB-8a in tests with the A-B translocation set. The phenotype is identical to that of v-A552 described above. Neither this mutant (v-E25) nor v-A552 is allelic to v_{16}. It is not unlikely that v-A552 and v-E25 are allelic, but test results are not yet available.

Because our v_{16} stocks are difficult to handle, v-A552 is being used in place of v_{16} as the tester for TB-8a.

In warm sandbench tests, v_{16} can rarely be separated from normal, while v-A552 can usually be classified easily. Conditions in an unheated sandbench favor ready classification of both virescents.

J. B. Beckett

M. G. Neuffer

7. A chlorophyll mutant associated with a_3 located on chromosome 3.

For some years we have had a stock of a_3 that carries a yellow-green or extreme golden type mutant. Crosses of a_3 golden by the A-B translocation series have produced progeny in which a_3 (recessive plant color) was uncovered by TB-3a and the golden mutant was uncovered by TB-3b. Our data confirm Earl Patterson's oral report to the Maize Genetics Conference in 1971 that a_3 is beyond the breakpoint of TB-3a. It is therefore on 3L rather than on chromosome 10 as reported in Emerson, Beadle, and Fraser (1935). Since the golden factor is distal to TB-3b, it lies

in 3S. We designate this gene golden-5 (g_5).

The stock from which a_3 and g_5 were derived was Coop. 54-1342, labeled a_3 g_1. Three backcrosses to normal failed to eliminate the golden when a_3 plants were extracted. Golden plants are usually weaker than normal; in some backgrounds they are semi-lethal. The leaves vary from yellow-green to pale or whitish golden; leaf sheaths are pale or nearly white. In contrast, g_1 plants derived from other Coop stocks are relatively vigorous, with much greener leaves and leaf sheaths when crossed to the same lines. Sandbench tests of $+/g_1$ X $+/g_5$ confirm that the two genes are not allelic. In our experience, g_5 is easier to classify in the sandbench than g_1.

J. B. Beckett
E. H. Coe, Jr.
M. G. Neuffer

8. New mutants located by A-B translocation method.

Following procedures described in previous years (MNL 45:144, 46:131), 156 mutants were tested with a set of A-B translocations that had been improved by the addition of TB4L,9S$_{6222}$ and 4L,9S$_{6504}$ (from Robertson). The collection included mostly seedling traits but some endosperm and mature plant mutants from various sources. Most were the result of chemical treatment.

60 of the 156 tested were located to chromosome. They are listed below by chromosome arm and can be added to those in Figure 1, MNL 46:131.

1S	1L	2S	2L	3S	3L	4S
1 wl	1 wl	1 v	2 v	1 v	1 gl	1 pg
1 v	2 v	1 pg		2 d		1 ad
1 ad	3 pg	1 nl				
2 et	1 d	4 d				
1 colorless defective kernel	4 ad	1 gl				
	1 nl	1 et				
1 rough kernel	1 colorless kernel	1 pitted kernel				
		1 collapsed kernel				

4L	5L	6L	7L	8L	9S	9L	10S
1 c_2-like	1 wl	2 w	1 ad	1 wl	1 w	1 pg	1 l
	1 v	1 v	1 gl		1 wl		1 str
	1 d		1 wrinkled kernel		1 pg		
	1 gl				1 gl		
					1 rough kernel		

10L
1 l

M. G. Neuffer
J. B. Beckett

9. Yg_4 allelic to oy, designated Oy^{yg}.

Yellow-green plants heterozygous for Yg_4 (MNL 46:136) were crossed on normal green plants heterogygous for oy to determine whether or not the two genes were allelic, since both were located on the short arm of chromosome 10. 280 kernels from this cross were planted. 277 grew and produced 130 normal green, 72 yellow-green, and 75 deep yellow seedlings. The latter seedlings resembled homozygous oy except that they were lethal and died at endosperm depletion. This is a close fit to the expected results assuming Yg_4 and oy are alleles and that the deep yellow seedlings are the heterozygotes carrying both mutants. More sophisticated tests for allelism are presently blocked by lethality of the double mutant heterozygote. Based on the assumption of allelism and our observations, the following relationships are evident:

Genotype	seedling color	viability
Yg Yg	yellowish white	lethal
Yg/-hypoploid	yellowish white	lethal
Yg +	yellow green	viable
+ +	normal green	viable
+ oy	normal green	viable
oy oy	deep yellow (greenish)	viable
oy/-hypoploid	deep yellow (greenish)	lethal?
Yg oy	deep yellow	lethal

In view of the above facts it is appropriate to change the designation of this mutant to a dominant yellow-green allele of oy and give it the symbol Oy^{yg}.

Another peculiar characteristic of this dominant allele is that homozygous kernels appear to lose their viability quite rapidly. Plantings made soon after harvest of randomly selected kernels from selfed ears from dominant/normal heterozygotes gave good 1 yellowish white: 2 yellow-green: 1 green ratios, while plantings from the same ears 1 year later gave 2 yellow-green: 1 green seedlings with an occasional yellowish white seedling. Approximately 1/4 of the kernels did not germinate. These would account for the missing yellowish white seedlings.

M. G. Neuffer

10. tn allelic to oy, designated oy^{tn}.

Crosses of tinged (tn tn) with yellow-green heterozygotes (Oy Oy^{yg}) and with oil yellow (oy oy) plants have given seedling progenies whose phenotypes clearly indicate allelism. The interactions are as follows:

parent genotypes	seedling phenotype
tn tn	green ⟶ yellow green
oy oy	oil yellow ⟶ yellow green
tn tn x oy oy	intermediate (yellow green)
tn tn x Oy Oy^{yg}	1 green: 1 lethal yellow
oy oy x Oy Oy^{yg}	1 green: 1 lethal yellow
Oy Oy x Oy Oy^{yg}	1 green: 1 yellow green

These observations demonstrate a complexity of activity at the oy locus that had not been suspected. More complete descriptions of phenotypes and better comparisons await conversion to a common background.

M. G. Neuffer
J. B. Beckett

11. Tan necrotic (nec-E409).

One of the tan necrotic mutants reported last year (nec-E409) has been located by selfing the hypoploids from each of the TB tests. Three out of four selfed ears from TB-5a hypoploids produced from the cross of

+/nec x N/TB-5a gave a total of 208 necrotic: 9 normal seedlings in progeny tests. Therefore, since hypoploids transmit only the normal chromosome (in this case chromosome 5) the mutant must be located proximal to the breakpoint of TB-5a. Excluding unlikely contaminants and the coincidental union of crossover gametes, the 9 normal seedlings represent 9 crossover and 9 noncrossover gametes among 434 chances or 2% exchange between the mutant and the breakpoint. Allowing for crossover reduction around the breakpoint, this would place the mutant near and possibly on the opposite side of the centromere.

For reasons as yet unknown, the mutant seedlings from the selfed hypoploid were not lethal initially, but gave green seedlings with tan necrotic crossbands. This contrasts with earlier observations that this mutant failed to develop chlorophyll after emergence.

M. G. Neuffer

12. Pale green mutable.

Two separate cases of pale green mutable seedling (frequent normal green sectors on pale green background) arose in a culture that also had Spm present. The mutants resemble Dr. Peterson's pg^{m}. Crosses by A-B translocations produced mutant hypoploids for both from crosses involving translocation 3b; therefore the mutants are tentatively located on the short arm of chromosome 3.

M. G. Neuffer

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1. Mass selection for seedling survival in a shrunken-2 (sh_2) population.

Our population of southern corn belt material has undergone 8 cycles of selection for seedling survival. Additional selection pressure was applied in the last 4 cycles for kernel weight and kernel density. The population now expresses greatly improved seedling survival, kernel weight, and kernel test weight when compared with corn belt inbred lines homozygous for the sh_2 gene or genetic stocks currently in use.

Seed stocks can be obtained from the Missouri Agricultural Experiment Stations.

	Seedling[+] survival	Kernel weight	Test weight
Mo sh_2 population	55%	.16g	54 kg/hl
(N15 sh_2 x B37 sh_2)F_1	19%	.08g	39 kg/hl
Corn Belt SX (dent)	86%	.60g	72 kg/hl

[+]Average of 13 planting date - corn belt locations in 1971.

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1. Complex regulatory scheme for catalase in early maize development*.

Maize catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) is a

*A portion of this work was completed at Michigan State University under AEC Contract AT(11-1)1338.

tetrameric enzyme containing four heme prosthetic groups. It catalyzes the breakdown of hydrogen peroxide to water and molecular oxygen although its precise physiological function is unknown. The enzyme exists in several isozymic forms, and has been well characterized genetically (Scandalios, 1968, 1969). In the liquid endosperm of the immature kernel, a single catalase species is present, and is the homotetrameric gene product of the $\underline{Ct_1}$ locus. At seed maturation, a second and distinct locus is activated ($\underline{Ct_2}$), and shortly after imbibition of the seed, five isozymes can be distinguished (the homotetramers of each locus plus three heterotetramers). The product of the $\underline{Ct_1}$ locus disappears during the first few days of development, and the $\underline{Ct_2}$ homotetramer becomes the primary species by days 7-10 (Scandalios, 1970). In addition to this differential activation of two distinct loci, there appear to be several other mechanisms controlling catalase expression during early maize development. The enzyme is subject to changing patterns of compartmentation (Longo and Longo, 1970), and the isozyme balance is controlled in part by differential rates of synthesis and degradation (Quail and Scandalios, 1971; Ganapathy and Scandalios, manuscript in preparation). Preliminary evidence indicates that at least two other mechanisms may be active during this same period, namely that one isozyme appears to be preferentially secreted from isolated scutella in response to gibberellic acid, and that there appears to be a catalase specific inhibitor present shortly after imbibition, but absent by the fourth day of germination. We are presently attempting to characterize this inhibitor, and relate it to the overall scheme of catalase regulation in maize.

Experiments in which crude day 1 and day 4 scutellar extracts were mixed showed that the catalase activity of the mixture was less than the sum of the activities added. Similar results were obtained in all three inbred lines tested (W64A, T21, 229). Dilution effects and proteolysis were ruled out as causes of the lowered activity, and the inhibitory factor was found to be in the day 1 extract. This factor has since been shown to be heat labile and non-dialyzable, leading to speculation that it may be a protein. The factor has been shown not to inhibit peroxidases (a group of catalytically related hemoproteins), indicating an apparently high degree of catalase specificity. An attempt is presently being made

to purify this inhibitory factor, and to determine if it differentially inhibits the various catalase isozymes.

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John C. Sorenson
John G. Scandalios

2. De novo synthesis of soluble and mitochondrial forms of genetically determined isozymes of malate dehydrogenase.

Three classes of malate dehydrogenase (MDH) have been identified according to their subcellular location: those found in the soluble fraction (s-MDH), those associated with the mitochondrial fraction (m-MDH) and those associated with glyoxysomes (g-MDH). Seven electrophoretic variants of m-MDH have been found among 35 inbred lines examined.

The developmental control of the two s-MDH's and the five m-MDH's has been studied using the inbred strain W64A. During early sporophytic development (dry seed - 10 days), all of the scutellar s-MDH's and m-MDH's follow the same developmental pattern; however, the total m-MDH activity is only 60% that in the cytosol. Chloramphenicol (CAP) and cycloheximide (CH), two known inhibitors of protein synthesis, were employed to determine whether the MDH isozymes are affected during the course of development. CAP (0.5-2.0 mg/ml) did not have an inhibitory effect on MDH, whereas CH (2-10 μg/ml) inhibited 60-65% of the MDH activity in scutella by 96 hrs. after treatment. Both s-MDH's and m-MDH's are inhibited to the same extent. It is thus apparent that protein synthesis in the cytoplasm is essential for the increase seen in both s-MDH and m-MDH activities during development. This result is quite consistent with our earlier findings that mitochondrial MDH's are controlled by nuclear genes (Longo and Scandalios, 1969, PNAS 62:104).

In order to test whether the increased MDH activities in the developing scutella result from activation of pre-existing MDH molecules or

from de novo synthesis of the MDH molecules, density labeling experiments were performed. Our results showed that both s-MDH's and m-MDH's extracted from scutella grown in 70% D_2O with 10mM $^{15}NH_4Cl$ do have higher buoyant densities than those grown in H_2O and $^{14}NH_4Cl$. This finding indicates that both s-MDH's and m-MDH's in the scutella of developing maize seedlings are de novo synthesized. These results suggest that in maize m-MDH isozymes are synthesized in the cytoplasm and then become associated with the mitochondria.

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1. Genetic studies of susceptibility to bacterial leaf freckles and wilt, Corynebacterium nebraskense.

A new bacterial wilt disease first discovered in Dawson County Nebraska in 1969 and later found in 23 counties in Nebraska, one in Iowa and one in Kansas, was observed to be much more severe on some hybrids than others in a demonstration plot in Dawson County in 1971. Therefore some preliminary studies have been conducted to obtain information on the genetic nature of susceptibility and tolerance in lines and their hybrids.

Twenty-three lines previously used in two diallel crosses (one 10 x 10 and one 13 x 13) were evaluated in the greenhouse in 1972. Two weeks after planting seeds in soil in pots, the plants were inoculated using a 25 gauge, 1 cc plastic tuberculin syringe. Two punctures were used, one just above ground level and the other about one inch above the first and at right angles to it. A total of 1 ml of inoculum containing approximately 1×10^8 bacterial cells from a mixture of 6 cultures was injected into each plant. Plants were each rated for susceptibility two weeks after inoculation using the following scale: 0 = no visible infection, 1 = slight infection, 2 = moderate infection, 3 = severe infection and 4 = dead. Since no plants were killed and the highest rating was 3, a

disease index was calculated by dividing the mean rating by 3 and multiplying by 100.

No resistant lines were evident and only a few 0 ratings were given. However, some lines were definitely more tolerant than others. Disease indexes ranged from 30 to 100. The most tolerant line and the two most susceptible lines from one diallel and the two most tolerant lines and the two most susceptible lines from the other along with their F_1 and F_2 hybrid and available backcross generations were then grown in the greenhouse in gravel beds flushed periodically with nutrient solution. Approximately the same inoculation and rating procedure was followed as in the first experiment except that inbred lines had not reached sufficient size to be inoculated until 18 days after planting.

In the second experiment, F_1, F_2 and backcross generations tended to be intermediate between their susceptible and tolerant parents. Ratings obtained were quite close to that expected based on an additive model.

The third experiment included the same lines and F_1, F_2 and backcross generations of Experiment 2 but it was grown in a field nursery in 1972. Planting date was June 3, plants were inoculated June 22, and disease ratings were determined July 14. A mixture of 6 cultures of the bacteria were again used in a sterile distilled-water suspension but in a higher concentration of about 2×10^8 bacterial cells per ml. One ml was injected into each plant using two injections as before--one at ground level and another one inch above the first and at right angles to it.

Some plants were completely killed and distinct differences between lines were evident. In addition to leaf blight and wilt symptoms, stunting of some plants was evident. In general, field ratings were in agreement with greenhouse ratings; however, the correlation coefficient between field ratings and greenhouse ratings was only 0.55. Hence, the predictive value of greenhouse studies is not as good as we would like. One experimental line which was completely susceptible in the first greenhouse experiment (Index = 100), and quite susceptible in the second (I = 82.4) was somewhat intermediate in the field (I = 68.5). Two of the most resistant lines, B37 and N10, appeared to be more susceptible in the field study than in the greenhouse. Nevertheless, the more tolerant lines based on greenhouse studies were also the most tolerant in the field, and they produced hybrids

which were also more tolerant than hybrids of susceptible x susceptible or susceptible x tolerant lines.

We can conclude from these studies that some lines are quite susceptible and some are reasonably tolerant but none were resistant when Corynebacterium nebraskense cells are injected into the plants at the dose levels used. Crosses of susceptible x susceptible, susceptible x tolerant and tolerant x tolerant lines tend to be intermediate between their parents. Probably more than one major gene locus controls disease reaction, but no definite conclusions can be drawn at this time. Further refinement of techniques are essential and further studies are needed to establish the genetic nature of disease reaction on corn plants.

We can definitely conclude that the use of resistant lines and hybrids and the breeding of even more resistant ones seems to be the best way to avoid farm losses due to bacterial leaf freckles and wilt of corn in Nebraska.

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1. Growth regulator induction of parthenocarpy in maize.

Natural parthenocarpy in maize is a common occurrence on partially fertilized ears. Growth substances from pollen or developing seeds stimulates parthenocarpy in unfertilized ovaries. This was first noted by Jones (1920) and investigated by Mangelsdorf (1926). Britton (1947, 1950) artificially induced parthenocarpy with alpha-naphthaleneacetic acid applied through a thistle tube in the cob or topically to the exposed ovaries. He included indole-3-acetic acid, indole-3-propionic acid, indole-3-butyric acid and beta-naphthoxyacetic acid in the 1950 study, but did not continue their use because of low or irregular response. He also tried applications of NAA to the silk, but got no ovary response to that method of application.

In 1971 we applied a range of growth regulators to maize silks in the course of a larger study of fertilization. An early-mid season hybrid (PA-290) was used with at least three ears per treatment. All growth regulators (see Table 1) were applied at a concentration of 10,000 ppm in 95% ethanol. One cc of each was applied to fresh silk with a

Table 1

Growth regulators applied to maize silks as 10,000 ppm solutions in 95% ethanol

Name	Symbols
B-(2-Furyl)-acrylic acid	B Acrylic
2-4 Dichloro phenoxyacetic acid	2-4,D
3-Indole butyric acid	IBA
3-Indole propionic acid	IPA
Naphthalene acetic acid	NAA
2,4,5 Trichloro phenoxypropionic acid	2,4,5 TP
N_6 Benzyladenine	BA
N_6 Benzyladenine plus 2,4,5 Trichloro phenoxyacetic acid	BA + 2,4,5 T

hypodermic syringe. The silk was protected from contamination with a standard glassine ear bag and, following treatment, the glassine bag plus a Kraft paper bag. Controls received one cc of 95% ethanol. All ears were harvested when normally pollinated ears showed full ripe kernels and frost had damaged the foliage to the point of preventing further seed or fruit developments.

The results are given in Table 2.

Cob development was obviously linked to fruit development and the full normal cob length was not attained with less than 50% of the possible fruits showing development. Also, as noted by Britton (1950), fruits began development from the tip, not the base of the ear. Apparently a gradient of action is established and at some distal point this is not sufficient to stimulate fruit development.

Table 2

Results of growth regulators applied to maize silks. Results are averages of at least three ears per treatment.

Growth regulator	Cob development	Percent possible fruit development
B Acrylic	small	none
2-4,D	normal length	75 - 80%
IBA	none	none
IPA	medium length	25 - 30%
NAA	normal length	75 - 80%
2,4,5TP	normal length	90 - 100%
BA	normal length	85 - 90%
BA + 2,4,5T	normal length	85 - 90%

The 2-4,D group of compounds were the most effective in inducing parthenocarpic fruits. One possible explanation of this is that they can remain at higher concentrations in the plant because plants do not contain enzyme systems for the natural breakdown of 2-4,D compounds. Benzyladenine was also an effective stimulator, but showed no synergistic increase when combined with 2,4,5T.

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2. Peroxidase activity, β-glucosidase activity and phenolic levels in monogenic resistant (Ht) and susceptible (ht) maize tissues inoculated with Helminthosporium turcicum.

The Ht gene conditions chlorotic lesion resistance to northern corn leaf blight, the causal agent of which is Helminthosporium turcicum Pass. Post-inoculation levels of peroxidase, β-glucosidase and phenolics were determined in isogenic susceptible (ht) and resistant (Ht) genotypes at daily intervals for four days and again after a period of 9-10 days.

Peroxidase activity in resistant inoculated tissues increased on day 1, rose sharply through day 3, decreased on day 4 and was only slightly higher than healthy tissue on day 10. Activity of susceptible inoculated tissues increased on day 2 and continued to rise through day 4 and decreased at day 10 with the onset of lesion desiccation. Electrophoresis showed that three peroxidase bands were produced in both resistant and susceptible inoculated tissue extracts. The bands were not detected in healthy tissue. No peroxidase was detected in culture homogenates. Increased peroxidase activities appeared to be correlated with degree of cellular disruption brought about by the infective process and was not directly associated with monogenic resistance.

β-glucosidase activities in resistant and susceptible tissues also increased following inoculation and decreased with desiccation of susceptible lesions and slowing down of lesion expansion in resistant tissues. H. turcicum cultures were shown to produce large amounts of β-glucosidase indicating that increases following inoculation may be fungal in origin.

Total phenolics increased between day 1-4 in both resistant and susceptible inoculated tissues. However, phenolic levels 10 days after inoculation were substantially higher in resistant inoculated tissue, any amount of which could be related to phytoalexin production since its identity is believed to be phenolic in nature.

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1. Characterization of chloroplast and nuclear DNA of maize.

The base composition of chloroplast and nuclear DNA was determined for two corn hybrids by two techniques, acid hydrolysis and melting point profile. The corn hybrids were Coker X210 and X210V, which are single cross hybrids differing only in that X210 contains normal cytoplasm while X210V has the Texas cytoplasm (Tcms). To the best of our knowledge, the base composition of chloroplast DNA (cDNA) has not been reported previously. Base composition of total cellular DNA has been determined by others (1, 2, 3, and 4) although their determinations were not always in complete agreement. In this study the cell organelles, nuclei and chloroplasts were first isolated and then DNA was isolated from the different organelles. Since total cellular DNA is comprised primarily of nuclear DNA (nDNA), a difference in base composition between total cellular and nDNA is unlikely.

Chloroplasts and nuclei were isolated from green leaves taken from plants 1-2 months of age. Leaves were ground in a Waring blender with buffered sucrose. Chloroplasts and nuclei were isolated by differential centrifugation in a sucrose gradient first and later in a discontinuous glycerol gradient. The chloroplast fraction was further purified by selective solubilization with Triton X-100. Nuclear DNA was isolated from nuclei by a modification of the Marmur method (5). The same method was employed for isolating cDNA from chloroplast except that some alcohol precipitation steps were omitted. Further purification of c and nDNA's was obtained by preparative CsCl density centrifugation (6). DNA was pelleted from CsCl solutions by centrifugation at 50,000 RPM for 18 hours at 25°C in the Beckman L2-65 ultracentrifuge (Type 65 rotor).

The thermal denaturation temperature (T_m) and the mole percentage of guanine plus cytosine were determined by the methods of Marmur and Doty (7). T_m measurements were made on a Gilford 2000 multiple sample absorbance recorder coupled with a Beckman DU spectrophotometer equipped with a temperature controlled cuvette chamber. DNA was melted in 1 SSC (.15 M NaCl, .015 M trisodium citrate). An acid hydrolysis technique (8)

was used for determining base composition. The molar percent GC was determined by "Differential Extinction Technique".

The means of the molar percentage of guanine and cytosine are given in Table 1 for the c and nDNA's of the two hybrids as determined by the two methods. An analysis of variance was used to test for significance. Determination of GC percentage by the acid hydrolysis technique (8) or the thermal denaturation (T_m) technique (7) gave results which were not significantly different. Nuclear DNA from X210 and X210V had similar GC percentages as expected. Chloroplast DNA from X210 and X210V also had similar GC percentages. This result was of interest because X210 carries the normal cytoplasm while X210V has the Texas type. Texas cytoplasm differs from normal in at least three factors: male sterility and resistance to two leaf diseases.

Table 1

Molar percentage of guanine and cytosine of maize DNA's from two hybrids determined by two methods

	nDNA		cDNA	
	T_m method	Acid hydrolysis method	T_m method	Acid hydrolysis method
X210 (normal cytoplasm)	42.9	43.0	40.9	38.2
X210V (Texas cytoplasm)	43.2	44.5	40.1	38.8

The overall mean GC percentage for nDNA is 43.3 as compared with 39.5 for cDNA. This difference is significant at the 1% level. The 43.3% GC for nDNA compares favorably with the result of 42% found by Rinehart and Sansing (1). No value is available for comparison of the GC percentage of cDNA. However, Kirk (9) contends that the cDNA of typical higher plants has a GC content of about 37-38%, which compares favorably with our estimate of 39.5% for maize.

The acid hydrolysis technique also allowed us to check for the presence of 5-methyl cytosine. None was found in cDNA, but 6.9% of the nDNA

was found to be 5-methyl cytosine. No data are apparently available on the amount of this base in maize DNA's. Our results are consistent with other higher plant DNA's since 5 to 6% 5-methyl cytosine is found in several other nDNA's of grasses and little or no methyl cytosine is found in the cDNA's of higher plants.

The GC content of total cellular DNA was determined by the thermal denaturation method for the two hybrids. A mean value of 43.7% GC was obtained which is not significantly different from 43.3% found for nDNA. Therefore, the organelle DNA's are either not present in sufficient quantities to influence the GC content or are identical. The former is known to be true for cDNA.

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1. Ribonuclease activity in half opaque-2 kernels.

The defatted mature endosperm powder (200 mg) of normal maize (CM 109), opaque-2 (CM 109), and half opaque ($S_{5}o_{2}$): half normal ($S_{5}+$) maize was extracted according to the procedure of Wolf (Experimentia 24:890, 1968). Acrylamide (7.5%) gel electrophoresis of the above extract was carried out at 5+ 2°C. The gels were incubated in an RNA solution for one hour. The reaction mixture included yeast RNA in a 0.2 M acetate buffer, pH 5.0. The fixation and staining was done in a 0.2 M acetate buffer containing 1% Lanthan acetate and 1% methylene blue. The stained gels were washed repeatedly with 0.1% acetic acid until the unstained transparent bands of RNase were clearly revealed.

The normal endosperm (CM 109) revealed a single broad but not very clean band, between 1.5 cm and 2.5 cm from the origin. The opaque-2 endosperm on the other hand, revealed four very clear (transparent) and distinct bands occupying a longer area of the gel than the normal counterpart. They extended from 1.5 cm to 3.0 cm from the origin. Among the four bands, the first and fourth were very prominent. The first two bands in the opaque-2 occupy the same position as the single band in the normal.

The normal and opaque tissues of S_5 kernels revealed a still different pattern with an increased number of bands. In $S_{5}+$ three bands were present, the first two being nearer to the origin. The third band occupied the same position as in the normal but was less broad. The opaque tissue of $S_5(S_{5}o_{2})$ revealed five bands, all except the first two being very transparent. The first three bands resemble the pattern of $S_{5}+$. Bands 4 and 5 of $S_{5}o_{2}$ were not observed in $S_{5}+$. In general, the pattern of $S_{5}o_{2}$ represented a combination of the patterns of opaque-2 and S_5.

The RNase activity seems to be much higher in opaque than in normal endosperm and its accumulation occurs within 16 days of pollination, the rates being smaller in both endosperms after sixteen days, suggesting

activity of the opaque gene. The presence of more bands and RNase isozymes in S_{5o_2} suggests a higher activity compared with S_{5+} or opaque-2; however, the S_{5+} exhibits a higher RNase activity compared to normal or opaque-2, which may be due to genotypic differences.

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2. Luteolinidin in aleurone tissue of the bz_1 mutant.

By using chromatographic (BAW, Forestal), spectrophotometric and chemical techniques, it was found that hydrolysates of methyl alcohol-HCl extracts of bz_1 aleurone contain an orange-red pigment, Luteolinidin (3-deoxycyanidin) and apigeninidin (3-deoxy pelargonidin), in addition to a dark brown pigment. However, apigeninidin was present only in trace amounts. These pigments were absent in the hydrolysates of the single mutants C^I, a_1, r, c_1, c_2, and a_2 and the double mutants C^I bz_1, c_1 bz_1, a_1 bz_1, and $a_2 bz_1$. The a_2 bz_1 hydrolysate yielded cyanidin chloride as a result of conversion of the Leucocyanidin. The double mutant, in bz_1, has shown about a fivefold increase in pigment as determined by a Klett Summersson photoelectric colorimeter.

A. R. Reddy
G. M. Reddy

3. Chemical nature of an induced salmon silk mutant.

A salmon silk mutant induced by DES in opaque-2 material was subjected to chromatographic, spectrophotometric, and chemical techniques and it was found that the hydrolysates of a methyl alcohol-HCl extract of fresh silks contain an orange-red pigment, Luteolinidin.

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1. Opaque-4 designation withdrawn.

The recessive endosperm mutant which was tentatively designated opaque-4 (MGCNL 40) has now been found to be an allele at the floury-1 locus. Our analyses indicate that both $\underline{fl}^{a}$ (supplied by Dr. Alex Paez) and $\underline{o}_4$ are normal in lysine levels. Also both apparently have the same phenotype when in similar backgrounds. Therefore, we propose to withdraw the opaque-4 designation in favor of the earlier (Mazoti, 1940, Anales del Institute Fit, de St. Catelina 2:17-26) designation $\underline{fl}^{a}$.

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1. Inheritance of date of pollen shedding in a corn diallel cross.

Pollen grain diameters measured on a number of inbred lines in 1970 (Maize Genetics Coop. News Letter 46:171-172, 1972) were used to select ten lines as parents for diallel crosses made in 1971. The parents represented a wide range of phenotype for pollen grain size but were not selected for any measurement of maturity. The complete diallel was planted in the field at Brookings, South Dakota, in 1972 with three replications of seven plant plots for each line and reciprocal cross. As the plants matured the date when four or more of the plants in a plot first shed pollen was recorded as the numerical day of the year.

Table 1 includes the means of the 100 test entries. The earliest parent was W629A which attained 50 percent pollen shedding on day 200. This was equaled by the cross W629A x A641 and its reciprocal. The latest maturing entries were the two parent lines Mo17 and WF9, both of which shed pollen on day number 218, and parent line B45 which shed on day 220.

Table 1

Day of 50 percent pollen shedding for the 10 x 10 corn diallel in 1972

	W64AHt$_1$B	Mo17	P254	SD9	SD12C	A641	WF9	Oh45	B45	W629A
W64AHt$_1$B	217	214	214	208	209	206	213	210	211	205
Mo17	212	218	210	209	207	207	211	210	216	205
P254	212	211	213	205	205	204	212	208	212	201
SD9	207	208	207	213	206	204	207	208	209	202
SD12C	207	211	206	207	213	203	210	207	213	203
A641	207	206	204	203	202	209	207	203	208	200
WF9	212	211	212	207	209	208	218	208	211	205
Oh45	209	210	208	207	206	204	208	214	212	202
B45	213	214	212	211	213	211	212	212	220	208
W629A	203	205	202	203	202	200	203	203	208	200
Mean	210	211	209	207	207	206	210	208	212	203

An analysis of variance of combining ability indicated that both general and specific combining ability effects were highly significant sources of variation. Reciprocal crosses were found to be dissimilar in their reactions. The analysis is summarized in Table 2.

Table 2

Analysis of variance of date of pollen shedding in 1972

Source	d.f.	m.s.
Total	269	
Replications	2	161.0
General combining ability	9	357.7**
Specific combining ability	35	8.5**
Maternal	9	3.0
Reciprocal	36	2.1*
Error	178	1.2

*P < .05
**P < .01

The statistical difference Wr-Vr (B.I. Hayman, Genetics 39:789-809, 1954) was homogeneous over arrays (Table 3). This indicated that an additive-dominance model with independent gene distribution was adequate to explain the data. We therefore proceeded further with the analysis.

Table 3

Analysis of variance of Wr-Vr for date of pollen shedding

Source	d.f.	m.s.
Total	29	
Replications	2	39.327
Arrays	9	4.441
Error	18	2.333

The dominance status of the parental lines was established by their relative positions along the regression line of Wr,Vr. There appeared to be no relationship between the dominance level and the actual date of maturity. A listing of the parents from most dominant to least dominant follows: A641, W629A, SD9, Oh45, B45, SD12C, WF9, W64AHt$_1$B, P254, and Mol7.

Theoretical genetic limits of maturity were obtained for each replication from the points in intersection of the regression line of Wr,Vr and the limiting parabolas. Table 4 shows that a recombination of this germplasm would permit the selection of inbred lines both earlier and later than those entering the diallel as parents.

Table 4

Limits of ranges of parental pollen shedding dates and estimated theoretical genetic limits in each of three replications

Statistic	Block I		Block II		Block III	
	Parent	Estimate	Parent	Estimate	Parent	Estimate
P_E	W629A	200	W629A	200	W629A	201
P_L	WF9	219	B45	221	B45	221
Y_E	----	189	----	185	----	196
Y_L	----	248	----	250	----	237

Both the additive and dominance components of genetic variation appeared to be substantial as judged by the relative magnitudes of their standard

errors. The components of variation are shown in Table 5 and some of the proportions of the components in Table 6.

Table 5

Components of variation for 50 percent pollen shedding in 1972

Component	Estimate	Standard error
D	31.8533	1.0847
F	4.3719	2.5027
H1	20.9500	2.3088
H2	18.9128	1.9622
LOWH2	114.8357	1.3134
D-H1	10.9034	1.9414
E	1.6212	0.3270

Table 6

Proportions of components of variation for 50 percent pollen shedding date in 1972

Proportion	Estimate
$(H1/D)^{1/2}$	0.8110
H2/4H1	0.2257
$[(4DH1)^{1/2} + F]/[(4DH1)^{1/2} - F]$	1.1849
LOWH2/H2	6.0719
Heritability (Crumpacker - Allard)	0.58

The mean degree of dominance was 0.81 or well within the partial dominance range. Although H2/4H1 was equal to 0.2257 and therefore suggestive of some asymmetry at loci showing dominance, the fact that H2 was not significantly different than H1 leads to the conclusion that the parents contain positive and negative genes in similar proportions.

The narrow sense heritability $h^2 = 0.58$ was sufficiently large

that the germplasm of the diallel should respond within a few cycles of mass selection.

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2. Measurement of pollen grain size in the diallel.

The same diallel used to study the inheritance of maturity was also used for an investigation of diameter of pollen grains. Bulk pollen samples from maturing plants in each plot were collected by tapping the tassels over a petri dish. The pollen was suspended in acetocarmine stain solution and examined with a light microscope. Diameters of at least 50 grains per plot with the grains randomly oriented in the microscope field were measured with an ocular micrometer. The data were analyzed in ocular micrometer units without transformation to metric scale.

A preliminary analysis of variance indicated highly significant differences among the parents for both general and specific combining ability effects. Also, the analysis of Wr,Vr indicated homogeneity of that statistic over arrays. However, upon proceeding further with the analysis it was evident that the genetic partitions of the components of variation did not differ significantly from zero. These components are shown in Table 7.

Table 7

Component	Estimate	Standard error
D	-0.2175	2.1563
F	-4.7487	4.9752
H1	2.7980	4.5899
H2	2.6978	3.9009
LOWH2	-0.5152	2.6111
D-H1	-3.0154	3.8595
E	4.2276	0.6501

Thus, despite the fact that the parents were selected to provide a maximum range of expression in pollen grain diameter, the differences observed in 1972 were not sufficiently large to permit a genetic analysis. Banerjee and Barghoorn (Maize Genetics Coop. News Letter 45:244-245, 1971) reported that position of the flower on the tassel, size of the anthers, time of anthesis and anther dehiscence and water deprivation cause variability in maize pollen grain size. Rumbaugh and Whalen (Maize Genetics Coop. News Letter 46:171-172, 1972) noted real differences in pollen grain size when the same plants were sampled on successive days. The results of the 1972 research indicated the sensitivity of this trait to environmental influences and the difficulties inherent in a genetic analysis of pollen grain size.

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1. Linkage relations of opaque-7 with marker loci in linkage group 10.

Linkage relations of the opaque endosperm, high lysine mutant previously reported to be in linkage group 10 (M.N.L. 45: 184, 1971), and now designated opaque-7, have been determined in testcrosses involving the marker loci : R (aleurone color), M^{st} (modifier of Stippled), G_1 (plant color) and Lc (leaf color). The locus of opaque-7 is situated about 25 recombination units to the right of the R locus on the linkage map of chromosome 10.

In testcrosses of $R^r o_7 / r^g o_7$ heterozygotes, the recombination value varied from .224±.02 to .333±.01. Differences in recombination value between families were significant and there was a significant tendency for recombination in microsporogenesis to be more frequent than recombination in megasporogenesis. Recombination values of .244±.02 and .264±.02, .245±.01 and .333±.01, .296±.01 and .319±.01 among female and male gametes,

respectively, were obtained in three sets of reciprocal testcross matings.

In plants heterozygous for abnormal 10 (K10), recombination between R and O_7 was greatly reduced, the recombination values being 0.004 in R^{nj}Cudu O_7 K10/r^g o_7 k10 heterozygotes and 0.0136 in R^g O_7 K10/$r^g o_7$ k10 heterozygotes.

Three point testcross data, involving R^r(Ecuador)Lc O /r^g lc o, and $R^{st}M^{st}O$ /r^g m^{st} o heterozygotes, and classifications of G R^r o / g r^g O selfed matings, which establish the sequence of loci in this region of the linkage map of chromosome 10, are given below:

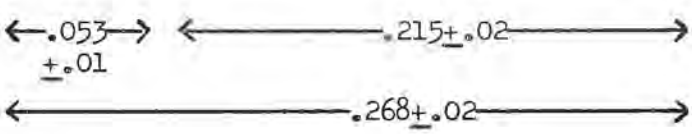
(a) Loci: R Lc and O

Mating: $\frac{R^r(\text{Ecuador})\ \text{Lc}\ O}{r^g \qquad \text{lc}\ o}$ ♀♀ x $\frac{r^g\ \text{lc}\ o}{r^g\ \text{lc}\ o}$ ♂♂

No. of families classified	No. of plants							
	R^rLc O	R^rLc o	R^rlc o	R^rlc O	r^gLc o	r^gLc O	r^glc O	r^glc o
2	106	37	6	1	nil	9	37	106
Total 302								

Linkage Map:

Recombination values:

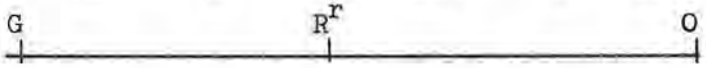


(b) Loci: G, R^r, and O

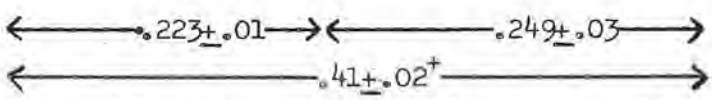
Mating: G R^ro /g r^gO selfed.

No. of ears classified	No. of seedlings*								
	G R^rO	G R^ro	g R^rO	G R^ro	G r^gO	G r^go	g r^gO	g r^go	Total
4	557	288	98	43	107	2	164	18	1277

Linkage Map:



Recombination values:



*Data obtained by classification of green (G) vs golden (g) plant color in the seedling stage.

+Recombination value not corrected for occurrence of double crossovers.

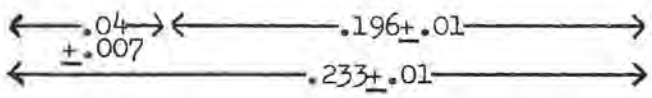
(c) Loci: $\underline{R^{st}}$ $\underline{M^{st}}$ and $\underline{O}$

Mating: $\frac{G\ R^{st}\ M^{st}\ O}{g\ r^{g}\ m^{st}\ o}$ ♀♀ $\times$ $\frac{g\ r^{g}\ m^{st}\ o}{g\ r^{g}\ m^{st}\ o}$ ♂♂

Family	No. ears classified	Number of kernels: $R^{st}M^{st}O$	$R^{st}M^{st}o$	$R^{st}m^{st}o$	$R^{st}m^{st}O$	$r^{g}O$	$r^{g}o$	Total
K765	7	445	116	27	3	136	479	1206
K766	3	206	48	5	-	71	222	552
Totals	10	651	164	32	3	207	701	1758

Linkage Map:

Recombination values:

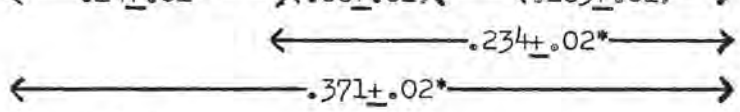


In addition, the following four point testcross data, obtained by classification of four ears produced by the above mating, confirms the sequence of loci as being G, $\underline{R^{st}}$, $\underline{M^{st}}$ and O.

Kernel phenotype:	Number of individuals of: $R^{st}M^{st}O$	$R^{st}M^{st}o$	$R^{st}m^{st}o$	$R^{st}m^{st}O$	$r^{g}O$	$r^{g}o$	Total
Plant color:	G g	G g	G g	G g	G g	G g	
	181 32	42 1	14 1	2 -	167	41 172	554

Linkage map:

Recombination values:



*additive recombination values

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1. Animal response to diets containing opaque-7 endosperm proteins.

Following establishment of the high lysine character of the opaque-7 endosperm mutant, a small feeding trial was conducted to obtain evidence of animal response to this maize mutant.

In the experiment, net protein utilization (N.P.U.) was measured for 3 diets each fed over a 10-day test period to three groups of 4 Wistar albino rats. The diets were meals prepared from opaque-2 maize (S.U. hi lysine synthetic), opaque-7 maize (W22 inbred stock) and normal endosperm maize (W22 inbred isoline). The diets were formulated to an equi-nitrogen level and each contained 7.806% protein. The opaque-2 diet contained 93% opaque-2 maize meal and 7% of a salts, vitamins, and cellulose mixture. The opaque-7 diet contained 85% opaque-7 maize meal, 7% salts, vitamins, and cellulose and 8% maize starch. The normal endosperm meal contained 79% of meal, 7% salts, vitamins, and cellulose, and 14% maize starch. Net protein utilization was calculated from the relation (Miller and Bender, 1955, Br. J. Nutrition 9: 382):

$$N.P.U. = \frac{B - B_k + I_k}{I} \times 100\%$$

where B = body nitrogen of the group of rats fed the test protein,
I = nitrogen intake of the group of rats fed the test protein,
B_k = body nitrogen of the group of rats fed a nearly nitrogen-free diet, and
I_k = nitrogen intake of the group of rats fed the "nitrogen free" diet.

The "nitrogen free" diet used in the experiment contained 0.308% protein, and was fed to two groups of 4 rats for the purpose of obtaining estimates of B_k and I_k.

N.P.U. values were calculated for each group of 4 rats separately, thus giving 3 estimates of N.P.U. for each diet. These estimates were averaged to give mean N.P.U. values for the three diets as follows:

Diet	N.P.U. Value (%)
Normal endosperm (W22)	46.3
opaque-7 endosperm (W22)	56.28
opaque-2 endosperm	59.89

Analysis of variance showed that N.P.U. value for normal endosperm maize was significantly lower than the values obtained for opaque-2 and opaque-7, but there was no difference between the opaque mutants.

The advantage of N.P.U. as a measure of biological activity of a protein is that diets may be fed over a short test period, and the N.P.U. value is not influenced by differences in feed intake. Diets are fed at the same level of protein, and differences in N.P.U. reflect differences in protein quality and not differences in protein quantity.

These data, therefore, suggest that opaque-7 is superior to normal maize and equal to opaque-2 maize in nutritive value.

This preliminary experiment does not establish the biological value of opaque-7 endosperm proteins, but it does suggest a thorough analysis of the feeding value of opaque-7 maize to be worthwhile.

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1. Effect of position within the tassel on crossover frequency in microsporocytes.

Using bridge and fragment frequencies at anaphase I in plants heterozygous for inversion 5083 as an assay of crossover frequency, the following results were obtained: In five out of five tests (four within first flowers and one within second flowers) significant differences ($P \simeq .025$) were found between sporocytes from main spike and lateral branches. In four of the five tests (three for first flowers, one for second) crossover frequency estimates were greater for cells from the main spike than for laterals, but in the fifth case (from first flowers) crossover frequency estimates were

significantly greater for lateral branches. Data from sessile and pedicellate spikelets were compared within lateral branches and either within first flowers (12 tests) or within second flowers (7 tests). Significant heterogeneity was found, but no signed chi-tests (where $\chi = \pm\sqrt{\chi^2}$) were significant, i.e., crossover frequency estimates were sometimes greater in sessile spikelets and sometimes greater in pedicellate spikelets. Data from first and second flowers were compared either among pooled lateral branches (11 tests) or within single lateral branches (3 tests) and within sessile spikelets only (2 tests), or within pedicellate spikelets only (1 test) with these latter three tests made up of pooled branches, or with pedicellate and sessile spikelets pooled. Three of the fourteen individual tests (one within lateral branches and two among pooled branches) showed significant differences in crossover frequency estimates between first and second flowers with first flower greater than second flower, but within a branch, values for second flowers sometimes exceeded those for first flowers. Data from proximal and distal spikelets on lateral branches were compared either within single lateral branches (7 tests), or between lateral pools composed of some (or all of the same branches), or between pools with no branch in common (24 tests), and simultaneously either within first flower (25 tests) or within second flower (6 tests) and simultaneously either within pedicellate spikelets (13 tests) or within sessile spikelets (18 tests). Very significant heterogeneity was found. Eight of the tests, all involving sessile spikelets showed significant differences between crossover frequency estimates from proximal and distal spikelets. In (very significant) signed chi-tests, crossover frequency estimates from sessile spikelets were found to be greater in distal spikelets where first flowers were used than in proximal spikelets where first flowers were used (three tests each within a single branch). Conversely, crossover frequency estimates from sessile spikelets were found to be greater in proximal spikelets where second flowers were used than in distal spikelets where second flowers were used (two tests each within a single branch).

M. Maguire

2. Effect of heat treatment on crossover frequency (bridge and fragment frequencies) in inversion 5083 at early synizesis (probably zygotene) and mid synizesis (probably pachytene).

Plants heterozygous for the inversion were removed from a growth chamber at 25°C for three hour treatment of meiotic tassel tissue at 36°C, and the crossover frequencies of their microsporocytes were compared to controls in three types of chromosome region: (1) where a single initiation of pairing can provide a site for a crossover without subsequent spreading of synapsis (crossover within the inversion), (2) where two independent events of pairing initiation are required for two coincident crossovers (double crossovers within and proximal to the inversion) and (3) where spreading of synapsis over a short distance from a single event of pairing initiation may provide the requisite pairing for two coincident crossovers (double crossovers within the inversion). Significant difference was found between treated and control for type (1) when treatment was applied at early synizesis but not at mid synizesis; difference of borderline significance was found between treated and control for type (2) when treatment was applied at early synizesis but not at mid synizesis; difference of borderline significance was found between treated and control for type (3) when treatment was applied at mid synizesis but not at early synizesis. Results are consistent with the interpretation that crossover sites are established for the most part at events of synaptic initiation and that nearby second crossovers occasionally follow the spreading of synapsis to adjoining regions.

M. Maguire

3. Crossover interference for regions within inversion 5083 and proximal to it.

The normal crossover map distance proximal to inversion 5083 is probably about 70 units; the map extent within the inversion is about 19 units if its cytological extent per map unit is average for the long arm of chromosome 1. Production of a fragment only at anaphase I requires coincident 3-strand double exchanges within the inversion and proximal to it. Using the table of Haldane (1919) for conversion of map distance to recombination percentage and assuming a proximal map extent of 70 units and no

chromatid interference, the expected frequency of recombination in the proximal region is 0.46. Since a total frequency of exchange in the inverted region of 0.36 was found, with no interference between the two regions, the expected frequency of cells at anaphase I with fragment only is 0.17. The frequency found, 0.07, suggests a substantial interference to simultaneous double exchanges within and proximal to the inversion. Double crossovers within the inversion, however, apparently occurred with a frequency near that expected in normal sequence material of the same estimated length (0.01). Crossing over within the inversion and/or the reversal of pairing which accompanies it seems somehow to be frequently inhibitory to proximal crossing over although the proximal region seems usually to be synapsed regularly at pachytene for most of its length. It is suggested that pairing initiation in the inverted region (which is distal) is likely often to occur earlier than in the proximal region and that in these cases the synapsis of the proximal region may result from extension of synapsis from the other arm. As proposed in the preceding report, crossover sites may usually be established at synaptic initiation, with nearby second crossovers sometimes following the spreading of synapsis to adjoining regions. Pairing of the proximal region, following homologous pairing in the inverted region, may take place in a manner less favorable for crossing over.

M. Maguire

4. Experimentally produced meiotic abnormalities.

Induced meiotic abnormalities so far observed in this laboratory have included synaptic failure, failure of chiasmate association to persist until metaphase, irregular chromosome contraction at diplotene, presence of multiple nucleolar-like bodies at diakinesis, end-to-end association of diakinesis bivalents, cytokinetic failure at first and second meiotic division, tripolar spindles, distorted spindles, decondensation of chromosomes at metaphase and anaphase, and reorientation and abnormal separation of metaphase I bivalents such that sister centromeres may separate equationally at anaphase I. These abnormalities were apparently first induced while pieces of 3 x 5 index cards were positioned next to the meiotic tassel during heat treatment (for mechanical support).

The cards were found to contain sizing composed of corn starch which had been treated with ethylene oxide. Similar heat treatment in the absence of foreign material or with filter paper only produces few or no abnormal cells, but such treatment in the presence of filter paper soaked with solutions of a number of substances has been found to produce some or most of the above abnormalities. These substances include NaOH, NH_4OH, ethylene glycol, glyoxal and ethanol. (Abnormalities have not been found after treatment in the presence of grocery store corn starch.) Observations suggest that treatments with some of these substances may provide useful techniques for studies of chromosome structure, synapsis and crossing over.

M. Maguire

5. Retardation of bivalent separation.

Some mid and late anaphase I cells of a maize-Tripsacum chromosome 2 substitution stock have been found to be "exceptional," in that they show some evidence of retarded bivalent separation. Abnormalities observed include: (1) retention of attenuated, but unbroken, terminal connections between half-bivalents, (2) half-bivalents under tension, joined by incompletely terminalized chiasmata, (3) pairs of half-bivalents, each with at least one chromatid under tension, although complete connections are not visible, and (4) "lagging" bivalents under little or no tension. Examples of (1) and (3) are illustrated in Figure 1.

To test whether the observed anaphase behavior is related to genetic and structural heterozygosity, frequencies of such "exceptional" cells were recorded for plants of different chromosomal constitutions. Table 1 compares these frequencies for (A) disomic plants with normal maize complements, (B) disomic plants of the same stock, carrying a normal maize chromosome 2 and a maize-Tripsacum interchange chromosome, (B') trisomic plants carrying a normal maize chromosome 2 and reciprocal maize-Tripsacum interchange chromosomes 2, and (C) plants of the KYS stock. No two samples could be shown to be significantly different (5% confidence level) by a t test.

One disomic plant, which had a normal maize complement (A), exhibited chromosome behavior which approached the "sticky" phenotype in about 15% of the anaphase I cells analyzed. There was considerable tension

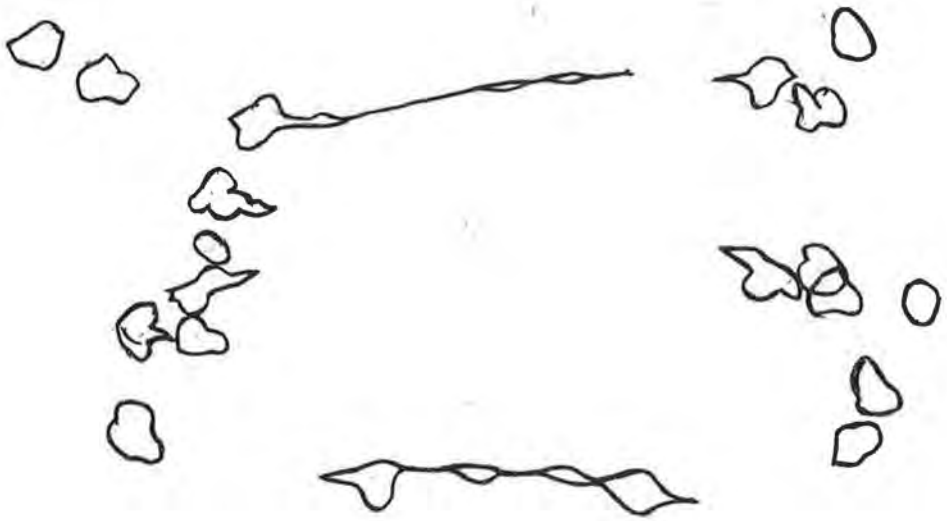

Figure 1. "Exceptional" mid-anaphase I. Retraced from a camera lucida drawing. Magnification 1905 X.

Table 1

Frequencies of "exceptional" cells at anaphase I

Stock*	No. plants	No. cells	Overall mean	Variance
	Mid and late anaphase I combined			
A	7	200	.23	.02
B	4	76	.32	.06
B'	4	182	.097	.0008
	Mid-anaphase I only			
A	4	71	.40	.03
B	7	128	.53	.05
C	3	57	.39	.01

*Described in text.

on most chromatids in each affected cell, although few complete connections between chromatids could be resolved.

The significance of these phenomena is not clear. Darlington (Recent Advances in Cytology, 1937) suggested that terminalization of chiasmata may be arrested when a region where there is a change of homology is encountered. The data presented here provide neither support for, nor disproof of, this hypothesis.

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1. The effects of light on pigmentation in developing maize seeds.

A. Increased aleurone pigmentation induced by light in developing seeds.

Plants of six strains of W22 differing only with respect to factors conditioning pigment formation in the aleurone were pollinated and cobs sampled at various time intervals after pollination (normally every two

days). Ten kernels were removed from a specified location on the cob. Five of these kernels were placed on a moistened filter paper in a closed petri dish, and exposed to Sylvania GRO-LUX fluorescent lights at a distance of twelve inches. The other five kernels were kept in the dark, but otherwise under identical conditions. At the end of four days, optical density measurements were taken of pigments extracted in 1% HCl in MeOH, and the kernels were dried and weighed.

Of the six strains, five showed significantly more pigmentation ($P > .95$) in the light than in the dark. Included in these five were the following: $\underline{R}^{sc}_{57}$; $\underline{R}^{sc}_{61}$; $\underline{R}^{s}$ (Arizona P.I. 218164); $\underline{R}^{s}$ (Canada P.I. 214199); and standard $\underline{R}^{r}$ (see Table 1). A strain with an aleurone pigmenting factor at the $\underline{B}$ locus (Peru 1497, described by Styles 1965, MGNL 39:172 as $\underline{R}^{s}$-2), was the only family that did not show a significantly greater amount of pigment in the light at the 95% level, although the trend was in the same direction.

Table 1

The effects of light and dark on aleurone pigmenting ability of six strains of W22. All values are in terms of optical density units per five kernels.

	$\underline{R}^{sc}_{57}$	$\underline{R}^{sc}_{61}$	$\underline{R}^{g}$ (Arizona)	$\underline{R}^{g}$ (Canada)	Std. $\underline{R}^{r}$	Peru $\underline{B}$ ($\underline{R}^{g}$-2)
Number of samples taken at even time intervals from initial pigment formation to harvest. (n)	31	31	18	18	23	26
Mean deviation between light and dark values ($\bar{D}$)	0.866	0.649	0.197	0.681	0.174	0.597
Standard error of the mean deviation ($S_{\bar{D}}$)	0.244	0.308	0.060	0.243	0.062	0.327
Paired comparisons t-test (light vs. dark) (t_s)	3.55**	2.11*	3.30**	2.81*	2.80*	1.83ns

*P > .95 **P > .99 ns P < .95

B. Rates of aleurone pigmentation in light and dark.

Graphs were made from the data obtained from the above experiment, plotting days after pollination (ordinate) against optical density (abscissa) over the period of time from the initial pigmentation to the time of harvest. Linear regression lines were computed and the goodness of fit determined by a t-test. The calculated regression lines were shown to be statistically valid ($P > .95$) for three of the R alleles only, and thus a linear regression may not represent the correct relationship of pigment formation with time. Such calculated regressions do allow some comparisons to be made between the light and dark treatments, however. The data for these three alleles are shown in Table 2.

Table 2

Rates of aleurone pigmentation in three strains of W22 as explained by linear regression

		R^{sc}_{61}	R^{sc}_{57}	Std. R^{r}
Sample size (n)		22	29	20
Y-intercept (a)	light	-0.2534	-0.4364	-0.1832
	dark	-0.1050	-0.2469	-0.0358
Slope (b)	light	0.0263	0.0269	0.108
	dark	0.0127	0.0144	0.0058
Goodness of fit (t)	light	2.81**	4.13**	5.35**
	dark	4.91**	4.73**	2.53*

*P > .95 **P > .99

For all three alleles the slope from the light treated seeds is greater than that from the dark treated seeds. More interestingly, the slope from the light treatment is consistently about twice that for the dark treatment. Thus, at any one point in time there is a doubling of pigmentation as a result of exposure to light.

C. Pericarp pigmentation in developing R^{nj} seeds exposed to light.

Two R^{nj} strains (R^{nj} cudu and R^{nj}_{6}, a compound allele derived by Brink from a stippled crown allele $R^{nj:st}$) were included in the experiment described above. Aleurone pigmentation in R^{nj} stocks is normally confined to the crown of the seed and usually pigment does not start forming until late in the development of the kernel. Although R^{nj} pericarp is normally colorless, it was found that when the seeds were removed from the cob and exposed to light for four days, anthocyanin forms in the pericarp. The potential for this light induced pigment formation is present at about twenty-five days after pollination and lasts until the time when aleurone pigment starts to form (approximately fifty days after pollination under our conditions). Pigment does not form in the pericarp of R^{nj} seeds kept in the dark.

Sastry (MGNL 39:178) has shown that although Pl is normally required for pigment formation in R^{ch} pericarp, pl R^{ch} plants can develop pericarp pigment if the husks are removed from the ears, thus exposing the pericarp to the light. Although it has been reported that R^{nj} does not produce pericarp pigment with Pl, it does seem to have the potential for pericarp pigmentation under certain conditions, as demonstrated in this experiment. The fact that R^{nj}_{6} showed the same pattern of pericarp pigmentation as R^{nj} cudu is worth noting, because R^{nj}_{6} is similar to R^{st} and R^{sc} in plant color distribution (i.e., green plant and anthers) and it is similar to R^{nj} only in aleurone pigment distribution. Other R^{nj} alleles, including R^{nj} cudu, have red seedlings, red anthers and deep red silks. If, as Sastry has suggested (MGNL 43:204), some R alleles have a component (Ch) for pericarp color, then it would seem that R^{nj}_{6} does contain such a component, perhaps normally inactive, and that this component was retained together with the aleurone pigmenting component when the $R^{nj:st}$ compound allele was derived.

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E. Derek Styles

2. Peonidin in W22 Pr pericarp and cob glumes.

The ratio of cyanidin to other anthocyanidins in pigmented tissues of W22 Pr strains is normally weighted very heavily towards cyanidin.

Pelargonidin and peonidin occur usually only as minor components. An exception to this rule seems to exist in the pericarp and cob glumes.

The following table shows the ratio of cyanidin (Cy) : pelargonidin (Pg) : Peonidin (Pn) in various W22 Pr stocks that give anthocyanin in pericarp and cob glumes. Also shown are the ratios of the same three pigments in the aleurone from the same ears and the relative concentration of the pigment in the pericarp in terms of relative O.D. units per gram of pericarp.

Strain (all Pr)	Tissue			Relative O.D./gm. pericarp
	Aleurone Cy:Pg:Pn	Cob glumes Cy:Pg:Pn	Pericarp Cy:Pg:Pn	
New Mexico $R^{ch}R^{ch}$ Pl Pl	1:.07:.06	1:.86:.23	1:.39:.29	719.8
Costa Rica $R^{ch}r^{g}$ Pl pl	1:.01:.03	not made	1:.07:.24	415.5
Stadler $R^{ch}R^{ch}$ Pl Pl	1:.01:.12	1:.04:.11	1:.03:.20	57.9
Ecuador R^{r} r^{g} Pl Pl	1:.01:.03	1:.08:.23	1:.04:.41	54.7
Ecuador R^{r} r^{g} Pl pl	1:.01:.03	1:.14:.21	1:.07:.34	30.9
$r^{ch}r^{g}$ Pl Pl	colorless	1:.09:.15	1:.02:.22	339.4
$r^{ch}r^{g}$ Pl pl	colorless	1:.06:.10	1:.05:.23	98.7
r^{g} r^{g} Pl Pl + leaf and pericarp factor from R^{r} Ecuador	colorless	1:.04:.11	1:.10:.22	11.4

Although there are some inconsistencies, there seems to be a definite tendency for quite high relative concentrations of peonidin in the pericarp and cob glumes. Also, the relative concentration of peonidin seems to be independent of the absolute concentration of all anthocyanins in the tissue. It could be argued that this is a Pl effect, because Pl is normally required for anthocyanin pigmentation in these tissues. However, we are inclined to think that it is more likely to be a tissue specific effect, because preliminary results indicate that pericarp pigment from pl R^{ch} ears exposed to light is high in peonidin, and also that other pigmented tissues in Pl plants are low in peonidin. It may be significant

that the P gene is expressed in these same two tissues, but as yet we see no obvious relationship between P controlled pigment and the production of peonidin, which is a methylated form of cyanidin.

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1. Genetics of photoperiodism in corn.

Genetic studies of photoperiodism of corn under natural conditions (field or greenhouse) have been carried out in the past two decades (Singleton 1946, Galinat and Naylor 1951, Shaver 1967, Brown 1967). The short-day corn, Indeterminate (id/id), and the long-day corn, Gaspe Flint, were well established genetic stocks. Observation of these genetic stocks under artificial light in controlled environment was made in this study in order to determine whether photoperiod alone initiates or inhibits the sexual differentiation and flowering process.

Two identical growth chambers (each illuminated with 12 150 W incandescent light bulbs and 34 96" fluorescent lamps) were used to grow the corn plants. Total intensity of the combined light sources was around 10,000 foot-candles at 4 feet distance. Temperature was maintained at 72°-75° F by cooling and heating systems of the growth chambers. Fresh air was circulated through the rooms to insure the normal content of CO_2. Indeterminate and Gaspe Flint seeds were sown in gravel beds and sub-irrigated with nutrient solution twice daily. One room had a 10-hour photoperiod (short-day) from 7 a.m. to 5 p.m.; the other had a 15-hour photoperiod (long-day) from 6 a.m. to 9 p.m.

One hundred seeds from the progeny of a selfed Id/id stock were sown in the short-day room on 12/26, 1967. The silks and tassels emerged on both Id/- and id/id plants on 2/8, 1968. A total of 42 days was required to reach the flowering stage. There were 80 Id/- plants and 20 id/id plants: roughly a 3:1 ratio. The id/id plants were easily

identifiable by the genetic marker, striped leaves and short stalk (8 inches at tasseling stage) under the short-day condition. Two seeds were obtained from one of the id/id tassel-seed plants by selfing. Another 100 seeds from self pollination of the monohybrid (Id/id) were sown in the long-day room on 4/15, 1968. Eighty plants which showed Id/- phenotypes produced tassels and silks on 6/14, 1968. A total of 60 days was required to reach the flowering stage. There were 12 id/id seedlings with typical characteristics on 7/1, 1968, but only 3 survived. At the end of 30 days, 3 id/id plants grew too tall (more than 7 feet) to be housed in the growth chamber and were moved into the greenhouse on 7/15, the normal long-day season. These 3 plants were grown in the greenhouse till 9/15, 1968 and reached 10 feet in height. No tassel or silk emerged at that time.

Kernels of the long-day stock, Gaspe Flint, were sown in the long-day room on 7/11, 1968. Tassels and silks emerged on 7/29, 1968. Only 18 days were needed to reach the flowering stage.

From these findings under the controlled environmental conditions, we may conclude that photoperiod alone regulated the gene action which in turn controls the physiology and differentiation of the plant.

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1. Mitotic inhibition and chromosome damage produced by 5-Bromodeoxyuridine (BUdR) in Zea mays L. root tip cells.

Bromodeoxyuridine (BUdR) is an analogue of thymidine and is incorporated, with concomitant thymine replacement, into the DNA molecule. According to Kit et al. (1958) and Szybalski (1959) 5-BUdR is incorporated into cellular DNA but does not interfere with other metabolic processes. Although Djordjevic and Szybalski (1960) were able to show that a partial substitution of BUdR for thymine in the DNA leads to an increase in U.V. radiosensitivity, they were unsuccessful in extending their observations to changes detectable at the chromosomal level. The present investigation is

designed to investigate UV sensitization with respect to mitotic inhibition and chromosome damage.

Preliminary experiments were performed to ascertain the suitable concentration for BUdR treatment. It was found that root growth was not visibly altered with 100 ug/ml BUdR. All treatments were carried out on attached 3 day old singlecross (Seneca 60) primary root tips at 25°C as described in earlier reports from this laboratory. In one experiment, all roots were treated with BUdR (100 ug/ml) for 10 hours. Following the treatment, the roots were washed thoroughly and were divided into two batches. One batch was returned to the germination chamber for further growth and fixed at 5 hour intervals up to 25 hours post-treatment. A second batch was exposed to UV for 15 minutes (51 uW/Cm^2 x 100) and then the roots were returned to the germination chamber and again fixed at 5 hour intervals up to 25 hours. After hydrolysis, the root tips were processed by the Feulgen smear technique (Verma, MGCNL 45: 214-217). A minimum of four slides, one root tip per slide, from each collection period was scored for the nuclear stage. The mitotic index was determined for each collection period. The values are recorded in Table 1.

The decrease in mitotic index to 1-2% was apparent after 10 hours of treatment with BUdR. Thus, it may be presumed that mitotic indices may be affected even during the incubation period. The mitotic indices returned to the control level 15 hours after treatment. It is apparent from Table 1 that the exposure to UV did not alter the mitotic indices. These results suggest that some interphase processes have been delayed or inhibited. BUdR was, however, introduced into cultured Chinese hamster cells in a concentration of 200 ug/ml for 1 hour by Zakharov and Egolina (1972). The results of their experiment suggested that there was no difference in total duration of the nuclear cycle and duration of its phases between cells under BUdR treatment and those in control; it should be noted that the incubation period was very short.

In order to record the chromosome aberrations, in a second experiment intact roots were treated, after BUdR incorporation and UV exposure, with 0.002M 8-Hydroxyquinoline for 2.5 hours prior to fixation. A sampling of these slides suggests that there has been produced in the treated material:

Table 1

Mean mitotic indices (M.I., with standard deviations, S.D.) after treatment with BUdR, with or without U.V. [(51 uW/Cm^2 x 100) (15 Minutes)] at 25°C.

Hours after treatment	BUdR without U.V.			M.I. with S.D.	BUdR with 15 Min. U.V.			M.I. with S.D.
	I*	M**	Total		I*	M**	Total	
0	3931	46	3977	1.2 ± 0.2	5613	119	5732	2.1 ± 0.20
5	3443	107	3550	3.0 ± 0.3	3731	138	3869	3.5 ± 0.30
10	2113	159	2272	7.0 ± 0.35	2771	150	2921	5.1 ± 0.41
15	2667	273	2970	9.2 ± 0.53	3084	276	3350	8.4 ± 0.47
20	1810	192	2002	9.5 ± 0.65	1651	169	1810	9.4 ± 0.68
C[a]	8439	844	9283	9.1 ± 0.29				

*Interphase

**Mitosis

[a]Control

1. Fragments at metaphase;
2. Bridges at anaphase;
3. Dicentric chromosomes;
4. Chromatid breaks;
5. Aberrant spindle fiber development.

To enhance the incorporation of BUdR into DNA, in a further experiment FUdR has been combined with BUdR (5 ug/ml + 100 ug/ml, respectively). We are in the process of recording mitotic indices and chromosome aberrations from this experiment.

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Ram S. Verma

2. Nuclear cycle: a parameter for selection?

During the last five years, we have reported extensive data on the nuclear cycle in 'Seneca 60', chromosome 9 tester, and W23 stocks of Zea mays L. The present report describes the duration of the nuclear cycle and its component phases in KYS. This stock was chosen as an exemplar, late maturity stock to complement 'Seneca 60', and W23 as early and medium maturity material, respectively.

The experiment was conducted at 25°C. Autoradiographs were prepared according to the schedule reported earlier (MGCNL 43: 186-190; 44: 192-195). A minimum of four slides, one root-tip per slide, from each collection period, were coded and scored blindly.

The classification data are presented in Table 1. Employing the proportion method, the nuclear cycle duration and its components were estimated and are presented in Table 2. Table 3 contains the S.D. of the nuclear cycle components. The duration of the nuclear cycle and its component phases of KYS were compared with 'Seneca 60', W23, and the 9 tester stock; it was found that the nuclear cycle in the several stocks was of similar duration.

Table 1

Frequency of labelled and unlabelled nuclei from primary root-tips following pulse labelling (^{3}H-TdR, 30 min) at 25°C in KYS

Stock	Interphase		Prophase		Metaphase		Anaphase		Telophase	
	Lab.	Unlab.	Lab.	Unlab.	Lab.	Unlab.	Lab.	Unlab.	Lab.	Unlab.
KYS	9093	17530	654	654	199	238	64	73	242	282

Table 2

Duration of the nuclear cycle in the root-tips of Zea mays L. (KYS) at 25°C

Phase		Duration	
		Hrs.	%
Interphase			
	G_1	1.91	18.2
	S	4.7	44.8
	G_2	2.67	25.4
	Sub-total	9.28	88.4
Mitosis			
	Prophase	0.66	6.3
	Metaphase	0.22	2.1
	Anaphase	0.07	0.6
	Telophase	0.27	2.6
	Sub-total	1.22	11.6
Total		10.5	

Table 3

Standard deviations of the nuclear cycle component estimates in the primary root-tips of Zea mays L. (KYS) at 25°C

Phase	Hrs.
G_1 + Mitosis	0.53
S	0.13
G_2 + ½ Prophase	0.05
Total nuclear cycle	0.55

Initially, consideration was given to the view that the nuclear cycle was under specific genetic (gene) control. It was proposed that maize was an excellent material in which to test the hypothesis of specific genetic control, inasmuch as a wide variety of markedly different agronomic and genetic stocks was available. If the duration of the nuclear cycle was related to the growth characteristics of a stock, judicious choice of a few stocks should permit the identification of different nuclear cycles under identical controlled conditions.

Starting with 'Seneca 60', KYS, 9 tester and W23 stocks, all possible F_1, F_2, BC_1 and BC_2 stocks were developed at our field station. Upon discovering that no differences in the nuclear cycle of the different stocks at 25°C could be described, the analysis of the F_1, F_2, BC_1 and BC_2 hybrids was discontinued. It remains to be shown whether or not stock differences can be described at temperatures other than 25°C, or under the influence of other environmental conditions, as a prerequisite for heritability studies; at the moment, we must conclude that these differences do not exist and that further information would not be contributed by analyzing the F_1 and subsequent generations.

Ram S. Verma

3. Reassociation of interchange and interstitial segments.

It has already been demonstrated (Jancey and Walden, 1972) that significant departures from an equidistribution of 'breaks' occur in the

chromosome arms of maize. In this report, it will be shown that a striking reassociation pattern also exists, beyond that attributable to the previous inequality. Data were drawn from the reports of Longley (1958, 1961) and Burnham (1969).

The hypothesis was erected that the reassociation between chromosome segments, pooled for long and short arms, did not differ from an equidistribution when corrected for known 'breakage' frequency per segment. Data were expressed in micron units from measurements made at pachynema; arms were divided into 5u classes. If the pooled data are divided into n frequency classes, and if f = the observed break frequency per class, then the expected frequency of reassociation (F), for the ith row and jth column intersection in the n x n symmetric matrix of reassociation frequencies, will be:

$$F_{ij} = \sum_{i=1}^{n} f_{ij} \left(\sum_{j=1}^{n} f_{ij} / \sum_{i=1}^{n} \sum_{j=1}^{n} f_{ij} \right)$$

From this, a test of significance of departures from expectation can readily be obtained using X^2 values.

The frequencies of reassociation of chromosome 'breaks' were used to compute deviations from expected values as described earlier. Chi square contributions resulting from these deviations are presented in three forms, in Tables 1, 2 and 3. In Table 1, the 10 columns of X^2 contributions correspond to class intervals along the chromosome arms. Thus 'breaks' occurring between 0.0u and 4.9u show a X^2 contribution of 107.6 resulting from an excess of observed reassociations with interchange segments whose residual, complementary interstitial segments were also of 0.0u to 4.9u. In the same column it will be seen that the identical interstitial class shows a X^2 contribution of 7.7, obtained from the deficit in observed reassociations in the class of interstitial segments which were 5.0 - 9.9u in length. Subsequent columns of Table 1 similarly show relatively large X^2 contributions resulting from excess reassociation where the interstitial segment (class I) is of the same length as the interstitial segment of the second chromosome involved in the translocation (class II). This relationship between segments of like length can be seen by examining the values around the top left to lower right diagonal of Table 1. Chi square con-

Table 1

Pooled data for total chromosome complement. X^2 contributions for reassociation frequencies. Classes based on mid pachytene lengths expressed in microns.

RELATED INTERSTITIAL SEGMENT LENGTH	INTERSTITIAL SEGMENT LENGTH 0.0-4.99	5.0-9.99	10.0-14.99	15.0-19.99	20.0-24.99	25.0-29.99	30.0-34.99	35.0-39.99	40.0-44.99	45.0-49.99	Total X^2 contribution interstitial segment
0.00-4.99	107.6°	7.7	3.0	7.6	5.0	2.0	0.0	0.4°	2.9	0.0	136.2
5.00-9.99	7.7	37.1°	0.3°	2.4	3.4	4.1	3.2	3.0	0.7	0.0	61.9
10.00-14.99	3.0	0.3°	0.6°	2.6°	0.2°	1.3	0.3	1.4	0.2°	0.0	9.9
15.00-19.99	7.6	2.4	2.6°	0.5	7.8°	2.7°	1.2°	0.1°	0.4	0.0	25.3
20.00-24.99	5.0	3.4	0.2°	7.8°	0.9°	3.6°	0.0	0.1	0.4	0.0	21.4
25.00-29.99	2.0	4.1	1.3	2.7°	3.6°	4.9°	0.2°	0.0	5.0°	0.0	23.8
30.00-34.99	0.0	3.2	0.3	1.2°	0.0	0.2°	0.0	10.3°	15.6°	0.0	30.8
35.00-39.99	0.4°	3.0	1.4	0.1°	0.1	0.0	10.3°	15.3°	0.2	0.0	30.8
40.00-44.99	2.9	0.7	0.2	0.4	0.4	5.0°	15.6°	0.2	0.1	0.0	25.5

°X^2 contributions resulting from reassociations in excess of expected frequencies.

tributions away from the diagonal tend to be small; the larger values are, without exception, derived from negative departures from expected values in the direction of fewer events than expected rather than the reverse.

Table 2 presents X^2 contributions derived from expected reassociation frequencies based on the same data, but expressed in terms of interchange segment classes reassociating with interstitial segment classes. In the first column it will be seen that the interchange class containing 'breaks' 0.0 - 4.9u from the telomere shows X^2 values resulting from an excess of reassociation over expected values for the larger interstitial segment classes, with a high X^2 contribution (13.0) from the interstitial segment class 25.0 - 29.9u. There is a corresponding lack of observed reassociations with the shorter interstitial segment classes, e.g., the X^2 contribution (9.4) for the interstitial class 5.0 - 9.9u. The distinction is less marked than that seen in the first column of Table 1. This may be attributed to the differing total lengths of chromosome arms, which, when breaking within 4.9u from the centromere, will give rise to a variety of interchange segment lengths. In other words, for both chromosomes involved, only the distance of the break from the centromere has relevance. This latter point is clearly made in Table 3, which is similar to Table 1 except that both segments are expressed in terms of distance of 'breaks' from the telomere, i.e., interchange segments. The pattern of excess and deficit of reassociation is still less clear in this case, except when close to the telomeres (top left of table) where the pattern of preferential reassociation of like lengths is again seen. This is as would be expected if a phenomenon involving interstitial segments is being twice diffused in its demonstration by the variability of arm length among the chromosome complement. The contribution of 18.3 from a positive deviation for class 25.0 - 29.9u corresponds to the top left cell of Table 1 as revealed by a reference system based on the telomere and observed by those based on the centromere.

The relationship between the data as recovered and original reassociation events has been discussed elsewhere (Jancey and Walden, 1972). The values derived from reassociation frequencies demonstrate that the most important feature in predicting the preferential areas of reassociation is the distance of the 'breaks' from the centromere (see Table 1). As would

Table 2

Pooled data for total chromosome complement. X^2 contributions for reassociation frequencies. Classes based on mid pachytene lengths expressed in microns.

INTERSTITIAL SEGMENT LENGTH	INTERCHANGE SEGMENT LENGTH 0.0-4.99	5.0-9.99	10.0-14.99	15.0-19.99	20.0-24.99	25.0-29.99	30.0-34.99	35.0-39.99	40.0-44.99	45.0-49.99	Total X^2 contribution interstitial segment
0.00-4.99	0.9	1.1	0.7	3.2	1.5	0.6°	13.2°	4.6°	5.0°	3.0°	33.8
5.00-9.99	9.4	6.5	2.5	0.2°	3.3°	13.9°	1.2°	0.1°	0.1°	0.0	37.2
10.00-14.99	0.3	0.0	3.2°	0.1°	0.0	0.1°	0.2	1.9	1.0	0.4	7.2
15.00-19.99	0.2°	3.4°	0.2	2.6°	0.7	1.8	1.1	0.0	0.4	0.4	10.8
20.00-24.99	1.4°	5.4°	2.4°	0.2°	3.6	4.4	4.6	0.1°	0.2°	0.4°	22.7
25.00-29.99	13.0°	0.5°	0.2	0.1°	2.4°	8.0	4.1	1.2	0.9	0.2	30.6
30.00-34.99	3.9°	0.8°	1.0°	0.7°	0.8°	4.9	1.4	0.3	1.3	0.2	15.3
35.00-39.99	3.2°	0.4°	0.4	0.0	3.1	0.0	0.2°	0.6	0.2	0.0	8.1
40.00-44.99	0.7°	0.4°	2.5°	0.0	0.1	0.7	1.7	0.6	0.2	0.0	6.9

°X^2 contributions resulting from reassociations in excess of expected frequencies.

Table 3

Pooled data for total chromosome complement. X^2 contributions for reassociation frequencies. Classes based on mid pachytene lengths expressed in microns.

RELATED INTERCHANGE SEGMENT LENGTH	INTERCHANGE SEGMENT LENGTH 0.0-4.99	5.0-9.99	10.0-14.99	15.0-19.99	20.0-24.99	25.0-29.99	30.0-34.99	35.0-39.99	40.0-44.99	45.0-49.99	Total X^2 contribution interstitial segment
0.00-4.99	12.1°	1.6°	1.3°	0.0	2.9	6.3	0.2	3.4	0.5	0.3	28.6
5.00-9.99	1.6°	0.8°	0.9°	0.4°	0.0	2.6	3.7	0.0	1.2	0.5	11.7
10.00-14.99	1.3°	0.9°	1.4	0.2	0.0	1.1	0.4°	0.0	0.1	0.4	5.8
15.00-19.99	0.0	0.4°	0.2	3.0°	0.1°	4.3	0.0	0.2	0.3	0.4	8.9
20.00-24.99	2.9	0.0	0.0	0.1°	0.2°	0.9°	0.3	0.2°	0.1	0.4	5.1
25.00-29.99	6.3	2.6	1.1	4.3	0.9°	18.3°	3.3°	0.4	6.3°	2.3	45.8
30.00-34.99	0.2	3.7	0.4°	0.0	0.3	3.3°	0.3	6.0°	0.5	1.6	16.3
35.00-39.99	3.4	0.0	0.0	0.2	0.2°	0.4	6.0°	2.1°	0.0	0.4	12.7
40.00-44.99	0.5	1.2	0.1	0.3	0.1	7.6°	0.0	0.0	0.5	0.0	10.3
45.00-49.99	0.3	0.5	0.4	0.4	0.4	2.3	1.6	0.4	0.0	0.0	6.3

°X^2 contributions resulting from reassociations in excess of expected frequencies.

be expected, reassociation frequencies, expressed wholly or partially in terms of interchange segment length, still show some significance because of the apparent correlation which exists between the lengths of interchange and interstitial segment. Were all the chromosome arms of the same physical length, the correlation would of course be perfect. Its imperfection permits the demonstration of the primary role played by the interstitial segment lengths, and also the importance of telomere related length for telomeric events.

The deviations of observed frequencies of reassociation from expected values are of particular interest. Along the whole length of the chromosome arm an excess of reassociations occurs between other chromosomes in which the 'break' has occurred at a similar distance from the centromere. Both of these results would be compatible with the hypothesis of chromosome attachment by their centromeres to some limited area of the nuclear membrane, and telomere attachment to the nuclear membrane with possibly a greater mutual spatial separation between them than in the case of the centromeres. Evidence from electron micrographs for such attachment has been presented recently from several laboratories for several species. The excess of reassociations involving breaks of similar distance from the centromere would also suggest that the chromosome arms are extended in the nucleus in a parallel manner rather than the classical concept of a complex, intertwined mass.

We have been tempted to use these reassociation data to predict an organizational 'geography' of the interphase nucleus. Computer simulation has been moderately successful and we hope to report on this extension of our analysis shortly.

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4. Intervarietal differentiation of maize pollen.

Considerable differences in values for quantitative characters of maize pollen and pollen of maize-related taxa have already been demonstrated (Tsukada and Rowley, 1964, Banerjee and Barghoorn, 1970). Attempts to discriminate between the pollen of maize and that of Euchlaena mexicana (teosinte) and Tripsacum spp. are complicated by the wide range of values for any given character. Pollen diameter, pore-axis ratio, spinule density and spinule distribution have proven to be useful characters when taken together. Little work has been done, however, on variation at the varietal level.

Rumbaugh and Whalen (1972) reported that significant size differences exist among pollen grains from some maize genotypes, particularly in the case of tetraploid varieties.

This report outlines some preliminary aspects of a study being carried out to determine the feasibility of characterizing the pollen on the basis of multivariate analysis of a number of characters.

Pollen samples were collected from plants of 11 stocks (see Table 1) grown in the field or in the greenhouse. Samples were immediately transferred to a deep freeze where they were stored at -10°C.

Micrographs of pores and of areas of the spore wall were taken at a magnification of 5000, 10,000 and 20,000 diameters in a Cambridge Mark 2a scanning electron microscope. For this purpose, samples were fixed to glass and coated with a gold-palladium alloy.

Spinule density was calculated for 5 to 8 pollen grains per stock by counting the number of spinules in a 176 sq. cm. area from 20,000X micrographs. This corresponded to an actual area on the pollen of $44u^2$. The mean basal diameter of spinules was calculated from 20,000X micrographs and based on 40 spinules per grain. Data for 5 to 8 pollen grains were recorded for each stock.

Table 1

Sample number	Stock	Growing conditions
1	'Seneca' 60 su_1/su_1	field August 1972
2	'Seneca' 60 su_1/su_1	field August 1972
3	'Seneca' 60 su_1/su_1	field August 1972
4	'Seneca' 60 su_1/su_1	greenhouse October 1972
5	'Seneca' 60 su_1/su_1	greenhouse October 1972
6	Seneca Chief su_1/su_1	field August 1972
7	Seneca Chief su_1/su_1	field August 1972
8	Seneca Chief su_1/su_1	field August 1972
9	9-tester yg c sh bz wx	field August 1972
10	W23 4N	field August 1972
11	W23 4N	field August 1972
12	9 tester yg C sh wx	field August 1972
13	9 tester yg C sh wx	field August 1972
14	ABPHYL	field August 1972
15	C0106	field August 1972
16	8174-6 st/st	field August 1972
17	8200-1 am/+	field August 1972
18	O_2	greenhouse October 1972
19	v_1	greenhouse October 1972

Pore diameter was defined as the maximum distance separating the spinules on either side of the pore. Measurements were made for 8 to 10 grains per stock at 10,000 or 20,000X.

Pollen samples were also examined with a Phillips 75 transmission electron microscope. For this purpose, they were stained with $KMnO_4$ and embedded in hard Spur plastic prior to thin sectioning.

Measurements of pollen size were carried out for several stocks. The maximum diameter in the plane of the aperture and perpendicular to it was recorded for pollen which had been allowed to imbibe in 0.5M sucrose for 1 hour.

To determine differences in properties of imbibition and strength of intine at the pore, data were collected on a number of pollen samples which showed extent of intine rupture after 1 hour in 4, 3.5, 3, 2.5, 2, 1.5 and 1M sucrose.

The values representing spinule density listed in Table 2 show considerable discrimination between stocks. Within a variety, density values did not show significant differences between plants either under the same or differing culture conditions (see samples 1-4); 63% of the sample pairs which might have been expected to differ were discriminated at $p \leq 0.05$.

Measurements of spinule diameter resulted in 26% of intervarietal sample pairs being discriminated at $p \leq 0.05$ (see Table 3). No significant differences were demonstrated between plants of the same variety resulting from the same or different growing conditions. There was, however, a difference between measurements taken from different electron microscope preparations of the same sample (see Table 3, sample pair 1a - 1b).

The character pore diameter (see Table 4) proved capable of discriminating at $p = 0.05\%$ or better, 43% of those sample pairs representing varietal differences. Sample pairs 1-2, 1-4, 2-3, 2-4, 3-4, 6-7, 6-8, 7-8, representing samples of a single variety from different plants grown under the same or differing environmental conditions, did not differ significantly at $p \leq 0.05$.

Of the characters analyzed in detail, spinule density and pore diameter proved useful in discrimination of pollen of the varieties tested. In only one instance did intra-varietal samples differ significantly. For the varieties studied, it appears that values for these characters are relatively consistent within a given variety and differ between varieties, to an extent which allows for discrimination among a number of varieties with a probability of 0.999.

While it was shown that the values for spinule diameter differed considerably between varieties, differences were significant primarily at the $p = 0.05$ and $p = 0.02$ levels. This might suggest that the varietal differences in spinule diameter values are insufficient to allow for confident discrimination between varieties. However, the level of resolution obtainable with this material on the scanning electron microscope was such that considerable error variance was accumulated in measuring this character.

Table 2

Spinule density on surface of pollen grain: expressed as number of spinules per 44 sq.u.

Sample number	$\bar{X}$	S^2	Sample number										
			1a°	1b°	4	5	6	9	10	12	14	15	16
1a	208.00	23.56											
1b	190.60	13.90	-										
4	220.60	34.39	-	-									
5	189.00	38.11	-	-	-								
6	130.60	20.71	.001	.001	.002	.01							
9	105.80	12.47	.001	.001	.001	.002	.1						
10	151.40	19.34	.01	.01	.01	.1	-	.01					
12	119.20	17.46	.001	.001	.001	.01	-	-	.05				
14	167.60	39.17	.1	-	.1	-	.1	.002	-	.05			
15	141.60	19.16	.002	.002	.01	.05	-	.01	-	.1	-		
16	114.40	16.99	.001	.001	.001	.01	-	-	.02	-	.05	.05	
17	147.80	12.19	.001	.001	.01	.05	-	.001	-	.02	-	-	.05

°Separate scanning electron microscope preparation of sample 1.

Table 3

Spinule diameter: expressed in microns x 10^{-1}

Sample number	$\bar{X}$	S^2	Sample number 1a°	1b°	4	5	6	9	10	12	14	15	16	17
1a	3.26	0.24												
1b	3.54	0.15	.05											
4	3.48	0.18	-	-										
5	3.64	0.53	.1	-	-									
6	3.29	0.14	-	.02	.1	.05								
9	3.53	0.58	-	-	-	-	-							
10	3.81	0.42	.05	-	.1	-	.05	-						
12	3.81	0.29	.02	.1	.05	-	.01	-	-					
14	3.26	0.44	-	-	-	-	-	-	.1	.1				
15	3.69	0.26	.05	-	-	-	.05	-	-	-	-			
16	3.65	0.18	.05	-	-	-	.02	-	-	-	-	-		
17	3.43	0.27	-	-	-	-	-	-	-	-	-	-	-	
19	2.97	0.01	-	.02	.01	.02	.01	-	.05	.01	-	.05	.01	.1

°Separate scanning electron microscope preparations of sample 1.

Table 4

Pore diameter expressed in microns: measured as the maximum distance separating spinules on either side of the pore

Sample number	$\bar{X}$	s^2	Sample number 1	2	3	4	6	7	8	9	11	13	14	15	16	17
1	8.18	0.58														
2	8.26	0.96	-													
3	8.97	0.90	.05	.1												
4	8.50	0.82	-	-	-											
6	8.76	0.95	-	-	-	-										
7	8.76	0.72	-	-	-	-	-									
8	9.30	0.76	.01	.05	-	.1	-	-								
9	8.15	1.18	-	-	-	-	-	-	.05							
11	10.29	1.81	.01	.02	.1	.05	.05	.05	-	.01						
13	7.64	0.82	-	-	.002	.1	.02	.01	.001	-	.001					
14	6.52	0.62	.001	.002	.001	.001	.001	.001	.001	.01	.001	.01				
15	7.93	0.86	-	-	.1	-	.1	.1	.01	-	.01	-	.01			
16	8.73	1.67	-	-	-	-	-	-	-	-	.1	.1	.01	-		
17	7.67	0.88	-	-	.01	.1	.05	.02	.02	-	.002	.002	.02	-	-	
18	7.09	0.98	.05	.1	.002	.05	.01	.01	.001	.1	.01	-	-	-	.1	-

It is felt that the character spinule diameter will prove to be useful, if an improved measuring technique can be found. Preliminary investigations of ultrathin sections of pollen viewed through a transmission electron microscope suggest that this may represent an improved technique.

Early investigations of pollen size and tendency towards intine rupture lead us to believe that these characters will also contribute to intervarietal discrimination.

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1. The taxonomy of Zea mays (Gramineae).

The origin of maize has long been disputed. Of the various theories, the oldest postulates its direct origin by ancient human selection from a wild grass of the genus Euchlaena, the "Teosinte" of Mexico and Guatemala; i.e., maize is simply regarded as a highly domesticated and variable cultivar of Euchlaena. The morphological steps, first clearly outlined by G. N. Collins (J. Agr. Res. 17: 127-135, 1919), were discussed but not accepted by P. C. Mangelsdorf (Bot. Mus. Leafl. Harvard Univ. 12: 33-75, 1945) and amplified by W. C. Galinat (An. Rev. Gen. 5: 447-478, 1971) and myself (H. H. Iltis, The Maize Mystique, 5 pp. mimeo. MS. 1970; cf.

Galinat, loc. cit. pp. 450, 462). The genetic reasons were first outlined by G. Beadle (J. Hered. 30: 245-247, 1939).

The close relationship of Euchlaena to Zea was taxonomically formalized by Reeves and Mangelsdorf (Am. J. Bot. 29: 815-817, 1942), who transferred its two accepted species to Zea. Of these, the perennial tetraploid, Z. perennis, is the most primitive and distinctive in the genus. The diploid annual Z. mexicana sensu lato, however, contains two major elements: the Tripsacum-like, more primitive "Florida" Teosinte (E. luxurians) from Guatemala, which does not cross as readily with maize and other Teosinte forms, and which deserves separate subspecific status (see below); and the remaining races (cf. H. G. Wilkes, Teosinte: the closest relative of Maize, Bussey Inst. Harvard, 158 pp., 1967) which cross with close to 100% fertility with maize, and are best considered, for the time being, under but one subspecies, conspecific with maize as suggested by C. D. Darlington (Chromosome Botany, Allen and Unwin, London, pp. 130-131, 1956):

> "The breeding and chromosome evidence ... agree in requiring that Euchlaena mexicana and Zea mays should be described as one species, the wild grass being today an authentic and scarcely changed representative of what the ancestor of Zea mays must have been."

The new combination, made informally by Iltis (loc. cit.), was validly published in Galinat (loc. cit., p. 450), who, however, neglected to cite the basonym date and place of publication, an oversight rectified below. Further study may in addition demand separate subspecific recognition of other Zea mays races, especially the "Rio Balsas" and "Chalco" populations (cf. Wilkes, loc. cit.).

Synopsis of Zea

1. Zea mays L. ssp. mays
2. Zea mays L. ssp. mexicana (Schrad.) Iltis, in Galinat, An. Rev. Gen. 5: 450, 1971
   (based on Euchlaena mexicana Schrader, Index Sem. Hort. Goett., 1832; Linnaea 8: 25, 1833).
3. Zea mays L. ssp. luxurians (Durieu) Iltis, stat. et comb. nov.
   (based on Euchlaena luxurians Durieu, Bull. Soc. d'Acclimat. 19: 581, 1872).

4. Zea perennis (Hitchcock) Reeves et Mangelsdorf.
(based on Euchlaena perennis Hitchcock).

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1. Androgenesis and the indeterminate gametophyte (ig) mutation: Influence of pollen parent on androgenesis frequency.

Embryo sacs which carry ig promote androgenesis whether or not the nucleus of the male gametophyte involved carries the mutation. Evidence in support of this inference derives from matings between strains of inbred W23 where the total androgenesis frequency in crosses utilizing ig ig ear parents was 2.3% (Science 166: 1422-1424, 1969). A smaller population employing a second inbred as male yielded only one-fourth as many cases, raising thereby the possibility of a major influence of pollen parent on androgenesis frequency.

Further evidence bearing to this point derives from experiments whose principal aim was to identify the source of the cytoplasm in the event of ig related androgenesis (cf. following item). The pollen parents were inbreds WA374, W23R and A632; the female parent was W23 ig ig R^{nj} R^{nj}. Standard as well as Texas-sterile cytoplasmic counterparts of both parents were employed. The results are summarized according to male parentage, with the 1971 and 1972 data combined so as to provide large enough numbers for a test of heterogeneity.

The incidence of androgenesis, monoploids and diploids combined, differs significantly among the three male parents ($X^2 = 6.6$, $P = 0.03$).

Of 151 cases of androgenesis in all, 15 were diploid. The data indicated unequal proportions of monoploid and diploid derivatives over the three inbreds (X^2, uncorrected for continuity, = 6.1; $P = 0.05$). WA374, it will be noted, had both the highest total androgenesis frequency and the largest proportion of diploids. A broader survey of pollen parents and

Frequencies of androgenetic monoploids and diploids obtained in matings of three inbreds as male to W23 ig ig females

Pollen parent	Population	Androgenetic derivatives		
		Monoploid	Diploid	Frequency ($\times 10^{-3}$)
WA374	3369	59	11	20.8
W23R	2304	28	3	13.5
A632	3583	49	1	14.0
	9256	136	15	16.3

insight into the mechanisms of androgenesis and chromosome doubling is needed in order to discover whether this relationship is other than fortuitous.

Jerry Kermicle

2. Androgenesis and the indeterminate gametophyte mutation: Source of the cytoplasm.

Texas cytosterility is being used as a marker to identify maternal or paternal origin of the cytoplasm in the event of ig associated androgenesis. From T x N crosses, androgenetic plants were established from inbreds WA374, W23R, and A632 and then backcrossed to pollen parents of the respective inbred. Offspring from the backcrosses, consisting of 202 individuals descended from 44 monoploids and 73 descended from 3 diploids, were observed for pollen fertility in 1972.

All 202 were male sterile.

If sterility results from derivation of cytoplasm from the maternal parent, then descendants of andronotes obtained through N x N matings should be fertile. Such cases also serve as a control over the possibility that the observed sterility resulted not from inheritance of T-cytoplasm but as some consequence of the androgenetic event itself.

Observations on 71 first-generation descendants of 7 andronotes of N x N ancestry argue against a trivial basis for the male sterility which

Male fertility of the first generation offspring of andronotes

Cytoplasm of W23 ig ig +	Pollen parent	Andronotes		Number of offspring		
		Monoploid or Diploid	Cases	Sterile	Partially sterile	Fertile
T-sterile	WA374	M	14	29	0	0
		D	1	1	0	0
T-sterile	W23R	M	6	8	0	0
		D	1	23	0	0
T-sterile	A632	M	24	165	0	0
		D	1	49	0	0
Fertile	WA374	M	1	0	0	1
		D	1	0	0	25
Fertile	W23R	M	2	0	0	2
		D	1	0	0	23
Fertile	A632	M	2	1	1	28
		D	0	0	0	0

characterized the material of T x N extraction. All but two plants, one completely sterile and the other partially so, were scored as fertile. The sterile plant was morphologically atypical relative to the inbred involved. Both exceptions proved partially female sterile, furthermore, indicating the male sterility had a basis other than that observed in the T x N series.

Jerry Kermicle

3. Nucleo-cytoplasmic interaction in the determination of a defective seed trait.

A strain whose maternal lineage traces to Euchlaena perennis through six successive crosses to maize was furnished by J. B. Beckett. The early parentage is complex, including a backcross of the hybrid with a stock of elongate; the final two crosses were with inbred W23. Second backcrosses with W23, made in 1971, gave an unexpected outcome. All of

the ears segregated kernels of defective as compared to normal endosperm in approximately 1:1 proportion.

Recurring segregation in each backcross population could not be reconciled with expectation based solely on a consideration of nuclear genes. It was postulated, accordingly, that the defective condition resulted from the combination of W23 nuclear material and E. perennis cytoplasm. The plump seed class, on this view, reflects action of a nuclear factor which can offset the effect of the perennis cytoplasm to restore normal seed development. Various tests performed in 1972 and summarized below support this conjecture.

1. Reciprocal crosses between standard (std.) and derived (E.p.) forms of W23 yielded defective kernels only when the derivative was ♀ parent (seven identical reciprocal cross pairs; plants having perennis cytoplasm grown from plump seed). The ratio of kernel types on ears of E.p. maternity was approximately 1 plump : 1 defective.

2. Plants grown from defective seed and then pollinated by W23 (std.) yielded only defectives (17 ears, 2 progenies).

3. Offspring from a W23 (std.) x W23 (E.p., plump seed selections) cross gave normal ears when pollinated with W23 (std.) (single progeny of 12 ears).

4. Four out of nine plants from the progeny referred to in (3) gave only defectives when tested as male on plants reared from defective seeds, whereas five when similarly tested yielded plump and defective, 1:1.

5. Self-pollinated offspring from the reciprocal cross, (E.p., plump seed) x (std.), produced ears that segregated approximately 3:1 for plump vs. defective (8 ears).

Two additional features of this material were revealed in the matings outlined above. Seedlings grown from seed of the defective class were small and retarded in development beyond the stage which would be expected due to the effect of reduced seed size alone. Field grown plants were pale green and although retarded, eventually gave fertile ear shoots when not grown in competition with normals. Pollen is shed. Secondly, all of the backcross plants that received the nuclear factor which restores normal kernel development also carried a recessive white-endosperm mutation (15 plants tested by selfing). Further indicating close linkage between

the two effects, four backcross plants which lacked the nuclear restoring factor did not segregate white-endosperm. Kernels of the white-endosperm class yield albino seedlings.

Plants of perennis cytoplasm and heterozygous for the nuclear restoring factor gave one of three reactions when pollinated by various inbreds: (a) plump and severely defective, such as is characteristic of the W23 material, (b) plump and only moderately defective (partial restoration), and (c) no defectives (full restoration). The 16 inbreds tested are assigned to the following respective categories:

Group (a): M14 and W22

Group (b): A632, C123, WA374 and W513

Group (c): A619, Oh43, W64A, W182E, W153R, W59E, W629A, W749, SA 1490 and N6

The widespread distribution of a nuclear factor (or factors) in maize populations which offsets the effects of perennial teosinte cytoplasm makes it plausible that the one carried by the W23 (*E. p.*) strain analyzed may have come from a maize stock involved in the derivation of this strain rather than from teosinte itself. The close linkage, or possible pleiotropism, of the restoring factor with the recessive white-endosperm effect may afford a means of tracing its source.

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ADDENDUM:

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1. Stalk rot studies in maize.

In 1970, and especially in 1971 and 1972, we had a strong stalk rot infestation of maize associated with lodging, causing a significant yield loss in Hungary. Stalk rot due to Diplodia, Gibberella and Fusarium is common in Hungary and has caused increased infestation and damage both in seed and commercial crop production in the past two years.

We studied resistance to stalk rot in a series of diallel crosses of four inbred lines, including the reciprocals. The lines were: 156, a resistant inbred of Martonvásár, N 6, susceptible, H Mv 850-2, also Hungarian, and W 17, moderately resistant to stalk rot of maize.

Observations were made at three times. Final evaluation was made at complete maturity in the middle of November. Individual plants (60) from each hybrid and its parental lines were evaluated. The observations on percentage of natural infection in the direct and reciprocal hybrids, as well as in their parental lines, are presented in Table 1.

From Table 1 it can be seen that the percentages of infected and lodged individuals in the inbred lines are practically the same in both years. It is remarkable that the percentage of stalk rot of inbred lines showed no change in 1972, when the natural infection and the consequent lodging was very severe.

In contrast to the above, the behavior of diallel hybrids is different. If we consider the occurrence of stalk rot disease as 100% in 1971, then it is 39?% in 1972. It is evident from Table 1 that the resistant and the moderately resistant lines give, on an average, worse hybrids than the parents themselves. At the same time, the very susceptible N 6 hybrid progenies are generally better than the N 6 parent. Moreover, there is a remarkable difference between the direct and reciprocal hybrids in stalk rot resistance.

Table 1

Percentage of stalk rot infection in a diallel cross and in the parental inbred lines

Pedigree	Percentage of stalk rot infection		Average	Reciprocal differences
	1971	1972		
H Mv 850-2 x 156	15.0	35.5	25.2	5.8
156 x H Mv 850-2	18.8	20.0	19.4	
H Mv 850-2 x N 6	5.2	65.5	35.3	19.9
N 6 x H Mv 850-2	10.5	100.0	55.2	
H Mv 850-2 x W 17	2.6	34.3	18.5	12.7
W 17 x H Mv 850-2	19.4	43.1	31.2	
N 6 x W 17	17.0	57.9	37.5	5.2
W 17 x N 6	13.1	72.3	42.7	
N 6 x 156	25.0	70.1	47.6	24.7
156 x N 6	11.1	34.8	22.9	
W 17 x 156	7.8	57.3	32.6	10.1
156 x W 17	11.7	33.3	22.5	
Average	12.1	53.4	32.8	13.1
	14.1	50.6	32.3	
156	0.0	0.0	0.0	
N 6	77.7	81.6	79.7	
H Mv 850-2	6.6	8.9	7.7	
W 17	2.8	5.0	3.9	
Average	21.8	23.9	22.8	

There is a significant positive correlation between the stalk rot of maternal parent lines and their hybrids. A correlation coefficient of $r = 0.7455$ ++ is observed, which is significant at the 1 per cent level. Similarly, we have obtained a significant relationship between stalk rot of the two parent lines and their hybrids. The correlation coefficient was $r = 0.6941$ ++, which is also significant at the 1 per cent level.

We have investigated the stalk rot resistance of some normal lines and their opaque analogues, as well as hybrids (Table 2). Among them, the best was the single cross hybrid, 156 x B 14, in both the normal and the opaque forms.

Table 2

Stalk rot resistance of opaque-2 and normal hybrids and their parents

Pedigree	Normal	Opaque-2	Average
156	0.0	4.1	2.0
W 153	3.8	7.1	5.4
156 X W 153	20.0	30.0	25.0
156	0.0	4.1	2.0
B 14	0.0	0.0	0.0
156 X B 14	3.3	3.1	3.2
N 6	81.6	75.1	78.4
C 103	0.0	2.0	1.0
N 6 X C 103	63.1	53.4	58.3
Inbreds average	14.2	15.3	14.7
Hybrids average	28.8	28.8	28.8

István Kovács

2. Heat unit differences for germination of maize.

The many year's experiences with cold testing of maize have led to significant achievements in maize breeding. Attention has mainly been concentrated on good germination percentages at low temperatures. Maize breeding, together with a good seed technology and effective fungicides, resulted in a good field stand even in northern regions of maize cultivation. But there are also great differences in germination processes among samples germinating well under low temperatures.

In the last ten years we tested a few hundred samples of breeding materials in Martonvásár and observed highly significant differences in the heat requirement of maize genotypes which have good germination per-

Table 1

Processes of germination and sum of temperatures

Number of days after planting		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Sum of temperatures		192	206	220	234	248	262	276	290	303	318	332	346	360	374	388	402	416
No.	Pedigree	Germination percentages																
1	6 oszv. S_2	50	80	90														
2	8 oszv. S_2	60	80	86														
3	5 oszv. S_2	60	80	83														
4	4 oszv. S_2	50	70	80	82													
5	187	16	31	52	80													
6	W 17		28	47	70	93												
7	H Mv 356		7	33	65	87												
8	W P14 (1970)		23	63	73	86												
9	W P14 (1966)			20	53	80												
10	7 oszv. S_2	15	20	30	60	80	90											
11	A			7	24	50	60	80										
12	014		7	30	53	63	70	77										
13	WF9				46	60	70	70	80									
14	N 6			17	40	60	66	68	70									
15	A 375		3	3	20	47	60	65	75	86								
16	A 264				7	30	53	-	83	86								
17	A 293				3	13	23	-	60	83								
18	R 4				10	30	43	47	50	60	83							
19	Oh 43				20	30	40	67	73	73	80							
20	Ia 153					3	13	27	47	67	70							
21	A 286					7	30	-	50	63	70							
22	4519 segr.			3	10	20	23	30	43	50	73	83						
23	40 a		7	17	47	60	60	60	63	67	67	73						
24	9 oszv. S_2 I			3	56	61	66	66	75	76	80	83	86					
25	W 23 (C5)							13	23	37	40	47	60	70				
26	4518 segr.			3	60	10	23	36	50	56	63	73	73	80	90			
27	9 oszv. S_2 II		1	33	40	40	53	53	60	60	63	63	63	66	70			
28	H Mv 850				16	23	43	53	60	60	63	63	63	63	66	70		
29	4504 segr.			3	10	16	20	23	30	33	36	56	56	63	63	66	66	80

centages. We studied germination under the following conditions: three replications of 20 seeds each were planted in 5-6 cm deep soil (taken from a maize field). During the first ten days, the temperature was 8°C and from the eleventh day, 13-14°C. In Table 1, we have summarized the data from 29 maize genotypes with germination percentages of 70-90. We also calculated the sum of temperatures. It is probable that there are strong differences in the heat requirement, because all maize samples were able to germinate to an almost similar per cent, but the dynamics and final stage were very different in time. For the best types, 220°C was enough for complete germination, while others only began to germinate and finished above 400°C. Samples marked "oszv" are progenies of seeds overwintered in the breeding nursery in Martonvásár. These S_2 plants showed less heat requirement for good germination than any others.

We hope that by this technique we can select maize genotypes which have much less "time-loss" under low temperatures during a cool spring.

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1. Screening for redundant segments in the Zea mays genome with monosomic maize plants.*

Reciprocal translocations are found in the progeny of monoploid X diploid crosses (Alexander, Nature 201:737-738, 1964). We (Weber and Alexander, Chromosoma 39:27-42, 1972) have recently identified breakpoints of 22 reciprocal translocations generated in this way. The points of translocation appear to be at points of interchromosomal redundancy because a) certain of the translocations were found repeatedly and b) my previous work (Weber, Genetics 60:235, 1968) indicated that illegitimate recombination between nonhomologously synapsed segments does not occur in

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maize. It would obviously be desirable to catalog a larger number of translocations from monoploid X diploid crosses; however, recovery of translocations from these crosses is an extremely arduous procedure because: a) monoploids occur with a low frequency, b) the number of progeny per ovule is low (1/113 ovules), and c) the frequency of translocations found in monoploid X diploid crosses is also low (1.3% of the survivors of monoploid X diploid crosses had a reciprocal translocation).

As an extension of this work, translocations from monosomic X diploid crosses are being isolated and their breakpoints are being cataloged in a study to map additional points of redundancy in the Zea mays genome. Only a single chromosome is unpaired in a monosomic plant; thus, the frequency with which translocations are generated in crosses between monosomic and diploid plants would be lower than the frequency observed in monoploid X diploid crosses. However, more offspring are viable and large progenies have been produced from crosses between monosomics and diploids.

Crosses of known monosomics 2, 7, 8, 10, and their diploid sibs with normal diploid lines were made in the summer of 1969, and the resultant progeny were planted in the summer of 1970. All plants were grown to maturity and at least one open-pollinated ear from each plant was examined. Of the 17,359 plants scored, 212 expressed varying degrees of semisterility. A sample from each semisterile ear was planted in the summer of 1971, and plants were scored for pollen and ovule semisterility. Families segregating for semisterility were crossed with KYS and a series of known translocations. The progeny were grown in the summer of 1972, microsporocyte samples were taken, and they are currently being analyzed cytologically for the presence of reciprocal translocations.

The results from this material are as follows:

Cross	Number of progeny tested	Number of translocations confirmed cyto-logically	Frequency of translocations
2N X 2N	6980	1	0.01%
Monosomic 2 X 2N	241	1	0.41%
2N X Monosomic 7	4083	2*	0.05%
2N X Monosomic 8	2787	6	0.22%
2N X Monosomic 10	3268	6	0.18%

*One additional line which carries female and male semisterility from a 2N X monosomic 7 cross has not yet been analyzed cytologically; thus, it is likely that another member is in this class.

It is significant that a much higher frequency of reciprocal translocations is found in progeny of all monosomic X diploid crosses tested than in progeny of diploid X diploid crosses (control population). This strongly suggests that the unpaired monosomic chromosome can occasionally pair with homologous segments found in other regions of the genome, and recombination can occur between the paired regions.

18,992 additional progeny from monosomic by diploid crosses (monosomics 6, 8, and 10) as well as crosses involving a diploid control were screened in the summer of 1971, and 322 ears expressing some degree of semisterility were recovered. 12,203 progeny of monosomic X diploid crosses were screened this past summer.

David F. Weber

2. Fatty acid profiles from maize scutella: a new genetic tool.*

Introduction: Most research involving the genetics of fatty acids in Zea mays has been conducted by extracting the lipids from either whole kernels or entire embryos. Since the kernels are destroyed by these methods, siblings must be used in subsequent crosses and their fatty acid profiles can only be inferred. This article describes a technique to

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analyze the fatty acid profiles from a single kernel without greatly reducing the viability of that kernel. We are currently employing this technique in our genetic studies on maize lipids (Plewa and Weber, 1972, Maize Genet. Coop. News Letter 46:46-48).

Materials and Methods: All kernels analyzed came from a single sib of inbred W22 carrying $\underline{R}^r$. Separate fatty acid profiles from five whole embryos (group A), five scutella samples from the right side (group B), and five scutella samples from the left side of embryonic axes (group C), were compared.

Lipid extraction for group A was conducted by removing the pericarp covering the embryonic tissue and hand dissecting the entire embryo. The isolated embryos were placed into separate, numbered testtubes.

Lipid extraction for groups B and C were conducted as follows. The pericarp covering the scutellum area to be sampled was removed. Approximately a 1.5 mg scutellum sample, distal and parallel to the embryonic axis, was hand dissected, removed, placed in a vial, coded, and stored at -22°C. Sampled kernels were saved for planting.

Each sample was macerated with a glass rod in 5 ml of lipid extraction solvent (redistilled petroleum ether and absolute methanol, 2:1 v/v) and the lipids were extracted overnight. Ten ml of methylating reagent (1% H_2SO_4 in absolute methanol) were added to each sample and kept at room temperature for 1 hr. The volume was reduced to 5 ml by passing a stream of dry nitrogen (N_2) over the liquid. Each sample was refluxed for 1 hr. at 63°C under an atmosphere of N_2. After cooling to room temperature, 5 ml of redistilled petroleum either was added and the sample was poured into a separatory funnel. Each sample tube was quantitatively rinsed with 5 ml of redistilled petroleum ether and the rinse was added to the separatory funnel. The petroleum ether fraction with the dissolved fatty acid methyl esters was separated from the methanol fraction. The volume of the petroleum ether fraction was reduced under N_2 and the sample transferred to a vial. Each sample was cleaned and all traces of the reagent were removed. The resulting sample contained the methyl esters of the maize fatty acids. The volume was reduced to approximately 0.5 ml; the vial was wiffed with N_2, sealed, and stored at -22°C.

The methylated fatty acids were analyzed with a Hewlett-Packard model 5750 gas-liquid chromatograph. The column was 6 ft x ¼ in OD stainless steel packed with 10% EGSS-X 100/120 gas chromatography-Q (Applied Science Laboratories). The column temperature was 180°C, injection port temperature was 250°C, and the flame detector temperature was 235°C. The carrier gas was N_2 and the flow rates for the gases were: N_2, 20 ml/min; H_2, 42 ml/min.; and compressed air 470 ml/min.

The relative amounts of the fatty acids were determined by using a Dietzgen Compensating Polar Planimeter. Two GLC runs were conducted per sample and the relative fatty acid concentrations presented in Table 1 are averages of the runs.

The mean of each individual fatty acid from each group (A, B, and C) was analyzed for departures from the null hypothesis ($H_o = u_A = u_B = u_C$) by Analysis of Variance, $\alpha = 0.05$. Four statistical tests were conducted, one for each fatty acid (palmitic acid, 16:0; stearic acid, 18:0; oleic acid, 18:1; and linoleic acid 18:2).

Results and Discussion: The relative individual fatty acid concentrations for each kernel of the three groups are presented in Table 1. The F values computed from the means of each separate fatty acid in the three groups did not indicate a departure from the null hypothesis (Table 1). There is no significant difference in the individual fatty acid profiles among the three groups. Therefore, a fatty acid profile from a scutellum sample is representative of the fatty acid profile of the entire embryo. Thus, data from whole embryo studies can be compared with the data from scutella samples.

Although there are differences in the relative concentrations of fatty acids in the embryonic axis as compared to the whole embryo or scutellum (E. Weber, personal communication; Plewa, unpublished), these differences are diluted by the larger mass of the scutellum.

This technique has obvious advantages for studying the genetics of fatty acid biosynthesis in maize. The sampled kernels may be planted and crosses can be performed. During the summer of 1972, over 1,500 kernels were sampled and field planted. Approximately 85% of the kernels germinated and it was possible to use them for crosses, and have fatty

Table 1

Relative amounts of fatty acids in groups A, B, and C.

Sample No.	Fatty acids				Groups
	Palmitic	Stearic	Oleic	Linoleic	
MP234-21	0.1448	0.0259	0.2986	0.5290	A
MP234-22	0.1692	0.0306	0.2898	0.5108	
MP234-23	0.2114	0.0298	0.2763	0.4826	(Whole
MP234-24	0.1843	0.0301	0.2584	0.5273	embryos)
MP234-25	0.1632	0.0421	0.2391	0.5558	
Means	0.1746	0.0317	0.2724	0.5211	
MP234-1	0.1971	0.0464	0.3333	0.4232	B
MP234-2	0.1402	0.0449	0.2876	0.5274	
MP234-3	0.2010	0.0300	0.2637	0.5054	(Scutella
MP234-4	0.1391	0.0487	0.2383	0.5739	samples right
MP234-5	0.1741	0.0278	0.2235	0.5747	side)
Means	0.1703	0.0396	0.2693	0.5209	
MP234-6	0.2037	0.0201	0.2361	0.5417	C
MP234-7	0.1827	0.0543	0.2641	0.4989	
MP234-8	0.1545	0.0320	0.3045	0.5091	(Scutella
MP234-9	0.1458	0.0292	0.2915	0.5335	samples left
MP234-10	0.2041	0.0204	0.3010	0.4745	side)
Means	0.1782	0.0312	0.2794	0.5115	
$\underline{F}$* values	0.0714	1.0890	0.1341	0.0913	

*$F_{95}(2,13) = 3.89$; retain Ho

acid profiles from embryos of the corresponding sporophytes. Under greenhouse conditions, germination rates of sampled kernels are over 90%. We believe that this technique is a valuable tool and we are currently employing it in our studies in mapping genes involved in fatty acid biosynthesis utilizing maize monosomics.

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1. Interchromosomal effects on chiasma formation in maize heterozygous for an inversion and an interchange.

Reduction in crossing over due to inversion heterozygosity in one chromosome is known to enhance crossing over in certain nonhomologous chromosomes in Drosophila. Cytological evidence of such compensation has been reported in maize. The problem is a bit difficult to study in the case of maize, because of a large number of chromosomes. Further, as pointed out by Sinha and Mohapatra (Cytologia, 34:523-527, 1969), the occurrence of the phenomenon may be obscured under certain conditions. We have now obtained further evidence for compensation of the loss of chiasmata in a different stock of maize, heterozygous for both an inversion and an interchange. The conditions under which the phenomenon can be clearly detected have been determined.

Material for the present study was heterozygous for a pericentric inversion in the second chromosome (In 2a) and a reciprocal translocation involving chromosomes 6 and 9 (T6-9b). Chiasmata were recorded at diakinesis separately for each of the following bivalents: the longest (presumably chromosome 1), the second longest (presumably chromosome 2) and the shortest (presumably chromosome 10). Further, the types of configuration (ring, chain or other) of the interchanged chromosomes were also noted along with actual chiasma counts. Chiasma number for the remaining five bivalents as a group was recorded separately. The total number of PMC's studied was divided into suitable groups, as discussed later, according to (1) the number of chiasmata in the second chromosome carrying the inversion and (2) the number of chiasmata in the interchanged chromosomes. This kind of grouping made possible an examination of the probable effect of reduction in chiasmata in one or more of the specified chromosomes on the chiasma frequency in the other chromosomes. This method hopefully should eliminate the possible complicating influence of either segregating genes or environmental variation on chiasmata and their distribution.

The main findings of this study will be presented in two different sections.

I. Differences between PMC's grouped according to chiasma frequency in the second bivalent:

The relevant data presented in Table 1 permit comparison of chiasma frequencies of (a) the longest bivalent, (b) the shortest one and (c) the interchanged chromosomes 6-9 in two distinct classes of PMC's. In one class the second longest, carrying the inversion, had only one chiasma suggesting reduction due to inversion heterozygosity. In the other class there were two chiasmata, indicating little effect of the inversion. Our earlier work (Sinha and Mohapatra, 1969) suggested the utility of studying the problem of compensation in PMC's with a varying number of total chiasmata per nucleus. Hence for a more meaningful comparison, PMC's were further grouped as follows: Gr. 1 or low number (13-14) of chiasmata; Gr. 2 = medium number (15-16) of chiasmata; Gr. 3 = moderately high number (17-18) and Gr. 4 = very high number (above 18). The following salient points may be noted from Table 1:

Table 1

Comparison of chiasma frequencies of specific chromosomes in (1) PMC's with varying number of chiasmata in the second bivalent (carrying inversion) and (2) in different groups with varying number of chiasmata / nucleus.

		Gr. 1 (13-14 chiasmata)	Gr. 2 (15-16 chiasmata)	Gr. 3 (17-18 chiasmata)	Gr. 4 (18 or more chiasmata)	All PMC's pooled together
Chiasma frequency in the 6-9 interchange.	In Gr. I* cells	3.38	3.62	3.79	4.00	3.58
	In Gr. II** cells	3.08	3.51	3.71	4.00	3.62
Chiasma frequency in the longest bivalent.	In Gr. I cells	1.94	2.06	2.29	2.75	2.09
	In Gr. II Cells	2.00	2.09	2.46	2.82	2.34
Chiasma frequency in the shortest bivalent.	In Gr. I cells	0.97	1.00	1.13	1.25	1.03
	In Gr. II cells	1.00	0.97	1.08	1.36	1.09

*Cells with 1 chiasma in chromosome 2 bivalent.
**Cells with 2 chiasmata in chromosome 2 bivalent.

(i) Only the 6-9 interchange shows increased chiasma frequency along with reduction in the second bivalent. (ii) The increase in the case of the 6-9 interchange is most clearly manifested in the low chiasmata group and is gradually and consistently less evident in the medium and moderately high chiasmata groups. In the very high chiasmata group no difference can be noted. (iii) Considering all the PMC's together, little difference in chiasmata in the 6-9 interchange can be noted between the two classes of PMC's with 1 or 2 chiasmata in the second bivalent. (iv) The longest bivalent (chromosome 1 ?) shows a consistent decrease in chiasmata accompanying reduction in the second longest. This decrease is also seen when all the PMC's are considered together. (v) The shortest bivalent shows a trend similar to the longest, though not as sharp and consistent.

Evidently, all chromosomes do not participate in the compensatory increase, even if it occurs. Further, the detection of this phenomenon may not be possible, if all the PMC's are pooled for analysis.

From the standpoint of compensation, chromosomes may be divisible into two classes: (1) competitive, that is, capable of showing compensation and (2) non-competitive. The 6-9 interchange behaves as a strongly competitive group, particularly under conditions when the total number of PMC chiasmata is low. In the presence of this competitor, the longest and the shortest bivalents fail to take advantage of compensation.

II. A test of independence of events leading to chiasma formation in chromosome 2 and 6-9 interchange:

The results presented above suggest the possibility that events leading to chiasma formation in the second chromosome and the 6-9 interchange may not be entirely independent. An alternative analysis was adopted to test this point.

The frequencies of single chiasma and double chiasmata in the second bivalent were calculated. Similarly, the frequencies of 2, 3 and 4 chiasmata in the 6-9 interchange were determined. From these figures the probabilities of different combinations of chiasmata in the second and the 6-9 interchanged chromosomes could be worked out. Next a comparison was made between the expected probabilities of these joint events and the observed frequencies. The results presented in Table 2, based on the data on all the PMC's, do not indicate a significant difference between the expected and observed.

Table 2

Frequencies of PMC's with varying numbers of chiasmata in the second bivalent and the 6-9 interchange.

	4 or > in chr. 6-9 and 2 in chr. 2 (class 1)	3 in chr. 6-9 and 2 in chr. 2 (class 2)	4 in chr. 6-9 and 1 in chr. 2 (class 3)	3 in chr. 6-9 and 1 in chr. 2 (class 4)	2 in chr. 6-9 and 1 in chr. 2 (class 5)
Expected	60.9	27.1	71.7	31.1	11.2
Observed	60.0	26.0	71.0	33.0	12.0

It might be inferred that the events underlying chiasma formation in chromosome 2 are independent of those in chromosomes 6 and 9. Thus, there is apparently no evidence of compensatory chiasma formation in these three chromosomes. It should, however, be recalled that compensation was most clearly suggested in the PMC's showing a low number of chiasmata (vide Table 1). Hence, it should be desirable to undertake the same analysis in the PMC's with a low or medium number of chiasmata. Table 3 contains the results of this analysis.

Table 3

Frequencies of PMC's with varying numbers of chiasmata in the second bivalent and the 6-9 interchange. (Only PMC's with 13-16 chiasmata are considered.)

	4 or > in chr. 6-9 and 2 (or rarely 3) in chr. 2 (class 1)	3 in chr. 6-9 and 2 in chr. 2 (class 2)	4 in chr. 6-9 and 1 in chr. 2 (class 3)	3 in chr. 6-9 and 1 in chr. 2 (class 4)	2 in chr. 6-9 and 1 in chr. 2 (class 5)
Observed	24.00	19.00	49.00	29.00	12.00
Expected	47.08	28.86	27.93	17.16	11.97
Deviation (O-E)	-23.08	-9.86	+21.07	+11.84	+0.03
$(O-E)^2/E$	11.29	3.37	15.89	8.17	

$\chi^2 = 38.72$ $\quad P < 0.005$

It is evident that deviation in some of the classes has contributed to a high Chi-square value with $P < 0.005$. In fact, class 3 shows the maximum departure from the expected. This class can be called compensatory since a high chiasma frequency in the 6-9 interchange is combined with a low number in the chromosome carrying the inversion. A high deviation from the expected is also observed in class 1, a non-compensating type of combination, in which high chiasma frequencies are observed in chromosome 2 as well as in chromosomes 6-9. There is an excess of PMC's of the class 3 or compensating type; but in the case of class 1, the non-compensating type, the observed frequency of PMC's is much below the expected. Thus, it appears that under conditions leading to low PMC chiasmata, events underlying chiasma formation in the nonhomologous chromosomes may not be entirely independent of each other.

Besides providing an evidence of compensatory chiasma formation, this study further suggests that the phenomenon can be detected easily under conditions rather stringent for chiasma formation.

S. K. Sinha
B. K. Mohapatra

2. <u>The distribution of bivalent chiasmata in maize plants heterozygous for two pericentric inversions.</u>

An investigation was undertaken in maize to determine the effect of inversion heterozygosity on the distribution of chiasmata in maize, since the information on this aspect appears to be meagre.

The material, heterozygous for two pericentric inversions designated Inv. 2a (2S 0.7; 2L 0.8) and Inv. 9a (9S 0.7; 9L 0.9), was synthesized through suitable crosses involving the inversion stocks and a highly inbred line, Ext. 355. Chiasma frequency was studied at diakinesis in PMC's. Data were recorded separately for the longest, the shortest, the sixth and the remaining bivalents. The PMC's were grouped into classes according to chiasmata per PMC. The class-wise distribution of chiasmata was worked out for the longest (presumably chromosome 1), the shortest (presumably chromosome 10) and the sixth bivalent. These distributions were compared with the expected values calculated on the basis of relative pachytene lengths (published data of Rhoades, 1955). Two kinds of

comparisons were attempted. Inversion heterozygotes were compared with normal sibs. Further, the pooled data from a number of inbred lines were used to give an idea of the minimum and maximum number of chiasmata in a specific bivalent in different groups of PMC's. Class-wise chiasmata records of the inversion heterozygotes were next compared with these values.

Table 1

Comparison of class-wise distribution of chiasmata in the sixth bivalent in a normal plant and inversion heterozygotes (figures inside parentheses indicate % of PMC's with a particular number of chiasmata).

	Mean bivalent chiasma frequency in PMC's with chiasmata numbering:						
	12	13	14	15	16	17	18
Expected	1.06	1.15	1.23	1.32	1.41	1.50	1.59
Observed in normal plant		1.00 (6.00)	1.00 (14.00)	1.16 (38.00)	1.20 (32.00)	1.50 (8.00)	2.00 (2.00)
Observed in inversion heterozygotes	1.00 (13.30)	1.00 (15.00)	1.20 (33.30)	1.37 (26.70)	1.57 (11.70)		
Minimum and maximum values observed in earlier studies*	0.95 1.07	0.90 1.02	1.00 1.09	1.09 1.19	1.18 1.29	1.29 1.71	1.42 1.71

*Pooled data from several lines (unpublished data of Sinha and Mohapatra, 1967; Sinha and Pany, 1968). Upper and lower figures represent minimum and maximum values, respectively.

The salient points that should be noted from the data in Table 1 are as follows:

(1) Compared with the normal plants, there is a general reduction in the range of total chiasmata numbers in inversion heterozygotes.

(2) As indicated by the pooled data, except in the 17- and 18-chiasmata classes of PMC's, the observed bivalent chiasmata are much fewer than expected.

(3) In normal plants only 2% of the PMC's, those having 18 chiasmata, show more than the expected bivalent chiasmata. In most of the PMC's, the observed frequency is much below the expected frequency.

(4) On the other hand, in plants heterozygous for the inversion quite a high percentage (38.4%) of PMC's show more than the expected frequency of bivalent chiasmata. In about half the PMC's (those with 13-14 chiasmata), the bivalent chiasma frequency almost approaches the expected values.

(5) Thus, there is a well-marked tendency for increase of chiasma frequency in almost all classes of PMC's.

In maize, Rhoades (1955) reported that a reduction in recombination within the limits of the inversion in heterozygous condition leads to a simultaneous increase in the adjacent regions. Bellini and Bianchi (1963) studied the influence of two pericentric inversions in chromosomes 2 and 9 of maize on recombination frequency in chromosomes 9 and 2, respectively. The results of these studies are comparable to those obtained in Drosophila. Thus, within the same chromosomes as well as in heterologous chromosomes, there is evidence of increased crossing over accompanying reduction due to inversion heterozygosity. Further evidence supporting such compensation in maize has been presented recently (Sinha and Mohapatra, 1969). However, as suggested by Sinha and Mohapatra (1969), the magnitude of the compensatory increase would depend on the genotype. In the present investigation, the loss of chiasmata due to heterozygosity for two long inversions appears to be only partly compensated since, compared with the normal material, average chiasma frequency is lower in the inversion heterozygote. There is evidence from the earlier work in this laboratory (Sinha and Mohapatra, 1969), that Ext. 355, the inbred line in which the inversions have been incorporated, does not show compensatory chiasma formation. The results of the present investigation appear to further support the inference regarding Ext. 355. It should be interesting to study the effect of inversion heterozygosity in a genotype showing a high degree of compensation.

S. K. Sinha
B. K. Mohapatra
M. D. Pany

IV. RECOMMENDATIONS FOR NOMENCLATURE CHANGES

The following set of tentative recommendations for changes in maize nomenclature and symbols have been formulated by a committee composed of C. R. Burnham, E. H. Coe, O. E. Nelson, E. B. Patterson, and M. M. Rhoades. It is hoped that these recommendations will be studied during 1973 in order that they may be discussed at the 1974 Allerton meeting.

The recommendations have been formulated with three major objectives in mind: 1) to provide a uniform method of designating the reference mutant allele for each locus and of designating independent mutational events. This applies also to wild-type alleles where they can be identified. 2) To make type-setting as economical as possible. This accounts for the on-line symbols which will also be important in information storage and retrieval systems. The deletion of the numeral 1 avoids the potential confusion of the numeral 1 and a lower case l as might otherwise happen with a(1) and al when printed on the same line. 3) To adopt a symbol system that will adapt with the fewest changes in information retrieval systems. It should be kept in mind that all print-outs from computers contain only capital letters and further handle subscripts and superscripts awkwardly if at all. In our system, bt2-7201 would be encoded and printed out as BT2-7201 with the dash (or minus) here indicating that this is a recessive allele. A wild-type allele, Bt2, would appear as BT2+ with the + sign indicating a dominant allele. If desirable to indicate a codominant allele, this could be done with an = sign in place of the - or +.

The superscripts that currently indicate different alleles at a locus will be written after the dash following the locus designation. As examples R^r would become R-r and P^{RR} would become P-RR.

RECOMMENDATION 1: Each locus be designated by a lower case italicized symbol. Traditionally, this has been a one or two letter symbol, but some three letter symbols have been used. We recommend that all be three letters in the future.

RECOMMENDATION 2: As previously, different loci at which mutations produce the same general phenotype are distinguished by italicized numbers

following the gene symbol, but the number one will be omitted. The number will appear on the line both when the gene name is written out and when the symbol is used, e.g.: brittle 2 and bt2.

RECOMMENDATION 3: A mutational site or event is designated by an isolation number, laboratory number, or previous designation following the gene symbol and set off by a dash: e.g., sh2-6801.

The wild type allele at a locus can be designated either by the gene symbol with a capital letter, Sh2, or by the lower case gene symbol followed by a plus sign: e.g., sh2+. Where it is desirable to designate a particular wild type, this can be done as Sh2-W22 or sh2+W22.

The mutation by which the locus was first detected should be designated by a capital R or Ref. as sh2-Ref. to indicate the reference allele.

RECOMMENDATION 4: A mutation at an unknown locus conditioning a phenotype similar to that conditioned by mutations at one or more known loci can be designated by an appropriate gene symbol, an * to indicate that the locus is unknown and a laboratory number as bt*-7011. After tests establish its allelism with mutations at a given locus, the number of that locus can be substituted for the * but the laboratory isolation number retained, as bt2-7011. It would be preferable if the mutations within the locus that appear to represent independent mutational events were designated only by isolation numbers that do not purport to furnish any information about the characteristics of the allele.

O. E. Nelson, Jr.

V. REPORT ON MAIZE COOPERATIVE

During 1972 the Maize Genetic Cooperative received 145 requests for stocks and 1211 packets of seed were sent to fill these requests. Domestic requests accounted for 72% of the total, and foreign requests the remaining 28%. Requests from Geneticists numbered 101 or 70% of the total, Physiologists 13%, Plant Breeders 5%, and stocks for educational purposes 12%.

Seed stocks of chromosome 9 were the chief cultures increased during the 1972 growing season. Certain stocks of chromosomes 1 and 4 along with T-B translocations and trisomics were also increased. Certain unplaced traits in chromosomes 1, 2, 3, 4, 5, 6, and 7 were grown to obtain linkage information and also to obtain new chromosome tester stocks. In addition, certain stocks were grown to confirm mature plant and seedling traits of chromosomal tester stocks. This procedure insures that confirmed stocks are available to fill seed requests.

This year we began the rather large task of increasing stocks of our reciprocal translocation collection. We grew approximately 20% (170 cultures) of the collection during 1972, and hope to have fresh seed of the entire collection by 1977.

All stocks maintained by the Co-op have now been refiled and an up-to-date inventory completed. A newly revised stock list appears at the end of this report.

A list of reciprocal translocation stocks available from the Co-op is published in the Co-op report in News Letter volume 43, 1969.

Requests for stocks or correspondence relative to the stock collection should be addressed to:

Dr. R. J. Lambert
S-116 Turner Hall
Department of Agronomy
University of Illinois
Urbana, Illinois 61801

Catalogue of Stocks

Chromosome 1

sr_1 zb_4 P^{WW}

sr_1 P^{WR}

sr_1 P^{RR} an_1 ad_1 bm_2

sr_1 P^{WR} an_1 gs_1 bm_2

sr_1 P^{WR} an_1 bm_2

sr_1 P^{RR} gs_1 bm_2

sr_1 P^{WR} bm_2

vp_5

zb_4 ms_{17} P^{WW}

zb_4 ts_2 P^{WW} br_1 f_1 bm_2

zb_4 ts_2 P^{WW} bm_2

zb_4 P^{WW}

zb_4 P^{WW} br_1

zb_4 P^{WW} br_1 f_1 bm_2

zb_4 P^{WW} bm_2

ts_2 P^{RR}

ts_2 P^{WW} br_1 bm_2

P^{CR}

P^{RR}

P^{RW}

P^{CW}

P^{MO}

P^{VV}

P^{RR} as br_1 f_1 an_1 bm_2

Chromosome 1 (Continued)

P^{RR} br_1 f_1 an_1 gs_1 bm_2

P^{RR} an_1 ad_1 bm_2

P^{RR} an_1 gs_1 bm_2

P^{RR} ad_1 bm_2

P^{WR} an_1 Kn bm_2

P^{WR} an_1 ad_1 bm_2

P^{WR} an_1 br_2 bm_2

P^{WR} an_1 bm_2

P^{WR} ad_1 bm_2

P^{WR} br_1 Vg

P^{WR} br_1 f_1 gs_1 bm_2

P^{WW} rs_2

P^{WW} rs_2 br_1 f_1

P^{WW} as br_1 f_1 bm_2

P^{WW} hm_1 br_1 f_1

P^{WW} br_1 f_1 ad_1 bm_2

P^{WW} br_1 f_1 bm_2

P^{WW} br_1 f_1 an_1 gs_1 bm_2

as

as rs_2

rd-Hy

br_1 f_1

br_1 f_1 bm_2

br_1 f_1 Kn

Chromosome 1 (Continued)

br_1 f_1 Kn Ts_6

br_1 f_1 Kn bm_2

br_1 bm_2

Vg

Vg an_1 bm_2

Vg br_2 bm_2

bz_2^m m

bz_2^m M

an_1 bm_2

an_1 bz_2 6923 (apparent deficiency including an_1 and bz_2)

br_2

br_2 an_1 bm_2

br_2 bm_2

tb_{8963}

Kn

Kn Ts_6

lw_1

vp_8

gs_1 bm_2

Ts_6

bm_2

id

nec_{8147}

ms_9

ms_{12}

Chromosome 1 (Continued)

ms_{14}

$mi_{8043} = mi_1$

D_8

TB-1a (1L.20)

TB-1b (1S.05)

Chromosome 2

ws_3 al lg_1 gl_2 B fl_1 v_4

ws_3 lg_1 gl_2 B

ws_3 lg_1 gl_2 B sk

ws_3 lg_1 gl_2 B sk fl_1 v_4

ws_3 lg_1 gl_2 B sk v_4

ws_3 lg_1 gl_2 B ts_1

ws_3 lg_1 gl_2 b

ws_3 lg_1 gl_2 b sk fl_1 v_4

ws_3 lg_1 gl_2 b sk v_4

ws_3 lg_1 gl_2 b fl_1 v_4

ws_3 lg_1 gl_2 b ts_1

ws_3 lg_1 gl_2 b v_4

ws_3 lg_1 gl_2 b v_4; wx

al

al lg_1

al lg_1 gl_2 B sk v_4

al lg_1 gl_2 B v_4

al lg_1 gl_2 b sk v_4

lg_1 gl_2 B

Chromosome 2 (Continued)

lg_1 gl_2 B gl_{11}

lg_1 gl_2 B gs_2

lg_1 gl_2 B gs_2 v_4

lg_1 gl_2 B gs_2 Ch

lg_1 gl_2 B gs_2

lg_1 gl_2 B sk v_4

lg_1 gl_2 B v_4

lg_1 gl_2 b

lg_1 gl_2 b gs_2

lg_1 gl_2 b gs_2 sk Ch

lg_1 gl_2 b gs_2 wt_1

lg_1 gl_2 b gs_2 v_4

lg_1 gl_2 b gs_2 v_4 Ch

lg_1 gl_2 b sk

lg_1 gl_2 b sk fl_1 v_4

lg_1 gl_2 b sk v_4

lg_1 gl_2 b wt_1 v_4

lg_1 gl_2 b fl_1 v_4

lg_1 gl_2 b fl_1 v_4 Ch

lg_1 gl_2 b v_4

lg_1 gl_2 b v_4 Ch

lg_1 gl_2 wt_1

lg_1 gl_2 wt_1; a_1 Dt_1 A_2 C R

lg_1 gl_2 w_3

lg_1 gl_2 w_3 Ch

Chromosome 2 (Continued)

lg_1 gl_2 Ch

lg_1 b

lg_1 b gs_2 wt_1 v_4

lg_1 b gs_2 v_4

lg_1 Ch

gl_2

$d_5 = d_{037-9}$

B gl_{11}

B ts_1

$gl_{11} = gl_{8712}$

$wt_1 = wt_{6-9421} = wt_{6-9241-1}$

mn_1

fl_1

ts_1

v_4

w_3

w_3 Ht_1

w_3 Ch

Ht_1 A source

Ht_1 B source

ba_2

$R_2{}^g$; r_1 A_1 A_2 C_1 Pr y_1 P^{WW} (Brawn)

R_2; r_1 A_1 A_2 C_1 (Kermicle)

Ch

Primary Trisomic 2

Chromosome 3

cr_1

cr_1 d_1

cr_1 d_1 Lg_3

cr_1 pm ts_4 lg_2 na_1

cr_1 ts_4 ba_1 na_1

cr_1 ts_4 na_1

d_1 Tall = d_{6016} = tn

d_1 rt_1

d_1 rt_1 Lg_3

d_1 rt_1 Rg ts_4 lg_2

d_1 rt_1 pm

d_1 Rf_1 lg_2

d_1 ys_3

d_1 ys_3 Rg

d_1 Lg_3

d_1 Rg ts_4 lg_2

d_1 pm

d_1 ts_4 lg_2

d_1 ts_4 lg_2 a_1^m; A_2 C_1 R Dt_1

ra_2

ra_2 ys_3 Lg_3 Rg

ra_2 ys_3 Rg

ra_2 Rg pm ts_4 lg_2

ra_2 Rg lg_2

ra_2 pm lg_2

Chromosome 3 (Continued)

ra_2 lg_2

Cg

cl_1

cl_1 Cl_2

cl_1 Cl_3

clp Cl_4

rt_1

ys_3

ys_3 Lg_3

ys_3 gl_6 lg_2 a_1^m et; A_2 C_1 R Dt_1

ys_3 ts_4

Lg_3

Lg_3 Rg

gl_6 pm lg_2 a_1^m et; A_2 C_1 R Dt_1

gl_6 lg_2 A_1; A_2 C_1 R

gl_6 lg_2 A^b et; A_2 C_1 R Dt_1

gl_6 lg_2 a_1^m et; A_2 C_1 R dt_1

gl_6 lg_2 a_1^m et; A_2 C_1 R Dt_1

ts_4

ts_4 ba_1 na_1

ts_4 lg_2 a_1^m; A_2 C_1 R Dt_1

ts_4 lg_2 gl_7

ts_4 na_1 a_1^m et; A_2 C_1 R Dt_1

ts_4 a_1^m; A_2 C_1 R Dt_1

ba_1

Chromosome 3 (Continued)

lg_2 A^b et; A_2 C_1 R Dt_1

lg_2 a_1^m sh_2 et; A_2 C_1 R Dt_1

lg_2 a_1^m et; A_2 C_1 R dt_1

lg_2 a_1^m et; A_2 C_1 R Dt_1

lg_2 a_1^{st} sh_2 et; A_2 C_1 R Dt_1

lg_2 a_1^{st} et; A_2 C_1 R Dt_1

na_1

A_1 sh_2; A_2 C_1 R B Pl dt_1

A_1 ga_7; A_2 C_1 R B Pl dt_1

A_1^d-31; A_2 C_1 R

A_1^d-31; A_2 C_1 R pr dt_1

A_1^d-31; A_2 C_1 R pr B Pl dt_1

A_1^d-31; A_2 C_1 R B Pl dt_1

A_1^d-31; A_2 C_1 R Dt_1

A_1^d-31; A_2 C_1 R pr Dt_1

A_1^d-31; A_2 C_1 R pr B Pl Dt_1

A_1^d-31; A_2 C_1 R pr B pl Dt_1

A_1^d-31 sh_2; A_2 C_1 R B Pl dt_1

A_1^d-31 sh_2; A_2 C_1 R Dt_1

A_1^d-31 sh_2; A_2 C_1 R B Pl Dt_1

A_1^d-31 sh_2 et; A_2 C_1 R Dt_1

A_1^d-31 et; A_2 C_1 R Dt_1

a_1^m; A_2 C_1 R dt_1

a_1^m; A_2 C_1 R pr dt_1

a_1^m; A_2 C_1 R pr B Pl dt_1

Chromosome 3 (Continued)

a_1^m; A_2 C_1 R B Pl dt_1

a_1^m; A_2 C_1 R Dt_1

a_1^m; A_2 C_1 R pr Dt_1

a_1^m; A_2 C_1 R B Pl Dt_1

a_1^m sh_2; A_2 C_1 R B Pl dt_1

a_1^m sh_2; A_2 C_1 R B Pl Dt_1

a_1^m sh_2 et; A_2 C_1 R Dt_1

a_1^m et; A_2 C_1 R Dt_1

a_1^{st}; A_2 C_1 R Dt_1

a_1^{st} sh_2; A_2 C_1 R Dt_1

a_1^{st} sh_2; A_2 C_1 R B Pl Dt_1

a_1^{st} sh_2 et; A_2 C_1 R Dt_1

a_1^{st} et; A_2 C_1 R Dt_1

a_1^{st} et; A_2 C_1 R pr Dt_1

a_1^{st} et; A_2 C_1 R B Pl Dt_1

a_1^p et; A_2 C_1 R dt_1

a_1^p et; A_2 C_1 R B Pl dt_1

a_1^p et; A_2 C_1 R Dt_1

a_1^p et; A_2 C_1 R B Pl Dt_1

a_1 - xl

a_1 Ga_7; A_2 C_1 R

sh_2 = bt_{60-156} = $sh_{Garwood}$

vp_1

Rp_3

gl_7

Chromosome 3 (Continued)

gl_{12}

TB-3a (3L.10)

TB-3b (3S.50)

Primary Trisomic 3

Chromosome 4

Rp_4

Ga_1

Ga_1 su_1

Ga_1^S

Ga_1^S bt_2

st

st Ts_5

st fl_2

st Ts_5 su_1

Ts_5

Ts_5 la su_1 bm_3 gl_3

Ts_5 fl_2

Ts_5 fl_2 su_1

Ts_5 su_1

Ts_5 su_1 zb_6

Ts_5 su_1 zb_6 o_1

Ts_5 su_1 gl_3 o_1

Ts_5 Tu

la su_1 Tu gl_3

la su_1 gl_3

Chromosome 4 (Continued)

la su_1 gl_3 c_2; A_1 A_2 C_1 R

la su_1 gl_3 o_1

fl_2

fl_2 su_1

fl_2 su_1 bm_3

su_1

su_1^{am}

su_1 bm_3

su_1 bt_2 gl_4

su_1 zb_6

su_1 zb_6 Tu

su_1 zb_6 Tu gl_3

su_1 zb_6 $C_2^{Idf\ (Active-1)}$; A_1 A_2 C_1 R

su_1 gl_4

su_1 gl_4 Tu

su_1 gl_4 Tu o_1

su_1 gl_4 j_2

su_1 gl_4 o_1

su_1 j_2

su_1 gl_3

su_1 gl_3 o_1

su_1 o_1

$bt_2 = bt_4 = bt_{60-158} = bt_{Williams}$

bt_2 gl_4

bt_2 gl_4 j_2

Chromosome 4 (Continued)

$gl_4 = gl_{16} = gl_{Stadler}$

Tu

Tu^1 1st

Tu^1 2nd

Tu^d

Tu^{md}

Tu gl_3

j_2

j_2 c_2; A_1 A_2 C_1 R

j_2 C_2; A_1 A_2 C_1 R

v_8

gl_3

gl_3 dp

c_2; A_1 A_2 C_1 R

C_2; A_1 A_2 C_1 R

$C_2^{Idf\ (Active-1)}$; A_1 A_2 C_1 R

v_{17}

gl_7

o_1

ra_3

TB-4a (4S.20)

Primary Trisomic 4

Chromosome 5

lu_1

lu_1 sh_4

Chromosome 5 (Continued)

ms_{13}

gl_{17}

gl_{17} A_2 pr; A_1 C_1 R

gl_{17} a_2; A_1 C_1 R

A_2 vp_7 pr; A_1 C_1 R

A_2 bm_1 pr; A_1 C_1 R

A_2 bm_1 pr ys_1; A_1 C_1 R

A_2 bm_1 pr ys_1 eg; A_1 C_1 R

A_2 bm_1 pr v_2; A_1 C_1 R

A_2 bt_1 pr; A_1 C_1 R

A_2 sh_3 pr ys_1; in A_1 C_1 R

A_2 v_3 pr; A_1 C_1 R

A_2 pr na_2; A_1 C_1 R

A_2 pr ys_1; A_1 C_1 R

a_2; A_1 C_1 R

a_2; A_1 C_1 R B Pl

a_2 bm_1 bt_1 bv_1 pr; A_1 C_1 R

a_2 bm_1 bt_1 pr; A_1 C_1 R

a_2 bm_1 bt_1 pr ys_1; A_1 C_1 R

a_2 bm_1 gl_8 pr v_2; A_1 C_1 R

a_2 bm_1 sh_4 pr v_2; A_1 C_1 R

a_2 bm_1 pr na_2; A_1 C_1 R

a_2 bm_1 pr ys_1; A_1 C_1 R

a_2 bm_1 pr ys_1 eg; A_1 C_1 R

a_2 bm_1 pr ys_1 v_{12}; A_1 C_1 R

a_2 bm_1 pr v_2; A_1 C_1 R

Chromosome 5 (Continued)

a_2 bt_1 v_3 Pr; A_1 C_1 R

a_2 bt_1 v_3 pr; A_1 C_1 R

a_2 bt_1 pr; A_1 C_1 R

a_2 bt_1 v_2; A_1 C_1 R

a_2 v_3 pr; A_1 C_1 R

a_2 pr; A_1 C_1 R

a_2 pr v_2; A_1 C_1 R

vp_2

vp_2 gl_8

vp_7

bm_1 yg_1

$bt_1 = bt_{Alex-Krug} = bt_{Krug6-1303-2}$
$= bt_{Vineyard} = bt_{6-783-7} =$
$sh_{Eldridge} = bt_{C103} = sh_3 = sh_5$

ms_5

$v_3 = v_{8983}$

td ae

ae

sh_4

$gl_8 = gl_{10}$

na_2

lw_2

ys_1

eg

v_2

Chromosome 5 (Continued)

yg_1

gl_5

ms_{13}

v_{12}

lw_3 lw_4

Primary Trisomic 5

Chromosome 6

rgd po y_1

rgd Y_1

po = ms_6

po y_1 pl

po Y_1 pl

$y_1 = pb_1 = w^m$

y_1 l_{10}

y_1 l_{4920}

y_1 w_{8896}

y_1 pb_4

y_1 pb_4 pl

y_1 pb_4 Pl

y_1 ms-si

y_1 at-si = ms-si

y_1 wi Pl

y_1 pg_{11}; Wx pg_{12}

y_1 pg_{11}; wx pg_{12}

Y_1 pg_{11}; Wx pg_{12}

Chromosome 6 (Continued)

Y_1 pg_{11}; wx pg_{12}

y_1 pl

y_1 pl su_2

y_1 Pl

y_1 Pl Bh; c_1 sh_1 wx A_1 A_2 R

y_1 su_2

y_1 l_{4120}

Y_1 l_{10}

Y_1 pb_4

Y_1 wi pl

Y_1 wi Pl

Y_1 pl su_2

Y_1 su_2

wi

$pg_{48-040-8}$ = pg_{11} pg_{12}

pg_{6656} = pg_{11} pg_{12}

yg_{6853} = pg_{11} pg_{12}

Pl Dt_2; a_1 A_2 C R

pl sm; P^{RR}

Pl sm; P^{RR}

Pl sm Pt py; P^{RR}

Pl sm py; P^{RR}

Pt

w_1

Chromosome 6 (Continued)

w_{8657} = w_{025-12} = w_{035-2} = w_{5946} = w_{8050} = w_{6853} = $w_{1-7_{4302}}$

Primary Trisomic 6

Chromosome 7

Hs o_2 v_5 ra_1 gl_1

In^D

In^D o_2 v_5 ra_1 gl_1 ij

In^D gl_1

o_2

o_2 v_5

o_2 v_5 ra_1 gl_1

o_2 v_5 ra_1 gl_1 Tp_1

o_2 v_5 ra_1 gl_1 ij

o_2 v_5 gl_1

o_2 ra_1 gl_1 ij

o_2 ra_1 gl_1 ij bd

o_2 gl_1

o_2 gl_1 sl_1

o_2 bd

in

in gl_1

v_5

vp_9

Chromosome 7 (Continued)

vp_9 gl_1

ra_1 gl_1 ij Bn bd

ra_1 gl_1 ij bd

gl_1

$gl_1{}^m$

gl_1 Tp_1

gl_1 o_5

gl_1 g_2

gl_1 mn_2

Tp_1

ij

Bn

bd

Pn

o_5

g_2

va_1

Dt_3; a_1 A_2 C_1 R

TB-7b (7L.30)

v_{8647}

yel_{7748}

Primary Trisomic 7

Chromosome 8

gl_g

v_{16} = v_{8661}

Chromosome 8 (Continued)

v_{16} j_1

v_{16} ms_8 j_1

nec_{6697} = sie_{7748} = nec_{025-4}

v_{16} ms_8 j_1 gl_g

TB-8a (8L.70)

Primary Trisomic 8

Chromosome 9

yg_2 C_1 sh_1 bz_1; A_1 A_2 R

yg_2 C_1 sh_1 bz_1 wx; A_1 A_2 R

yg_2 C_1 sh_1 bz_1 wx; A_1 A_2 R $gl_{(\ )}$

yg_2 $C_1{}^I$ sh_1 bz_1 wx; A_1 A_2 R

yg_2 C_1 sh_1 bz_1 wx K^L9; A_1 A_2 R

yg_2 C_1 bz_1 wx; A_1 A_2 R

yg_2 c_1 sh_1 bz_1 wx; A_1 A_2 R

yg_2 c_1 sh_1 bz_1 wx; A_1 A_2 R pr y_1

yg_2 c_1 sh_1 wx; A_1 A_2 R

yg_2 c_1 sh_1 wx gl_{15}; A_1 A_2 R

yg_2 c_1 sh_1 wx gl_{15} K^L9; A_1 A_2 R^g

yg_2 c_1 bz_1 wx; A_1 A_2 R

wd-Ring $C_1{}^I$; A_1 A_2 R

C_1 sh_1 bz_1; A_1 A_2 R

C_1 sh_1 bz_1 wx; A_1 A_2 R

C_1 sh_1 bz_1 wx gl_{15} bm_4; A_1 A_2 R

C_1 sh_1; A_1 A_2 R

C_1 sh_1 wx; A_1 A_2 R

Chromosome 9 (Continued)

C_1 wx ar; A_1 A_2 R

C_1^I sh_1 wx v_1; A_1 A_2 R

C_1 sh_1 wx K^L9; A_1 A_2 R

C_1 sh_1 ms_2; A_1 A_2 R

C_1 bz_1 wx; A_1 A_2 R

C_1 Ds wx; A_1 A_2 R y_1

C_1 Ds wx; A_1 A_2 R pr

C_1^I Ds wx; A_1 A_2 R

C_1^I; A_1 A_2 R

C_1; A_1 A_2 R

C_1; A_1 A_2 R B Pl

C_1 wx; A_1 A_2 R

C_1 wx; A_1 A_2 R B Pl

C_1 wx; A_1 A_2 R b Pl

C_1 wx; A_1 A_2 R B pl

C_1^I wx; A_1 A_2 R y_1

C_1^I wx; A_1 A_2 R y_1 B pl

C_1 wx ar da; A_1 A_2 R

C_1 wx v_1; A_1 A_2 R

C_1 wx v_1; A_1 A_2 R Pl

C_1 wx gl_{15}; A_1 A_2 R

C_1 wx gl_{15}; A_1 A_2 R pr

C_1 wx Bf_1; A_1 A_2 R

c_1 sh_1 bz_1 wx; A_1 A_2 R y_1

c_1 sh_1 bz_1 wx; A_1 A_2 R pr y_1

Chromosome 9 (Continued)

c_1 sh_1 wx; A_1 A_2 R

c_1 sh_1 wx v_1; A_1 A_2 R

c_1 sh_1 wx gl_{15}; A_1 A_2 R

c_1 sh_1 wx gl_{15} bk_2; A_1 A_2 R

c_1 sh_1 wx gl_{15} Bf_1; A_1 A_2 R

c_1 sh_1 wx bk_2; A_1 A_2 R

c_1; A_1 A_2 R

c_1; A_1 A_2 R B

c_1 wx; A_1 A_2 R y_1

c_1 wx; A_1 A_2 R Pl

c_1 wx v_1; A_1 A_2 R

c_1 wx gl_{15}; A_1 A_2 R

c_1 wx Bf_1; A_1 A_2 R

c_1 wx bk_2; A_1 A_2 R

sh_1 = sh_{6349} = $sh_{60\text{-}155}$ = $sh_{67\text{-}Vineyard}$

sh_1 bp_1 wx; P^{RR}

sh_1 bp_1 wx; P^{RW}

sh_1 wx d_3

sh_1 wx pg_{12} gl_{15}; y_1 pg_{11}

sh_1 wx v_1

bp wx; P^{RR}

bp wx; P^{RW}

bp wx; P^{WW}

lo_2

wx

Chromosome 9 (Continued)

wx^a

wx d_3

wx d_3 gl_{15}

wx d_3 l_6

Wx pg_{12}; y_1 pg_{11}

wx pg_{12}; y_1 pg_{11}

Wx pg_{12}; Y_1 pg_{11}

wx pg_{12}; Y_1 pg_{11}

wx v_1

wx bk_2

wx bk_2 bm_4

wx Bf_1

wx Bf_1 bm_4

$d_3 = d_{015-12} = d_{072-7} = d_{fg} = d_{8054} = d_{x-ray}$

$v_1 = v_{8587}$

gl_{15}

gl_{15} bm_4

bk_2 Wc

Wc

bm_4

l_6

l_6; l_1

l_7

l_7; l_1

Chromosome 9 (Continued)

w_{11}

yel_{034-16}

$pg_{8925} = pg_{12}$; pg_{11}

yg zb_{5588}

w_{4889}

w_{8889}

w_{8951}

w_{8950}

w nl_{034-5}

w_{9000}

TB-9a (9L.40)

TB-9b (9S.40)

Primary Trisomic 9

Chromosome 10

oy

oy bf_2

oy bf_2 R; A_1 A_2 C_1

oy bf_2 r; A_1 A_2 C_1

oy bf_2 ms_{10}

oy du R; A_1 A_2 C_1

oy du r; A_1 A_2 C_1

oy zn_1

Og

Og nl li g_1 R; A_1 A_2 C_1

Og du R; A_1 A_2 C_1

Chromosome 10 (Continued)

bf_2

bf_2 li g_1 r; A_1 A_2 C_1

bf_2 g_1 R sr_2; A_1 A_2 C_1

bf_2 g_1 r sr_2; A_1 A_2 C_1

nl g_1 R; A_1 A_2 C_1

y_9

li

li du g_1 R; A_1 A_2 C_1

li du g_1 r; A_1 A_2 C_1

li zn_1 g_1 r; A_1 A_2 C_1

li g_1 R; A_1 A_2 C_1

li g_1 r; A_1 A_2 C_1

li g_1 r v_{18}; A_1 A_2 C_1

du

du g_1 r; A_1 A_2 C_1

zn_1

zn_1 g_1

zn_1 g_1 r; A_1 A_2 C_1

zn_1 g_1 sr_2

Tp_2 g_1 r; A_1 A_2 C_1

Tp_2 g_1 R sr_2; A_1 A_2 C_1

g_1

g_1 R sr_2; A_1 A_2 C_1

g_1 r; A_1 A_2 C_1

g_1 r sr_2; A_1 A_2 C_1

Chromosome 10 (Continued)

g_1 r sr_2 l_1; A_1 A_2 C_1

g_1 R^g sr_2; A_1 A_2 C_1

g_1 R^g sr_2 v_{18}; A_1 A_2 C_1

g_1 r^g; A_1 A_2 C_1

g_1 R^g K10; A_1 A_2 C_1

g_1 R^r sr_2; A_1 A_2 C_1

g_1 R^r K10; A_1 A_2 C_1

g_1 r^r sr_2; A_1 A_2 C_1

E^j

E^j r^r; A_1 A_2 C_1

E^j r^r sr_2; A_1 A_2 C_1

r sr_2 l_1; A_1 A_2 C_1

R^g; A_1 A_2 C_1

r^g sr_2; A_1 A_2 C_1

r K10; A_1 A_2 C_1

r^g; A_1 A_2 C_1

r^r; A_1 A_2 C_1

R^{mb}; A_1 A_2 C_1

R^{nj}; A_1 A_2 C_1

R^r; A_1 A_2 C_1

R^r_{Boone}; A_1 A_2 C_1

R^{lsk}; A_1 A_2 C_1

$R^{sk\ mc.2}$; A_1 A_2 C_1

R^{sk}; A_1 A_2 C_1

R^{st}; A_1 A_2 C_1

Chromosome 10 Continued)

Lc

w_2

w_2 l_1

w_2 l_2

l_1

l_2

v_{18}

Mt

yel_{8962}

l_1 yel_{5344}

yel_{8721}

yel_{8454}

yel_{8793}

$w_{7748} = w_{8905}$

TB-10a (10L.35)

Primary Trisomic 10

Unplaced Genes

dv

dy

el

gl_{14}

h

l_3

l_4

Rs_1

Unplaced Genes (Continued)

v_{13}

ws_1 ws_2

ub

zb_1

zb_2

zb_3

zn_2

l_{4923}

"necrotic 8376" (seedling)

Multiple Gene Stocks

A_1 A_2 C_1 R^g Pr B Pl

A_1 A_2 C_1 R^g Pr B pl

A_1 A_2 C_1 r^g Pr B Pl

A_1 A_2 C_1 r^g Pr B pl

A_1 A_2 c_1 R^g Pr B pl

A_1 A_2 C_1 R^r Pr B Pl

A_1 A_2 C_1 R^r Pr B pl

A_1 A_2 C_1 R^r Pr b Pl

A_1 A_2 c_1 R^r Pr B Pl

A_1 A_2 C_1 r^r Pr B Pl

A_1 A_2 c_1 r^r Pr B Pl

A_1 A_2 C_1 R Pr

A_1 A_2 C_1 R Pr wx

A_1 A_2 C_1 R Pr wx gl_1

A_1 A_2 C_1 R Pr wx y_1

Multiple Gene Stocks (Continued)

A_1 A_2 C_1 R pr

A_1 A_2 C_1 R pr y_1 gl_1

A_1 A_2 C_1 R pr y_1 wx

A_1 A_2 C_1 R pr y_1 wx gl_1

A_1 A_2 c_1 R Pr y_1 wx

A_1 A_2 C_1 r Pr y_1 wx

a_1 su_1 A_2 C_1 R

bm_2 lg_1 a_1 su_1 pr y_1 gl_1 j_1 wx g_1

colored scutellum

lg_1 su_1 bm_2 y_1 gl_1 j_1

su_1 y_1 wx a_1 A_2 C_1 R^g pr

y_1 wx gl_1

hm_1 hm_2

ts_2; sk

Popcorns

Amber Pearl

Argentine

Black Beauty

Hulless

Ladyfinger

Ohio Yellow

Red

South American

Strawberry

Supergold

Popcorns (Continued)

Tom Thumb

White Rice

Exotics and Varieties

Black Mexican Sweet Corn (with B-chromosomes)

Black Mexican Sweet Corn (without B-chromosomes)

Knobless Tama Flint

Knobless Wilbur's Flint

Gaspe Flint

Gourdseed

Maiz chapolote

Papago Flour Corn

Parker's Flint

Tama Flint

Zapaluta chica

Tetraploid Stocks

P^{RR}

P^{VV}

Ch

B Pl

a_1 A_2 C_1 R Dt_1

su_1

pr; A_1 A_2 C_1 R

y_1

gl_1

Tetraploid Stocks (Continued)

ij

Y_1 sh_1 wx

sh_1 bz_1 wx

wx

g_1

A_1 A_2 C_1 R

A_1 A_2 C_1 R B Pl

Cytoplasmic Steriles and Restorers

WF9 - (T)	rf_1 rf_2
N6 (S)	
WF9	rf_1 rf_2
N6	rf_1 Rf_2
R213	Rf_1 rf_2
Ky21	Rf_1 Rf_2

These combinations are also available in other inbred backgrounds.

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VII. RECENT MAIZE PUBLICATIONS

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