MAIZE GENETICS COOPERATION

NEWS LETTER

48

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Department of Plant Sciences
Indiana University
Bloomington, Indiana

M. G. Neuffer
202 Curtis Hall
Columbia, Missouri 65201

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I. FOREWORD

The 1974 issue of the Maize Genetics Cooperation News Letter is the 48th volume of the series which began in 1932. The first of these News Letters consisted of a call for useful genetic stocks to be sent to Cornell, where they would be maintained and distributed, and of a summary statement of the informal meeting of maize geneticists held during the International Congress of Genetics at Ithaca. Subsequent News Letters at first appeared sporadically, but soon an annual issue was published regularly under the auspices of Cornell University. In 1955, Cornell expressed the desire to be relieved of the responsibility of publishing the News Letter and the maize geneticists gathered at the 1955 Meeting of the A.I.B.S. agreed to transfer the News Letter to the University of Illinois. The 1956, 1957, and 1958 volumes appeared from Illinois and subsequent volumes including the 1974 issue were sponsored by Indiana University as a consequence of my accepting a position there. Inasmuch as I retire formally this spring, Indiana will no longer publish the News Letter and next year's volume will appear under other auspices. As many of you know, the Maize Genetics Cooperation News Letter was the first of its kind. Its immediate success led Demerec to begin the Drosophila Information Service and, in following years, many other groups initiated News Letters. It is with some regret that I terminate my connection with the News Letter. I feel that it has played an important and significant role in furthering the progress of maize genetics. My involvement in the editing and arranging of the News Letter has in recent years been little more than serving in an advisory role. It is to Ellen Dempsey that all of us are indebted for her truly magnificent services to the News Letter. She and she alone in recent years has been responsible for all of the various tasks involved in publication.

Acknowledgment should be given to Marsha Altschuler, Marian Beremand, James Birchler and Margaret Walsh for their assistance in proof reading volume 48.

The cost of publishing the 1974 issue has been met from a grant by the National Science Foundation to the Maize Genetics Stock Center at the University of Illinois. This assistance is indispensable and we are indeed grateful to the N.S.F.

M. M. Rhoades

Announcement:

The first Annual Colloquium of the College of Biological Sciences, The Ohio State University will be held September 5-7, 1974. The title of the Colloquium is "Genetics and Biogenesis of Chloroplasts and Mitochondria." The program includes the following:

G. Attardi: Mitochondrial Biogenesis in HeLa Cells.
C. W. Birky, Jr.: Mitochondrial Genetics in Yeast and Ciliates
D. E. Griffiths: Utilization of Nuclear and Mitochondrial Mutations in the Analysis of Oxidative Phosphorylation
J. K. Hoober: Regulation of Chloroplast Membrane Synthesis
J. R. Laughnan: Cytoplasmic Pollen Sterility and Plant Breeding Applications
H. R. Mahler: Mitochondrial Biogenesis in Fungi
P. S. Perlman: Mutation Induction by Intercalating Dyes
R. Sager: Maternal Inheritance and Evolution of the Chloroplast Genome
R. A. E. Tilney-Bassett: The Genetics of Plastid Variegation
S. G. Wildman: Organelle Genes in Evolution

Information about registration may be obtained from C. W. Birky, Jr., Department of Genetics, College of Biological Sciences, Ohio State University, Columbus, Ohio 43210.

A note on "Low magnification examination of seeds" from G. W. Beadle:

I have found the Bausch and Lomb Illuminated Stand Magnifier, catalogue number 813480, useful and convenient in examining individual teosinte seeds for fruit-case mutations. It magnifies about 1.5 diameters, has an adjustable built-in light-source with a 7 watt, 110 volt bulb, and can be moved over a row of seeds at constant distance and illumination. Chicago retail price, $9.95.

II. OBITUARY

Walter August Huelsen, 1892-1973

Professor Huelsen was a faculty member in the Department of Horticulture at the University of Illinois from 1921 to 1960. He was born in Brooklyn, New York in 1892. His life from 1906 to 1926 sets a pattern for his professional career. After graduation from the Commercial High School of Brooklyn, he attended two night schools, one summer school, obtained a B.S. from Cornell University, and M.S. from the University of Illinois. The period was also interspersed with employment as a truck farm manager and plant breeder.

He was a member of Sigma Xi, Gamma Sigma Delta, and the American Society for Horticultural Science. He was a Fellow of the American Association for the Advancement of Science. He is credited with 55 publications that range in subject matter from fertilizer practices to breeding for disease resistance, to seed corn injury, to popcorn conditioning, and popping expansion. He was also the author of a reference book on sweet corn.

He introduced 38 cultivars - 22 sweet corns, 12 tomatoes, and 4 lima beans. These resulted in two All America awards. Four of his cultivars introduced between 1936 and 1951 are still in use, which is a high compliment in these days of changing commercial and consumer values. He also had an impact that is less discernible but equally important in the introduction of sweet corn inbreds carrying the genetic potential for producing multiple ears. His gene pool is still used as a source of breeding material.

A partial quote from a friend in the industry rounds out the picture of his professional career: "Professor Huelsen was one of the few early pioneer plant breeders who contributed to the improvement of varieties useful to the canning and freezing industries. He was dedicated, industrious, and cooperative, kept up with related literature, maintained contacts with management and plant breeders of both seed companies and processors. A great deal of credit is due him for the survival of the processing industry in Illinois."

Typically he spent part of the last day of his career at the U. of I. at the Vegetable Research Farm, and, if he had not been intercepted, would have missed his farewell party. He never returned to the campus.

He retired to Deerfield Beach, Florida in August, 1960. He maintained his contacts with industry as a consultant, but other interests dominated the picture. He joined the Kiwanis, expanded his collection of paintings, puttered in the garden, used his excellence in woodworking - a life-time hobby - to assist neighbors, and he repaid favors with a gift of a handmade lamp, fruit or flower bowl. At the time of his death, he had a cabinet full of candy - potential gifts for the neighborhood children.

Walter A. Huelsen, an excellent researcher with a philosophy that his job was not done until his efforts had an impact on the industry he felt he served, a man that could be hard as nails, soft as a pussywillow, or anything in between, died February 15, 1973.

C. Y. Arnold

IV. REPORTS FROM COOPERATORS

AGRICULTURAL RESEARCH INSTITUTE
Hungarian Academy of Sciences
Martonvásár, Hungary

1. Cold tolerance of opaque-2 versus normal maize with and without fungicidal seed treatment.

Analysis of various o_2 recessive homozygous genotypes for their ability to tolerate low temperatures at the time of germination in comparison with their normal analogues is underway. The cold tolerance of these genotypes is examined in the laboratory by means of the procedure developed at this institute by Herczegh (1970) described briefly below. Ten seeds in 5 replicates each are sown 5 cm deep in soil from a maize field in plastic boxes, watered equally and kept at 8°C in incubators for 10 days and at 14°C thereafter. The emergence is recorded daily till about the 32nd day. The percent emergence of a given genotype on a particular day is divided by the number of days since starting incubation and the quotient is termed the CT value. This was calculated for each genotype every day and only the maximum CT value observed for a given genotype is used in calculating the overall averages.

The CT values of these genotypes were also determined after treatment with Quinolate V4X, an experimental fungicide manufactured by Budapesti Vegyimüvek under a French license.

A brief summary of the results obtained on CT values of 21 o_2 genotypes and their normal analogues, with and without seed treatment with 0.2% Quinolate V4X, has been presented in Table 1. It will be seen that the o_2 recessive homozygotes show only 44 percent tolerance of low temperatures in comparison with their normal counterparts, indicating pleiotropic effects of this mutant gene. The CT value of the o_2 genotypes improved by 34 percent following treatment with Quinolate V4X, whereas the increase was only 8 percent in the case of the normal counterparts. However, the performance of the o_2 types, even after fungicidal treatment of the seeds, was only 55 percent of the normal, a far poorer response.

Table 1

Averages of cold tolerance (CT) values of 21 opaque-2 and normal genotypes treated with 0.2% Quinolate V4X and the control

Type	Control	Treated with Quinolate V4X	Treated as % of control
Opaque-2	1.38	1.85	134.02
Normal	3.11	3.36	108.22
Opaque-2 as % of normal	44.37	55.06	

The results presented above seem to demonstrate that the o_2 gene alters the seed anatomy in such a way that the physiology of the kernel is greatly influenced. This might be due in part to a greater thickness of the pericarp in o_2 recessive homozygotes as was demonstrated in our earlier studies (Gupta and Kovács, 1973).

References:

Gupta, D. and I. Kovács (1973) Pericarp thickness in opaque-2 maize (Zea mays L.) and its normal analogue. Acta Agronomica 22: 400-405.

Herczegh, M. (1970) Some problems of cold tolerance. In: Some methodological achievements of the Hungarian hybrid maize breeding, pp. 271-281 (Kovács, I. ed.), Akadémiai Kiadó, Budapest.

D. Gupta
I. Kovács

2. A study on some characteristics of the heterozygous opaque-2 sister line crosses.

All of the parents of the opaque-2 heterozygote sister line crosses included here are of American origin, except for the line 156, which was developed by Dr. E. Pap from "Mindszentpusztai," a yellow dent improved open pollinated variety. Heterosis has been expressed as "heterosis index," which is a ratio of the observed value of the sister line hybrid to the average of its two parents.

Observations on some of the characters of the related normal and opaque-2 inbred lines and their $+/o_2$ heterozygote sister line hybrids have

Table 1

Studies on some properties of related normal and opaque inbred lines and their heterozygous opaque sister line crosses (Martonvásár, 1973)

Pedigree	Days to 50% male flowering	Moisture content %	Dry grain yield per plant g	1000 grain weight g	
				Normal	Opaque
C153 x W153 R o_2	75.0	18.1	88.4	245.2	231.7
C153	79.7	17.5	68.5	230.0	-
W153 R o_2	78.6	20.9	76.1	-	234.7
Heterosis index	94.8	94.3	122.3	105.5	99.7
W64 A x WF9 o_2	82.1	29.6	134.7	230.3	242.0
W64 A	85.2	24.7	89.6	195.6	-
WF9 o_2	87.8	38.0	64.8	-	238.4
Heterosis index	94.9	94.4	174.5	106.1	111.5
C123 x C123 o_2	82.1	28.2	125.0	226.3	221.0
C123	84.2	25.5	86.2	224.7	-
C123 o_2	86.5	33.3	63.0	-	209.1
Heterosis index	96.2	95.9	167.6	104.3	101.9
WF9 x WF9 o_2	84.4	31.1	96.9	218.3	223.4
WF9	87.1	27.5	58.6	191.0	-
WF9 o_2	87.8	38.0	64.8	-	227.7
Heterosis index	96.5	95.0	157.0	104.2	106.7
B14 x B14 o_2	86.7	36.8	71.1	259.5	238.0
B14	89.4	36.8	57.8	270.5	-
B14 o_2	87.2	39.6	53.3	-	245.7
Heterosis index	98.2	96.3	128.0	100.5	86.6
156 x 156 o_2	77.2	23.8	84.0	182.4	164.8
156	80.4	21.8	69.6	189.5	-
156 o_2	81.0	28.7	67.0	-	162.7
Heterosis index	95.7	94.2	123.0	103.7	93.6
Mean heterosis index	96.0	95.0	145.4	104.0	100.0

been presented in Table 1. There is a remarkable heterosis for earliness in the heterozygous hybrids as measured both by the days to 50% male flowering (96 percent of the parents) and the moisture content at harvest (95 percent). Further, it can be seen in Table 1 that there is a favorable heterosis for dry grain yield, the mean heterosis index of the hybrids being 145.4 percent of the parental lines. In other words the heterozygous hybrids demonstrate an average dry grain yield of 1.45 times that of their related opaque-2 and normal parental lines. Likewise, the 1000 grain weights demonstrate a considerable heterosis, at least for the normal grains (104%) obtained on these heterozygous hybrids.

On the basis of Mendel's law of segregation, such opaque-2 heterozygous hybrids are expected to yield a mixture of opaque and normal grains. The opaque grains are expected in the following ratios in the types of crosses mentioned below.

Possible combinations	Ratio of opaque grains
(W64A x WF9) x N6 o_2	25%
(W64A x WF9 o_2) x N6 o_2	50%
(W64A x WF9 o_2) x (A632 o_2 x A636 o_2)	50%
(W64A x WF9 o_2) x Opaque variety	50%

Breeding of such heterozygous opaque-2 hybrids is, thus, suggested as an alternative breeding procedure for developing modified opaque-2 hybrids.

I. Kovács

3. Comparison of some properties of the heterozygous opaque-2 hybrids and their normal analogues.

We studied the yielding ability as well as the earliness properties (days to 50% male flowering time and the moisture content) of the commercial hybrids 156 x N6 and 156 x B14 and their heterozygous opaque analogues. The results are summarized in Table 1.

Forty plants from each hybrid were examined individually for maturity and dry grain yield. From the Table 1 it can be stated that the values of two earliness properties, days to 50% male flowering and the moisture content at harvest, do not lead to the same conclusions in comparisons of the normal and the heterozygous opaque analogues. Data on flowering time

Table 1

Comparison of the heterozygous opaque hybrids 156 x N6 o_2 and 156 x B14 o_2 and their normal analogues (Martonvásár, 1973)

Combinations	Days to 50% male flowering	Moisture content %	Shelling percentage	Dry grain yield per plant g	1000 grain weight g	
					Normal	Opaque
156 x N6	76.1	30.6	84.1	211.7	257.3	-
156 x N6 o_2	76.7	32.8	83.0	202.4	251.4	247.7
156 x B14	77.3	33.1	85.2	231.9	321.3	-
156 x B14 o_2	77.6	36.8	84.2	223.4	317.9	304.8
Ratio in percentage of the normal hybrids						
156 x N6	100.0	100.0	100.0	100.0	100.0	100.0
156 x N6 o_2	100.8	107.2	98.7	95.5	97.7	-
156 x B14	100.0	100.0	100.0	100.0	100.0	100.0
156 x B14 o_2	100.4	111.2	98.8	96.3	98.9	-

show little if any difference between the normal hybrids and the heterozygote opaque forms. However, the moisture content of the heterozygous opaque hybrids is higher by 2.2 and 2.7% than in the normal forms. These data prove that only the "days to 50 percent male flowering" is not usable as an index of the earliness of the hybrids.

It is remarkable that the yielding ability of the normal and the heterozygous opaque single crosses is practically the same. The difference in the case of 156 x N6 o_2 is 4.5 percent, while in the case of 156 x B14 o_2 it is 3.7 percent. These properties are supplemented by the expected increase in lysine content, by nearly 25 percent, due to 25% o_2 grains among the normals.

I. Kovács

4. Evaluation of heterozygous opaque hybrids of the Mv Syn A-3 O_2 sublines.

The Syn A-3 opaque-2 inbred line was developed from the open pollinated variety, Illinois Synthetic A o_2. The sublines of Syn A-3, which are related to each other, were selected from their large population and crossed with the normal unrelated tester N6. The heterozygous opaque single hybrids of different Syn A-3 sublines were planted in comparative yield trials in two replications, with 40 plants per plot in 80 x 30 cms spacing. In the experiment with heterozygous opaque hybrids we used two standards. One of them was the Mv Syn A x Syn B opaque varietal hybrid and the other was the normal hybrid of 156 x B14.

Data on the most important properties of heterozygous opaque single crosses are summarized in Table 1. The data show considerable differences both in the moisture content at harvest and yielding ability, as well as the 1000 grain weight of the tested hybrids. The moisture content at harvest (earliness) varied from 29.4 to 33.1 percent showing a 3.7% difference. Similarly remarkable is the finding that the mean value of the moisture content in the heterozygous opaque hybrids in less by 15.6 percent than the same value in the opaque varietal hybrid and is 5 percent less than in the normal standard single cross.

The greatest differences were found in the yielding ability of the Syn A-3 o_2 sublines. The lowest yield was 92.1 percent of the opaque

Table 1

Yielding ability of the heterozygous opaque hybrids produced on N6 testers by sublines of Syn A-3 o_2 (Martonvásár, 1973)

Combination	Moisture content %	Dry grain yield		1000 grain weight		Yield %	
		kg/plot	q/ha	g Normal	g Opaque	Mv Syn A o_2 x Syn B o_2	156 x B 14
Syn A-3-4 o_2 x N6	29.4	12.8	66.7	270.2	259.7	101.6	88.9
Syn A-3-6 o_2 x N6	29.8	11.6	60.4	234.8	216.9	92.1	80.6
Syn A-3-11 o_2 x N6	30.0	13.4	69.8	244.3	223.5	106.3	93.0
Syn A-3-18 o_2 x N6	30.1	11.8	61.4	237.4	222.2	93.6	81.9
Syn A-3-24 o_2 x N6	31.1	13.2	68.7	265.8	248.7	104.8	91.7
Syn A-3-28 o_2 x N6	29.4	13.2	68.7	276.7	256.8	104.8	91.7
Mv Syn A o_2 x Syn B o_2	35.5	12.6	65.6	-	225.6	100.0	87.5
156 x B 14	32.2	14.4	75.0	324.8	-	114.3	100.0
Syn A-3-29 o_2 x N6	30.9	14.8	77.1	244.7	229.5	117.5	102.8
Syn A-3-30 o_2 x N6	32.9	14.2	74.0	277.9	258.1	112.7	98.6
Syn A-3-36 o_2 x N6	30.4	15.7	81.8	284.5	267.8	124.6	109.0
Syn A-3-38 o_2 x N6	33.1	13.0	67.7	242.3	223.3	103.2	90.3
Syn A-3-43 o_2 x N6	30.6	12.7	66.1	251.1	236.3	100.8	88.2
Mean	30.7	13.3	69.3	257.2	240.2	105.6	92.4
L.S.D. 5 per cent level		1.5	7.8				

standard hybrid, the highest 124.6 percent, indicating a 32.5% difference. The same value in the case of normal standard is 28.4 percent. It is remarkable that the yielding ability of some heterozygous opaque hybrids reaches or approaches the normal standard. At the same time, Table 1 shows significant differences in specific combining ability of the hybrids of Syn A-3 o_2 sublines. In this respect, the hybrids of Syn A-3-36 o_2, Syn A-3-29 o_2 and Syn A-3-30 o_2 seem to be promising. These properties are supplemented by the expected increase in lysine content by nearly 25 percent.

M. Herczegh

BHABHA ATOMIC RESEARCH CENTRE
Bombay, India
Biology and Agriculture Division

1. Hollow endosperm in maize.

Hollow endosperm, an allele of sh_1, was isolated from a single kernel on the cob where ACRShWx (coloured, non shrunken and non-waxy) plants from EMS-treated seeds were crossed with csh_1 wx pollen.

The kernel was colorless, non-shrunken and had a hollow cavity in the center. The phenotypic expression of the changed kernel indicated a possible loss of an intrachromosomal segment including the C and Sh_1 loci (2.8 map units apart). The mutant on selfing gave fifty hollow, seventy intermediate and 132 shrunken type seeds. The waxy and non-waxy were equal in number. When further selfed (Table 1) and reciprocally crossed to the pollen parent (Table 2), plants from hollow seeds showed only 20 to 30% hollow kernels. The hollow mutant, as well as the linked marker Wx, failed to be transmitted through the pollen. The Wx gene showed equal segregation in the hollow cob with slightly significant deviation. This suggests that there was a loss of a small chromosomal segment including C and a portion distal to it, which prevented functioning of gametes through the pollen. The hollowness indicated a possible mutational event at the Sh_1 locus. Prolonged selfing of plants from

Table 2

Reciprocal cross of hollow, non-waxy and c sh_1 wx plants

S. No.	Cross	Kernel type				♀ csh wx x hollow ♂ Kernel type	
		non-waxy			waxy	non-waxy	waxy
		Hollow	Inter-mediate	Shrunken	Shrunken	Shrunken	Shrunken
1	♀ Hollow non-waxy x ♂ csh wx	52	16	20	108	32	268
2	"	64	28	21	112	36	284
3	"	48	20	12	94	48	256
4	"	36	31	30	98	44	296
5	"	42	19	10	123	62	416
6	"	69	32	40	115	41	291
7	"	61	20	19	136	31	241
8	"	49	41	29	126	26	216
9	"	65	32	24	136	24	262
10	"	41	29	32	148	18	196
11	"	55	34	46	98	36	284
12	"	23	26	32	114	39	301
13	"	41	39	23	88	28	205
14	"	49	28	46	139	54	324
Total		695	395	384	1635	531	3741
Percent expression		22.35	12.70	12.35	52.60	12.42	87.58

Table 1

Kernel types produced by selfed individuals of the hollow kernel progeny

S.No.	Kernel type selfed	Kernel type			
		Non-waxy			waxy
		Hollow	Inter-mediate	Shrunken	Shrunken
1	Hollow, non-waxy	50	35	40	125
2	"	42	26	20	105
3	"	62	27	21	131
4	"	41	21	20	68
5	"	34	22	24	94
6	"	50	32	38	110
7	"	56	24	23	105
8	"	36	24	30	112
9	"	74	10	21	98
10	"	44	16	28	108
11	Intermediate types	34	32	22	99
12	"	84	26	20	124
13	"	24	20	26	92
14	" (6 cobs)	-	-	1184	1164
15	Shrunken (10 cobs)	-	-	1546	1464

hollow kernels resulted in a few cobs with a higher percent of hollowness. The test for the Sh_1 protein was negative, resembling the results found with the recessive $\underline{sh_1}$ (P.S. Chourey).

Chandramouli

2. Jointed seed syndrome in maize.

Jointed seeds, which spontaneously occur as a freak in maize, were not commonly inherited. A high frequency of jointed seeds, 2.5 per cent, was observed on a cob obtained from ethyl methanesulfonate treated seeds. On further analysis, by selfing the plants, it was found that 45 plants had jointed seeds ranging from 0.3 to 5.4 per cent and in 21 plants it was completely absent (Table 3). It seems that the character was inherited in

Table 3

Frequency of jointed seeds from selfed plants

S.No.	Number of plants	Seed type		Range-percent jointed
		Normal	Jointed	
1	2	685	42	above 5
2	3	362	17	4 - 5
3	16	4012	160	3 - 4
4	9	1800	41	8 - 3
5	10	2365	35	1 - 2
6	5	1689	10	below 1
7	21	6257	Nil	Nil

simple Mendelian manner plant-wise and its expression was quite limited. The induced change suggests a possible dominant expression and a maternally inherited one. The orientation of the two embryos in the jointed seeds was either opposite, alternate or adjacent and sometimes one of the embryos was in between the seeds. It is inferred from the cross that the seeds of jointed types arose from two independent pollen grains. The nature of the formation of these kernels is not yet clear and it is assumed that they are produced from the sterile flower of the spikelets, which becomes fertile and fuses to the normal seed.

Chandramouli

3. Induced dominant mutant Ce (curled-entangled).

Previously (MNL 47:17) it was reported that the pattern of segregation of the induced dominant mutant, curled-entangled, was abnormally deviating from a simple Mendelian ratio in selfed progenies. When selfed progenies were grown under controlled conditions in a sand flat (Table 4), the segregation was clearly three dominant and one normal type. The significant deviation was due to the homozygous and some heterozygous mutant plants which were severely entangled during the germination period and were incapable of emerging in field soil as against sand.

Table 4

Segregation of mutant plants in progenies derived from crosses of Ce/N heterozygotes

Pedigree No.	Genotype	No. seed grown	Plant type			
			Normal	Mutant		
				Possible homozygous		Heterozygous
				Severely entangled	Not severely entangled	
Co 72-41	[Ce x N] ⊗	148	36	31	1	80
-42	"	70	16	3	7	44
-44	"	125	30	20	20	50
-47	"	129	27	16	6	68
-48	"	79	18	11	5	38
-49	"	164	43	18	11	88
-59	"	176	44	28	6	96
-51/46	Sib	139	40	28	0	70
-51/43	"	154	34	26	8	86
-58/52	"	101	22	28	0	70

BOSTON COLLEGE
Chestnut Hill, Massachusetts
Department of Biology

1. Additional studies of the microsporocytes of Chalco (Mexico) teosintes.

Microsporocytes of five more Chalco teosintes were studied with the light microscope. Slides were prepared by following the standard aceto-carmine squash technique. These teosintes were grown from open-pollinated seeds. It was observed that the pachytene chromosomes were generally well spread. Individual chromosomes, as well as their knobs and other gross characteristics, could be readily identified. A total of eight knobs was found. All knobs were homozygous, except for the terminal knob on the short arm of chromosome 9. A small internal knob was present on the long arm of chromosome 1, medium-sized internal knobs were found on the long arms of chromosomes 2, 3, 4 and 6 and large internal knobs were observed on the long arms of chromosomes 7 and 8. Chromosomes 5 and 10 were knobless.

These teosintes differ from the Chalco teosintes previously studied by the author (1964) in having five fewer knobs. The internal knob positions on the long arms of chromosomes 2, 4, 5 and the first knob position on the long arm of chromosome 6 were not occupied in these teosintes. The small terminal knob on the short arm of chromosome 4 and the second type of chromosome 10 were not found.

Even though several hundred sporocytes were carefully examined, no In8 or any other structural alteration were observed. This finding is believed to confirm the report by the author (1964) that In8 is homozygous in Chalco teosintes. Homozygous inversions do not form any visibly abnormal configurations at the pachytene stage.

At diakinesis, 32% of 164 sporocytes studied were found to have nine bivalents and two univalents; 65%, 10 bivalents; 3%, eight bivalents and four univalents. The two univalents present in the first class of sporocytes are probably homologues of chromosome 9. The heterozygous terminal knob of this chromosome might interfere with chiasma formation.

Microsporocytes from two of the above teosintes were also examined with the electron microscope following the standard sectioning and staining procedures. A synaptonemal complex was consistently observed at the

pachytene stage. The three components of this complex, two lateral elements and one central element, were well differentiated. No complex was found to be attached to the nuclear envelope. The average diameter of the complex was 2200 Å. Both knobs and centromeres of the different chromosomes could also be recognized.

In addition to the normal nucleolus, one to several nucleolar bodies were frequently present. One of the nucleolar bodies was found to have several vacuoles arranged in an orderly form. Details of these studies will be reported later.

Y. C. Ting

2. Fine structure of the nucleolus of a diploid maize.

Under the light microscope a cup-shaped structure could be identified at the pachytene stage in the nucleolar organizer region of the microsporocytes of a diploid inbred maize (Strain A158). This structure persisted through diakinesis, even though its shape and size might vary somewhat. In addition, nucleolar bodies, ranging from one to four, were also frequently found. Their diameter was, on the average, five microns. With standard procedures of electron microscopy, the cup-shaped region of the nucleolus was seen to be comprised of fibers in a spiral arrangement. These fibers measured approximately 400 Å in diameter. They were as darkly stained as the rest of the nucleolus throughout the prophase of meiosis. No membrane-like structure enclosing either the nucleolus or the nucleolar bodies was observed. However, both organelles consistently showed a vacuole or vacuoles in the middle region. The vacuoles appeared to be free from any inclusions.

Y. C. Ting

3. Preliminary studies of normal and male sterile cytoplasm in maize.

Root tips from WF9T male sterile maize and its maintainer (WF9) were prepared for electron microscopic observation. Inclusions were not usually present in male sterile cells, although some inclusions were noticed in the cells of the future vascular cylinder. These inclusions, which have not been seen in the maintainer cells, appear to be membrane-bound and often contained three or four dark staining granules. The

average diameter of these inclusions is 6500 Å. In addition, it was noted that the cells of the male sterile root tips contain fewer chloroplasts than those of the maintainer. Studies of the inclusions and of the decreased number of chloroplasts will be continued using various lines of T-type cytoplasmic male sterile maize.

Rita Ryan

4. Effect of streptomycin on chlorophyll content in maize seedlings.

Currently an attempt is being made to induce cytoplasmic sterility in maize. Seeds (C103N) were immersed in 25 ml of streptomycin solution (Petrov, Fokina, and Zheleznova, U.S. Pat. #3,594,152) at 24°C. using the following doses: .0005, .001, .005, .01, .05, .1, .5, 1, 5, 10, 50, 100, 500, 750, 1000, 2500, 5000, and 10,000 micrograms/milliliter. At the same time, control seeds were soaked in distilled water.

The seeds were then placed in pots and allowed to germinate. Marked variations were noted when comparing the treated seedlings with the controls. Some seedlings that had been treated with 50 ug/ml (.005%) were albino and those which had been treated with higher doses either had completely white leaves or showed a variegated pattern of green stripes on white leaves. This demonstrates an effect of streptomycin on the chlorophyll content at lower doses than was expected.

Differences in the growth of the plants were also noted with an enhancement of growth occurring with doses between .1 and 1 ug/ml and with a retardation of growth occurring in seedlings treated with higher doses.

Although the albino plants are not expected to survive, all available plants will be checked later for pollen production. It is hoped that streptomycin will have had a more subtle effect at the lower doses and that this effect will result in cytoplasmic sterility.

Rita Ryan

5. G bands in haploid maize.

Haploid seeds were germinated and actively growing root tips were excised and pretreated in .05% colchicine for four hours. The root tips were then fixed in aceto-alcohol fixative for 18-24 hours and stored in 70% ethanol at 0°C until use. A modification of Sumner's technique for human chromosomes was applied. When the prometaphase and metaphase chromosomes were studied, it was found that the long arm of chromosome 7, the terminal region of the short arm of chromosome 9 and the short arm of chromosome 6 had strikingly prominent bands. In addition, less intense bands were visible along the lengths of different chromosomes. Chromosome 1 had three bands, one of which appeared at the proximal end of the long arm and the others in the middle of each of the two arms. Chromosome 2 had bands on both sides of the centromere. The preparation of a complete karyotype is currently in progress.

Parallel with this treatment, the aceto-carmine squash technique was also employed. The darkly staining G bands on chromosomes 7 and 9 were found to occupy regular knob positions. The G band on the short arm of chromosome 6 was in the nucleolar organizer region. The other G bands were not shown by this technique.

Since the Giemsa technique brings out more bands than the common aceto-carmine squash technique, new knowledge of the relationship among different varieties of maize may be gained as more studies are carried out.

Lorraine Sartori

UNIVERSITA' CATTOLICA DEL S.CUORE
Piacenza, Italy
Istituto di Botanica e Genetica vegetale

1. Collapsed endosperm-1 (cp_1) location.

An endosperm mutant linked to gl_1 was previously described under the symbol cl (M.G.C.N.L. 40: 77-78, 1966). In a further report (M.G.C.N.L. 44: 93, 1970) the mutant symbol has been modified from cl

to cp_2. Since Neuffer et al. (The Mutants of Maize, Crop Sci. Soc. Amer., 1968) reported this mutant as cp_1, this last symbol will be hereafter maintained.

1972 backcross data obtained from 16,715 kernels show a 8.0 ± 0.01% recombination between o_2 and cp_1. On the basis of the 1962, 1964 and 1972 data, the recombination values and the order of the chromosome 7 markers o_2, gl_1 and cp_1 should be as follows:

$$o_2 \quad (8.8\%) \quad cp_1 \quad (8.7\%) \quad gl_1$$

$$o_2 \qquad\qquad (14.9\%) \qquad gl_1$$

C. Lorenzoni
M. Pozzi
F. Salamini

CORNELL UNIVERSITY
Ithaca, New York
Department of Plant Breeding and Biometry

1. Evaluations of sources of cytoplasmic male sterility for use in multiplasm hybrid production.

A series of corn inbreds adapted to the Northeastern United States has been crossed onto 39 sources of cytoplasmic male sterility and backcrossed during 3 generations/year for the last three years. The backcross conversions have reached the 8th backcross generation. A list of cytoplasms that were fully male sterile in each inbred background in trials performed in New York in the summer of 1972 and in Florida in the winter of 1972-1973 is presented in Table 1. All of the cytoplasms listed are resistant to Helminthosporium maydis, race T, and Phyllosticta maydis leaf blights. Some of these cytoplasms are currently being incorporated into multiplasm hybrids. A multiplasm version of the single cross hybrid Cornell 101 has been produced using various combinations of male sterile cytoplasms. A limited amount of seed of the cytoplasmic sources is available for distribution.

In addition to the fully male sterile cytoplasms, several cytoplasmic sources form partially male sterile combinations with inbred lines

Table 1

List of *Helminthosporium maydis*, race T resistant male sterile cytoplasms for 24 inbred lines*

Inbred	Male sterile cytoplasms
A239	C,CA,D,EK,J,K,ME,ML,MY,PS,SD,TA,TC
A619	EP
A632	G,M,RB
A636	RB
AyX138	EK,H,M,SC,SD
AyX145A	CA,H,I,J,L,ME,ML,MY,PS,R,TA,TC,W
AyX157	C,CA,EK,ES,G,H,I,IA,K,ME,RB,SC,SD
AyX187y-1	C,CA,K,RB
Ay49w329	CA,EK,G,H,I,IA,J,K,ME,ML,PS,SD
Ay191-71	B,CA,EK,G,H,I,J,MY,PS, R,TA,W
Ay490-2A	C,CA,EK,G,H,IA,K,M,PS,RB,SD
CO150	B,EK,G,H,I,IA,K,L,ML,MY,PS,TA,VG,W
CrS4HLA	CA,EK,ES,F,G,H,I,IA,J,K,M,ME,PR,PS,R,S,TA,TC,VG
MS89A	CA,EK,G,H,I,J,M,ME,MY,PS,S
MS1334	CA,EK,G,H,I,IA,J,K,ML,PS,TC
MYD410	C,CA,D,G,H,I,IA,K,L,ME,ML,MY,PS,RB,SD,TC
NY63-71-1	D,EK,G,H,I,IA,K,ML,MY,PS,RB,TC
MY821	EK,F,G,I,IA,J,ME,ML,MY,PS,R,S,SD,TC,W
Oh51A	C,RB
Pa884p	CA,G,H,IA,K,M,R
SD10	CA,EK,MY,PS,RB
Va20	C,RB
W64A	J,K,PS,TA
W182BN	B,C,CA,D,EK,ES,F,G,H,I,IA,J,K,L,M,ML,PS,R,RB,S,SC,SD,TA,TC, VG,W

*All cytoplasms gave a rating of 1 or 2 on the 1-5 scale for sterility. (1 = most sterile).

(Table 2). The partially fertile plants shed less pollen and often pollen shedding is delayed after tassel emergence. Although these partially sterile cytoplasms may not be suitable for use as male steriles, they are attractive since they can be detasselled more effectively. The delay in pollen shedding makes it easier to detassel female plants before they shed pollen and the partial male sterility results in less pollen being shed by tassels that are missed.

Table 2

List of Helminthosporium maydis, race T resistant cytoplasms that are partially male sterile in inbred combinations*

Inbred	Partially male sterile cytoplasms
A239	S
A495	F,G,H,IA,TC,VG,W
A632	EK,CA,I,IA,J,K,ME,ML,MY,TC
A636	CA,IA,J,K,M,ME,ML,MY,PS,SD,TA,TC
AyX145A	D,EK
AyX187y-2	B,CA,D,M
Ay303E	CA,EK,G,I,K,RB,VG
B8	C,CA,G,H,I,J,M,ML,S,TA,TC,VG,W
C153	EK,F,G,I,K,M,PS,R,S,SD,TA,TC,VG,W
CO113	EK,H,I
CO192	CA,EK,F,G,H,I,IA,J,K,M,R,S,SD,TC
CrS4HLA	D,MY
MS89A	D,L
MS1334	MY
Oh43	CA,G,I,M,ME,PS,RB
Oh51A	EK,G,H,J
Pa33	CA,F,I
Pa884p	EK
Va20	EK

*All cytoplasms gave a rating of 3 on the 1-5 scale for sterility (1 = most sterile).

Our studies of fertility restoration of the male sterile cytoplasms demonstrate the 3 groups (C, S & T) of cytoplasms described by Dr. Jack Beckett at the University of Illinois. In addition, we have identified several cytoplasms that don't fit into any of the groups (Table 3). We have also detected a diversity of fertility reactions within the S group. Certain cytoplasmic sources previously included in this group give the opposite fertility reactions of other members of the group in certain inbred backgrounds (Table 3). Additional groups or subgroups could be developed if partially fertile reactions are considered. Further studies of the genetic and physiological diversity of these cytoplasmic sources of male sterility are in progress.

Table 3

Groups of male sterile cytoplasmic sources with similar fertility restoration reactions

C group:	C,RB
T group:	HA,P,Q,RS,T
S group:	F,H,I,IA,J,MY,R,SD,VG,W
	- cytoplasms similar to S group but which give differential reactions in some inbred backgrounds:
	CA,EK,G,K,L,M,ME,ML,PS,TA
Other male sterile cytoplasms:	B,CH,D,EP,LF
Probably non-male sterile cytoplasms:	NT,OY,SG,181,234

V. E. Gracen
C. O. Grogan

2. Heat induced autotetraploids of maize.

A series of maize inbreds homozygous for endosperm mutants were exposed to heat and cold shock in an attempt to duplicate their basic chromosome complement. Crosses with known tetraploid stocks indicate the treatment was successful.

Inbreds W64A $\underline{fl_2fl_2}$ and W153R $\underline{fl_2fl_2}$ were exposed to 42°C for 30 minutes, 48 hours after pollination. The heat shock was followed by

packing the treated ears in ice for 15 minutes. The offspring from the treated ears were grown out, selfed, and crossed to Syn B. Several of the cross pollinations were successful. These tetraploid stocks are being expanded.

M. J. Forster

CORNNUTS, INCORPORATED
Salinas, California
Plant Breeding Department

1. Cryptic earliness in maize.

To produce an early maturing hybrid, one commonly uses at least one very early inbred as a parent in the cross. The degree of difference between maturities of parental inbreds is often limited by the ability of the breeder to provide for adequate "nick" of anthesis and silk extrusion. The concept of using "cryptically early" germplasm to bring earliness to the progeny of a cross was proposed in the 1972 Maize Genetics Cooperation News Letter. The term "cryptic earliness" describes a latent genetic potential for earliness which is masked by the id/id genotype. An id/id plant without effective earliness genes is vegetatively indeterminant and barren, but with proper selection of earliness and fertility genes in the id/id background, adequate fertility has been obtained in the inbred line B37 id/id. B37 id/id is taller and later than B37 +/+. However, the cross of B37 id/id x A619 is shorter and earlier than B37 +/+ x A619. The data presented here demonstrate the effect of the id gene in masking extreme genetic earliness and associated agronomic characters so that those genes for earliness can be utilized without the usual wide divergence in maturity of the parental inbreds. Earliness, shortened plants, and lowered ears are unmasked in heterozygous +/id offspring. Any effect of cryptic earliness conversion on yield remains to be established in replicated trials, but the effect on maturity and ear and plant height appears evident in this pilot study. Cryptically early inbreds may prove useful in producing hybrids in a location with longer growing season than the area of intended

Table 1

Agronomic data on inbreds and hybrids illustrating the effects of the id/id genotype

Entry	No. of nodes		Ear node		Ear height		Plant height		Days to silk		Yield		K. row no.		Ear length	
	$\bar{x}$*	s*	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
A619	10.1	0.57	4.2	.63	8.6	1.90	49.7	2.11	86.8	1.32						
B37 +/+	13.0	0.67	6.2	.42	19.3	2.50	61.9	3.54	93.3	1.49						
B37 id/id	17.0	0.94	9.0	1.15	37.8	6.46	97.7	6.50	115.4	2.59						
B37 +/+ x A619	13.1	0.57	5.8	0.42	27.4	3.53	75.2	4.13	95.0	1.25	90.7	17.79	14.6	1.35	5.21	0.79
B37 id/id x A619	10.7	0.67	3.5	0.53	14.5	1.96	66.9	4.77	78.8	1.62	86.4	22.71	16.2	1.14	4.91	0.97

*Mean and standard deviation statistics are based on one replication, ten plants per entry.

production, or for the incorporation of earliness genes by standard backcrossing into very late maturing inbreds without delayed planting of the early parent.

D. L. Shaver
C. L. Prior

2. Defective cytoplasm in Zea.

In 1956 an experiment was begun to insert the Wf9 nucleus into a number of exotic cytoplasms, among these, that of perennial teosinte, Zea perennis. Two isolates of perennial teosinte cytoplasm were made. In the 1966 winter nursery at Homestead, Fla. one of these was found to cause defectiveness in plant phenotype: male sterility, chorophyll striping, reduced germination and plant size. This was discovered in the backcross-7 progeny of the transfer process.

Upon reaching the backcross-8 level of recovery of Wf9 with teosinte cytoplasms, each of three Wf9 cytoplasmic types were inserted into two different single cross hybrids in order to evaluate the effects of the defective cytoplasm upon hybrid performance, and these were tested in a 4-replication yield trial at Greenfield, Calif. in 1968.

Entry	Hybrid	Type of cytoplasm	Acre yield	No. days to ½ silk	Inches above ground ear height
1	Wf9xC103	Maize	14,221	102.2	51.2
2	Wf9xC103	Normal teosinte	14,084	101.2	51.0
3	Wf9xC103	Defective teosinte	11,377	104.2	49.0
4	Wf9x644	Maize	14,389	103.2	51.5
5	Wf9x644	Normal teosinte	14,649	103.8	51.0
6	Wf9x644	Defective teosinte	11,432	104.2	46.5

Analysis of variance

Source	D/F	SS	MS	F	5%	1%
Total	23	19.0687				
Hybrids	5	13.7151	2.7430	14.892**	2.90	4.56
Reps	3	2.5904	.8635	4.688*	3.29	5.42
Error	15	2.7632	.1842			

Entry 1 vs. entry 2 = .02
Entry 1 vs. entry 3 = 1.53**
Entry 2 vs. entry 3 = 1.51**
Entry 4 vs. entry 5 = .14
Entry 4 vs. entry 6 = 1.40**
Entry 5 vs. entry 6 = 1.74**

Probability	T	σ MD
5%	2.13	.646
1%	2.95	.895

In both types of single cross hybrids the defective cytoplasm isolate markedly depressed yield in comparison with the same hybrid made with either of the other two cytoplasm types, but hybrids having maize and normal teosinte cytoplasm did not differ from each other.

Since both isolates of perennial teosinte cytoplasm were made from the same original classical clone of teosinte, E16515, it seems apparent that a cyto mutation must have occurred during the process of transferring the Wf9 nucleus, but examination of remnant seeds and field records gives no further information.

Further work is in progress to insert the B37 nucleus into the two teosinte cytoplasm types, and this work has reached the BC-3 level of recovery. Since recoveries of B37 having the defective cytoplasm show essentially the same phenotype noted in Wf9, it is evident that the defective character of the cytoplasm is not restored by association with B37 nuclear factors. However, some differences are evident in the two backgrounds: male sterility and nearly all the chlorophyll striping are relieved in the B37 material. The defective cytoplasm described here differs from the classical S cytoplasm in its drastic reduction of plant size in all environments, and also in its production of partial kernel abortion.

D. L. Shaver

3. Inheritance of resistance to Sugar Cane Mosaic Virus (SCMV) in Calif.

SCMV is a serious threat to corn production in the Great Central Valley. In parts, resistance is a simple necessity. Johnson Grass is the principal alternate host, from which it is transferred to corn by the Green Peach Aphid. It is also easily transferred by mechanical inoculation.

In breeding for resistance, it is found to be fairly easy to develop multigenically resistant strains as derivatives from parents having tolerance and/or partial resistance. Other strains of corn seem to have a single dominant gene type of resistance. An experiment was undertaken to establish data from which to test the single gene idea, and to determine if allelism exists between the presumed single gene types.

On the basis of breeding experience, two susceptible, and four single gene resistant lines were selected:

Susceptible inbreds		Resistant inbreds	
01	02	11	33
		22	44

The following F_2 and backcross data were obtained in the 1973 disease nursery at Tulare:

Progeny	Resistant	Susceptible	P (Based on single gene idea)
01 x 44 F_2	357	270	.001**
11 x 22 F_2	674	6	
11 x 33 F_2	683	3	
11 x 44 F_2	470	11	
22 x 33 F_2	510	13	
22 x 44 F_2	463	7	
33 x 44 F_2	666	20	
(01 x 02)01	0	322	
(01 x 02)02	1	381	
(11 x 01)01	122	141	.300
(22 x 01)01	201	210	.200
(33 x 01)01	177	191	.500
(44 x 01)01	165	190	.200
(11 x 02)02	265	275	.999
(22 x 02)02	219	192	.200
(33 x 02)02	231	242	.200
(44 x 02)02	175	212	.100

There is a tendency towards an excess of susceptible plants which tends to make the P values seem too high, and made one very high indeed. But it should be explained that many of the plants scored as susceptible were in reality only slightly infected. "Resistant" plants, especially when heterozygous at the resistance locus, often show mosaic symptoms at times, but they still have essential resistance and usually recover later. The heterozygosity factor is more than sufficient to account for all the low P values reported here.

F_2 data indicate that all four resistant parents have allelic genes for resistance.

Added evidence for the single gene idea comes from numerous projects in which resistance is routinely transferred to susceptible lines. In extended, continuous backcrossing, the resistance factor continues to segregate as a unitary, essentially dominant gene. However, since, for example, resistance deriving from inbred 11 is clearly more potent during the advanced backcross generations than that from the other three sources, it seems clear that there is an allelic series at the resistance locus.

In recovering a few lines with resistance, the potency of the added gene seems to fade away upon reaching about BC_4 or BC_5. This is thought to be due to the fact that these exceptional lines lack complementary genes necessary for the expression of resistance. Added evidence for this explanation comes from the fact that inbred 44, while it has been found to be a source of excellent single gene resistance, is uniformly susceptible in its original state.

The dependence of the expression of the SCMV gene upon complementary genes is exactly parallel to the dependence of the $\underline{Rf}_1$ - $\underline{Rf}_2$ restoration system upon complementary loci, first noted by Shaver (MNL 30:160, 1956). It is again interesting to note that the inheritance of resistance to SCMV can appear perfectly simple, or relatively complex, depending entirely upon the type of material being observed. We propose the designation, Scm for the SCMV resistance locus.

D. L. Shaver

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH
PLANT PHYSIOLOGY DIVISION
Palmerston North, New Zealand

1. Maize genetics studies by protoplast fusion.

Use has been made of several lines of the well-documented chloroplast mutations and nuclear controlled chloroplast deficiencies of maize in studies to demonstrate genetic complementation by protoplast fusion. Because of the difficulty of initiating mitosis in fused protoplast heterokaryons, it was hoped that the appearance of chlorophyll

in chlorotic mutants could be used as a marker for complementation. Plants homozygous for the recessive iojap (ij ij) gene are striped and yield three types of protoplast upon treatment with 1% cellulase: those containing normal green chloroplasts, those with only mutant white ones, and those with a mixture of normal and mutant plastids. White deficient plants, homozygous for a terminal deletion of chromosome 9, (wd wd), are devoid of both carotenoids and chlorophyll and hence germinate as albinos. Fusion of green iojap protoplasts with white deficient protoplasts caused the formation of small light green plastids in the white deficient cytoplasm, as did the fusion of iojap protoplasts with a mixed plastid population to a wd wd protoplast. The fusion of green iojap protoplasts with white iojap protoplasts failed to induce chlorophyll synthesis in the white plastids. This is probably due to the fact that the double recessive ij ij induces white stripes by causing a high frequency of irreversible plastid mutations and the occurrence of cells with mixed plastid populations suggests that complementation in such a case is impossible. Fusion of white iojap and white deficient protoplasts also failed to bring about greening, and suggests that some chloroplast factor, only present in normal green plastids, is necessary for the complementation, as well as nuclear material.

Because of the possible diffusion of chlorophyll that might have occurred in the case of a fusion involving protoplasts with green plastids, similar experiments have been performed using protoplasts from the seedlings of plants homozygous for the recessive genes w_1, w_2 and w_3. Each is located on different chromosomes, 6, 10 and 2 respectively, and each gives rise to a white or off-white seedling. When protoplasts of w_3 seedlings were fused with either w_1 or w_2 protoplasts, complementation occurred, giving rise to pale green plastids, but fusion between protoplasts of w_1 and w_2 failed to result in any complementation. This would suggest that w_1 and w_2 are rather similar in their effect, and it has not escaped notice that it may even represent a reciprocal translocation.

The plastids which are formed as a result of this type of complementation do not green up to the extent that normal chloroplasts do, and contain only 15-20% of the normal amount of chlorophyll. Nevertheless,

it does seem that genetic complementation can occur; in the case of iojap/white deficient heterokaryons the deleted terminal portion of chromosome 9 is complemented, and the $\underline{w}_1$ and $\underline{w}_2$ genes can be complemented by $\underline{w}_3$. These findings open up the possible use of protoplast fusion studies in the dissection of gene expression and controlling gene-structural gene interactions in maize.

K. L. Giles

ESTACION EXPERIMENTAL REGIONAL AGROPECUARIA, INTA
Pergamino, Prov of Buenos Aires, Argentina

1. Mutant "branched silkless" found in flint inbred P578.

As described by Kempton (1) in 1934, the character "branched silkless" in corn was first discovered by E. B. Brown of the Office of Corn Investigations, USDA. Subsequently, the same character was found in sweet corn received through A. E. Longley from Nova Scotia, Canada.

The appearance of the present case of the mutant character "branched silkless" was observed first in 1967-68 in a strain of inbred line P578 of flint corn. As far as we know, this mutant was not observed before in corn from Argentina (2 & 3). It is postulated that the character appeared in inbred P578 by simple Mendelian segregation on continued selfing in a supposedly uniform and homozygous inbred line, or by natural spontaneous mutation in its genetic constitution.

This character behaves as a recessive in crosses with normal plants or supposedly non-branched silkless plants, giving first generation plants which are all normal. So far, the studies on its genetic inheritance and allelism are not completed. Its principal genetic effect is similar to that already described by Kempton (1), in that there is a characteristic modification in ear branching, florets, glumes and suppression or non-development of silks, resulting consequently in female sterility. On the contrary, there is a duplication of spikelets and florets with normal development in the tassel, giving a thicker and larger tassel than in normal plants.

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S. Bokde

FUNK SEEDS INTERNATIONAL, INC.
Bloomington, Illinois

1. Cytoplasmic male sterility research: M_2 generation from streptomycin treatments.

Streptomycin was used in an attempt to induce cytoplasmic male sterility in maize (Briggs, 1973). In this research streptomycin was used in concentrations of .001, .005, .01, .05, .10, .150% and a control. 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. Briggs (1973) can be consulted for further details on this research.

Plants from the streptomycin treated seeds were self-pollinated in the M_1 generation and good seed set was obtained from most plants in the treatments. The material was self-pollinated in order to eliminate any sterile plants that may have been in the population which could have arisen spontaneously or by seed mixtures. Seed from the

self-pollinated M_1 ears was planted out ear-to-row in the M_2 generation.

If a cell were mutated to cytoplasmic male sterility in the M_1 generation and if this cell along with nonmutated cells made up the ear (female inflorescence), a chimeric situation would exist and if this material were planted in the next generation, (M_2), male sterile and male fertile plants would be detected. These two types of plants would be found in a ratio which would be dependent on the ratio that the mutated and nonmutated cells made up the ear. If the cell that was mutated to cytoplasmic male sterility went to make up the tassel (male inflorescence), this male sterile sector would not produce pollen; hence it would not be transmissible and would be lost.

Data from these experiments are in Table 1. The sterile plants found in the M_2 generation were sib-pollinated by fertile plants from the same row.

Table 1

Data from M_2 generation of streptomycin treated seeds

Type of streptomycin seed treatment (see text)

	Germinated				Germinated submerged				Dry			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	0				0				0			
.001%	1	0	.0005	1.5	0				14	9	.50-.60	21.9
.005%	0				0				0			
.01%	0				18	10	.50-.60	28.1	0			
.05%	16	3	.999	25.0	0				0			
.10%	11	11	.10-.20	17.2	13	13	.30-.40	20.3	0			
.150%	0				0				0			

1 = No. sterile plants out of 64.
2 = No. of ears with seed.
3 = P value from X^2 (3:1 ratio)
4 = Percentage of sterile plants.

Stein and Steffensen (1959) and Steffensen (1968) indicated that there were 5 to 6 cells in the apical meristem of maize seeds. In the paper by Stein and Steffensen (1959) this was determined by irradiation of seeds where they found a sector that included about 18% of the leaf. More extensive sector data and cell number counts were presented by Steffensen (1968).

The ear is borne at the axil of a leaf; hence it might be assumed that, ontogenetically speaking, the ear and leaf arise similarly from the meristem of the seed. Therefore, if these 5 to 6 cells give rise to the leaves in the upper part of the plant, then a similar situation could also exist for the ear and 16.6 to 20.0% (for 6 and 5 cells, respectively) of the seeds on an ear would carry mutations for cytoplasmic male sterility. This assumes that one cell is mutated in the seed and the seeds from the M_1 ear are properly sampled and planted in the M_2 generation. If seed were heterozygous for a recessive gene for male sterility that arose spontaneously and the plant were self-pollinated or if the streptomycin induced a recessive gene for male sterility, in both instances the male sterile plants would occur in 25% (3:1) of the plants in the M_2 generation. In this research as reported no male sterile plants or plants with male sterile sectors were noted in the M_1 generation in any of the treatments, but the seed that was treated with streptomycin was from a lot that had been sib-pollinated and could carry genes for male sterility in the heterozygous condition.

The data in Table 1 give the probability values from the X^2 test. The cases that have P values of .999 and the two that have P values of .50-.60 are the best fits to a 3:1 ratio. However, the P values of .10-.20 and .30-.40 with 17.2% and 20.3%, respectively, do not give as good a fit to a 3:1 ratio as those previously discussed, but do fit rather closely to the 16.6% and 20.0% that might be obtained if one cell in the M_1 generation had mutated to cytoplasmic male sterility. Sixty-four seeds were planted and this is taken as the number of plants. Even though the field stands were good, a reduction in stand could modify these results. The treatment with one sterile plant (P = .0005) does not fit the above hypothesis, but could be easiest explained by poor sampling. However, since no seed set was obtained it will not be

possible to determine whether it was a genetic or cytoplasmic male sterile or a sterile caused by something not readily explained. These explanations of the data do not take into account any restorer genes that might be present in the material.

However, to determine if these sterile plants are genetic male steriles or cytoplasmic male steriles they will be planted out in this next generation ear-to-row.

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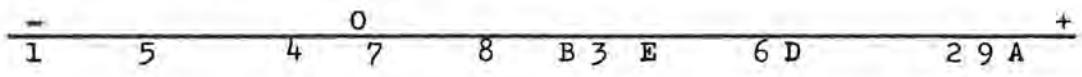
Robert W. Briggs

UNIVERSITY OF HAWAII
Honolulu, Hawaii
Department of Horticulture

1. Nine maize peroxidase loci and their tissue specificities.

We reported previously (1972 MNL 46:29-33) on seven loci governing peroxidase polymorphisms in corn. Two additional loci have been identified and will be reported here, together with observations on the tissue specificities of these and other peroxidases in maize.

Two new peroxidases have also been localized on vertical and horizontal gels since our 1972 report, bringing to a total of 13 the number of peroxidases we have identified. The approximate order of these enzymes is indicated below as they appear on horizontal 7% acrylamide gels at pH 8.1 (O = origin):



Enzymes are currently given numbers in the order in which loci controlling their genetic polymorphisms are recognized, while enzymes designated by alphabetic symbols have not yet shown genetic polymorphisms in our

studies. Thus, three locus designations in our 1972 report have been changed, from $\underline{Px_8}$ to $\underline{Px_A}$, from $\underline{Px_9}$ to $\underline{Px_B}$ and from $\underline{Px_{10}}$ to $\underline{Px_8}$. The nine loci and their alleles are listed below:

Locus	Alleles
Px_1	1,2,3,null
Px_2	1,2
Px_3	1,2,3,4,5,6 (=1+2)
Px_4	1,2,3
Px_5	1,null
Px_6	1,null
Px_7	1,2
Px_8	1,2
Px_9	1,null

$\underline{Px_8}$ genetic polymorphisms were studied in steles or in young mesocotyls, prior to synthesis of the neighboring $\underline{Px_3}$ band, which is restricted to cortex cells (see below). At least four isozymes (or "allozymes") occur in this region in different inbreds, but our present data unequivocally define only two of these as allelic, $\underline{Px_9}$ is present in comparatively few inbreds (e.g., AA8, CI64, CI66, H55, Oh51A, R168), and absence segregates as a recessive allele. $\underline{Px_9}$ action was also studied in stele of mesocotyl and in the pericarp; it is an exceptionally heat-labile enzyme among the maize peroxidases (band A-5 in Chenchin & Yamamoto, 1973, J. Food Sci. 38:40).

Additional studies of the $\underline{Px_7}$ locus, aided by electrophoresis at high pH and low gel concentrations, confirm allelism of the fast and slow bands; however, data obtained from progenies with null types (inbreds B37 and CI66) do not presently permit us to distinguish between allelism or separate locus control mechanisms. Further studies have also given no convincing evidence that allele 6 of $\underline{Px_3}$, which displays bands identical to both alleles 1 and 2 in homozygotes, is separable by crossing over.

With the exceptions of enzymes A and B, all of the peroxidases of maize have distinct tissue specificities. These specificities are given below, unique among them being the endosperm tissue culture results (T. T. Yu, unpublished data).

Peroxidases of maize

Tissue	Locus known									Locus unknown			
	1	2	3	4	5	6	7	8	9	A	B	D	E
Leaf	+	0	+	+*	0	+	+	0	0	0	0	0	0
Coleoptile	+*	0	+*	+	v	+	+*	+	+	0	0	0	0
Mesocotyl	+	0	+*	+	v	+	0	+	+	0	0	0	0
Stele	+	0	0	0	v	0	0	+	+	0	0	0	0
Cortex	+	0	+	+	v	+	0	0	0	0	0	0	0
Stem apex	0	0	0	+	0	+	+	0	0	0	0	0	0
Pericarp	+	0	+v	+	+	0	+	+	+	0	0	+	+
Embryo	0	0	0	+	0	0	0	+	+	0	0	+	+
Endosperm	0	0	0	+	+	0	0	0	+	0	0	+	+
Silk	+*	0	0	+	0	0	+	0	0	0	0	0	0
Pollen	0	+	0	0	+	0	0	0	0	0	0	+	0
Root	+*	0	0	+	0	+	0	v	0	+	+	0	0
Endosperm tissue cult.	+	0	0	+	0	+	0	0	0	+	+	0	0

* Bands prominent only in mature tissues

v Expression variable

James L. Brewbaker
Yoichi Hasegawa

2. Aphid resistance under apparent monogenic control.

In the autumn of 1972, aphid resistance was observed among a series of related shrunken-2 inbreds of the general pedigree (RR) AA8sh BC3 S1. There were 18 resistant lines and 21 lines that were susceptible or segregating. Further studies confirmed the homozygosity of resistant lines, and their aphid resistance under a wide variety of infections. Sorghum plants have been used to provide severe aphid infestations, minimizing escapes, and aphids are only rarely seen on the resistant plants.

The origin of the resistance is as yet unclear, with several possibilities. The basic inbred under conversion, a shrunken-2 version of Hawaiian inbred AA8, has no resistance. Conversion was being made

simultaneously to the Ht_1 gene (chromosome 2-121) and rust-resistant gene Rp_1 (chromosome 10-0), from different sources. The Ht source, a conversion of Illinois sweet corn inbred 101t, is not aphid resistant. The Rp source, a hybrid of W22 x B14A, is the more probable origin of resistance.

Resistance is recessive in all crosses we have made, and segregates monogenically in the crosses evaluated to date. A preliminary gene designation is the symbol aph. The possibility of linkage to the Rp_1 locus is suggested in one set of testcrosses, which segregated as follows: 43 resistant to both, 27 susceptible to both, 90 resistant only to rust and 80 resistant only to aphids.

James L. Brewbaker
Siew H. Chang

UNIVERSITY OF ILLINOIS
Urbana, Illinois
Provisional Department of Genetics and Development

1. Mutations that restore fertility to S male-sterile maize.

Exceptional male-fertile plants arising from crosses involving S male-sterile shrunken-2 inbred lines and their corresponding isogenic maintainer lines are under continuing study. The majority of these male-fertile exceptions involve cytoplasmic "mutations" in that the male-fertile trait is not pollen transmissible (Genetics 71: 607, 1972). However, we have recently reported on four independently-occurring mutations which restore fertility in S sterile cytoplasm and are pollen transmissible (MGNL 47: 50, 1973, Theoret. Appl. Genetics 43: 109, 1973). Analyses of these four nuclear restorer mutations have continued and two additional cases have been identified and are also being characterized. These six changes arose in the same strains in which the numerous cases involving cytoplasmic "mutations" were identified. The mode of restoration observed for the six newly-arisen nuclear restorers is gametophytic, as it is with the standard S restorer Rf_3, rather than sporophytic, which is characteristic of the T restorers Rf_1 and Rf_2.

We have examined the progeny of crosses designed to determine whether the six nuclear restorer genes, designated I through VI, are allelic with the standard S restorer gene of inbred line CE1. If a particular newly-arisen restorer gene in question is allelic with $\underline{Rf_3}$, crosses of plants carrying the new restorer gene in S cytoplasm with pollen from inbred line CE1 should produce some progeny with all normal pollen. Nonallelism, on the other hand, would be indicated by the presence of plants with 25% aborted pollen. On the basis of pollen analyses of these progeny, none of the six new restorer genes is allelic with $\underline{Rf_3}$. Testcross progeny from plants with 25% pollen abortion are now being grown to confirm this observation.

Studies were also carried out to determine whether the six nuclear restorer genes resulted from mutation of the same gene locus. Crosses were made between plants carrying the different new restorer genes in S sterile cytoplasm. Here again, allelism would be indicated by the occurrence of plants with all normal pollen and nonallelism by the occurrence of plants with 25% aborted grains. Of the 15 possible combinations involving the six new restorers, pollen checks of all but two combinations indicated that plants with 25% aborted pollen were present and thus that the restorer genes involved in these crosses are nonallelic. The two remaining combinations, II with V, and II with VI, were poorly represented in the test and are subject to further analysis. As in the cases involving the $\underline{Rf_3}$ hybrids, it is expected that progeny tests will confirm the suspected genotypes of plants with 25% pollen abortion.

Further indication of differences among the new restorer genes is apparent from a study of their patterns of fertility restoration in F_1 hybrids with various inbred line female parents carrying S male-sterile cytoplasm (Table 1). Restorers I and II are clearly different from restorers III through VI; they are distinguished from one another on the basis of their fertility restoration patterns in crosses with R853, WF9 and I153 S male-sterile inbred lines. Further tests are underway to obtain a more complete picture of the fertility restoration patterns of these new restorers.

In the process of preparing seed for the 1973 summer planting, reduced kernels were observed on many of the ears which also segregated

Table 1

Restoration patterns of six new S restorers. Testcross progeny indicated as F (male-fertile) or S (male-sterile).

cmsS inbred line female parent	Male parent restorer strain					
	I	II	III	IV	V	VI
R839	S	S	F	F	F	F
M825	F	F	F	F	F	F
R853	S	F	F	F		
WF9	F	S	F	F	F	F
K55	S	S		F	F	
M14	S	S	F	F		
I11A	S	S	F			F
N6	S	S	F	F	F	F
I153	S	F	F	F		F

for a new restorer gene. In fact, reduced kernels were found on ears segregating for each of the new restorer genes except restorer IV. This is of special interest because, of the six cases under discussion here, restorer IV is the only one in which the restorer mutation occurred in nonsterile cytoplasm. Reduced kernels from some of the ears were planted separately from the normal ones in order to determine whether the reduced kernel phenotype could be correlated with the presence of a new restorer gene. A positive correlation was found for restorers II, III and VI. Restorers I and V require further testing.

Through analyses of progeny of self pollinations, attempts have been made to obtain plants homozygous for each of the new restorer genes in sterile cytoplasm. These could be identified as plants, with all normal pollen, whose crosses with maintainer pollen yield all male-fertile progeny. Such plants have been identified for restorer IV only. The new restorer genes carried by strains II and III are apparently lethal when homozygous; the others require further tests. Again, restorer IV, the only one of the six that arose in nonsterile cytoplasm, is the only one, of those so far tested, that exhibits normal behavior.

As noted above, preliminary evidence strongly suggests that the new restorer genes I through VI are not allelic. Linkage characteristics of these restorers are currently being investigated using the waxy translocation series. We are encouraged to believe that what would ordinarily be a laborious procedure may be greatly simplified through the use of a technique designed to identify linkage relationships in the pollen system. A brief description of this technique is presented elsewhere in this report.

Our search for additional new restorer genes has led to the preliminary identification of six new cases, and these are currently under investigation.

So far we have not been able to distinguish between two alternatives for the origin of male-fertile exceptions in plants with S-type male-sterile cytoplasm. We assume they arise either as a result of a qualitative change in a cytoplasmic entity of S male-sterile cytoplasm, or as the result of occasional transfer of normal cytoplasm through the male germ cells of maintainer pollen parents. In any case, from what we now know it is clear that the male fertile exceptions involve either a change at the cytoplasmic level, which is most often the case, or a change in the nucleus. Because the two kinds of male-fertile exceptions have arisen in the same strains, and in both cases are expressed initially as either entirely male-fertile plants or as sterile-fertile chimeras, we consider them to have a common origin. According to this scheme the male-fertile element has the characteristics of an episome. If the latter is fixed in the cytoplasm, the newly arisen male-fertile behaves as a maintainer strain; if it is fixed in the nucleus it behaves as a restorer strain. This interpretation is supported by preliminary evidence indicating that none of the six newly-arisen restorers is allelic with standard Rf_3, and that all appear to be nonallelic with each other. The differential behavior of the six new restorers may be the result of qualitative differences in the integrated "episome" or of modulations in its message based on differences in integration sites in the chromosomes. We are currently searching for evidence of transposition of the male-fertile element from chromosome to cytoplasm, and vice versa. We are also undertaking an intensive analysis of male-

fertile chimeras when they first make their appearance. There is some indication that both cytoplasmic and nuclear fixations of the male-fertile element are involved in individual chimeras. If this is confirmed in more extensive studies it will add strong support to the episome hypothesis.

J. R. Laughnan
S. J. Gabay

2. Sensitivity of pollen with Texas group male-sterile cytoplasms to Helminthosporium maydis race T pathotoxin.

The reaction of maize pollen carrying T or P male-sterile cytoplasm to the presence of Helminthosporium maydis race T pathotoxin in the germination medium has been reported previously (MGCNL 47: 49, 1973; Crop Science 13: 681, 1973). Pollen germination and growth are inhibited in the presence of the race T pathotoxin. Smith et al. (Crop Science 11: 772, 1971) have reported that plants carrying T, P, Q or HA male-sterile cytoplasm are susceptible to H. maydis race T. These four cytoplasms are members of the Texas group of male-sterile cytoplasms (Beckett, Crop Science 11: 724, 1971). Gracen (Plant Disease Reptr. 55: 938, 1971) has reported that RS male-sterile cytoplasm, found at Cornell in 1967, is also susceptible to race T.

We have studied the reaction of pollen of these five susceptible male-sterile cytoplasms carried in the two inbred lines NY821LERf and AyX187Y-2, to the race T pathotoxin. These inbred lines, with normal cytoplasm, and their five restored Texas group male-sterile versions were kindly provided by Dr. V. E. Gracen. Concentrations of race T pathotoxin which allow normal growth of pollen grains from NY821LERf and AyX187Y-2 inbred lines with normal cytoplasm inhibit germination and growth of pollen grains from the T, P, Q, HA and RS restored male-sterile versions of these two lines.

The T, P, Q, HA and RS male-sterile cytoplasms were identified in different and, so far as we are aware, unrelated strains of maize, and each is associated with enhanced susceptibility to race T of H. maydis. As noted above, race T pathotoxin inhibits germination of pollen from plants carrying these sterile cytoplasms. This would seem to indicate that the susceptibility and the male sterility associated with these

cytoplasms are the result of a single alteration in the cytoplasm rather than the result of two independent genetic defects. In other words, there is no evidence that the Texas-type cytoplasmic male sterility can exist separately from sensitivity to H. maydis race T and to its pathotoxin.

S. J. Gabay
J. R. Laughnan

3. Growth of pollen from various inbred line and F_1 sources on Cook and Walden basal medium.

In the course of studies on the reaction of germinating maize pollen to Helminthosporium maydis pathotoxins (MGCNL 47: 49, 1973; Crop Science 13: 681, 1973) many inbred lines and their different cytoplasmic versions were tested along with some F_1 hybrids. As one of the controls in these studies, we used the Cook and Walden basal medium (CWBM) (Can. J. Bot. 43: 779, 1965), the only modification being an increase in agar content from 0.7 to 1.0%. It was noted that inbred lines vary widely with respect to growth of pollen tubes on CWBM. Since information on the performance of inbred line pollen may be of use to those studying the physiology of maize pollen or other phenomena involving pollen germination, we report here the relative performance of 30 inbred lines in classes ranging from poor to excellent.

Eight inbred lines were noted to have excellent pollen germination on CWBM: W23, Mo17, CI21E, Oh51A, N6, Hy2, C103, and B14. Pollen germination of six inbred lines was good: 38-11, NY821, AyX187Y-2, CE1, Oh43 and A632. Eleven lines exhibited satisfactory pollen germination on CWBM and, while they were not outstanding, they could certainly be used in pollen germination studies: SK2, R138, Ky21, B37, Oh07, Tr, N28, K4, K61, N28 and A619. Pollen from five inbred lines tested grew poorly on CWBM; WF9, W64A and Oh545 gave consistently poor germination while M14 and K55 varied somewhat but usually grew poorly.

The three commercially available F_1 hybrids, C123/C103, B37/B14A and A619/Oh43, gave good to excellent pollen tube growth on CWBM. While pollen grains obtained from F_1 hybrid plants vary in genotype, these sources may nevertheless be useful for some studies.

The restored cms-T versions of most of the inbred lines and the three F_1 hybrids mentioned above were also available and their pollen was tested on CWBM. Pollen from restored cms-S and cms-C versions of some of the inbred lines was also tested. These studies indicate that conversion to male-sterile cytoplasm does not appreciably alter the performance of inbred line pollen on CWBM.

S. J. Gabay

4. Linkage analysis in the male gametophyte.

As indicated in one of the above reports, we are attempting, through use of the waxy translocation technique, to identify the linkage groups of newly-arisen genic restorers of S male-sterile cytoplasm. The conventional procedure would be laborious as there are no less than six restorers to deal with and it would be necessary to score relatively large testcross progenies for a mature plant trait, male sterility. We propose to simplify the task by taking advantage of the fact that genic restoration of S cytoplasm occurs at the gametophytic level. Since plants with S cytoplasm that are heterozygous for a restorer gene produce equal numbers of normal (Rf) and aborted (rf) pollen grains, it should be possible to obtain at least a preliminary indication of the linkage group for a particular restorer through analysis of iodine-stained pollen samples from plants heterozygous for both the restorer and a particular wx-linked reciprocal translocation.

The procedure involves an initial cross of a plant with S cytoplasm that is heterozygous for a genic restorer, as female parent, with a plant that is homozygous for wx and a particular reciprocal translocation. The male parent in the cross should be in M14 background as this inbred line does not restore S. All F_1 offspring should be heterozygous for the translocation and approximately half of these, having received the rf allele from the female parent, should be male-sterile. The remaining half, those carrying the restorer allele from the female parent, should be semi-sterile, with about 25% normal pollen grains. If a particular restorer gene being tested is located on a chromosome other than the two that are involved in the waxy translocation carried by the male parent, blue and red staining normal pollen

grains should occur with equal frequency. If linkage is encountered, however, more than 50% of normal pollen grains should stain blue, the proportion of blue and red being a function of recombination between the restorer and wx loci.

There is some indication that the method described above may be employed successfully. In connection with his Ph.D. thesis study, Dr. Arjun Singh used this method in an attempt to identify the linkage group of the standard restorer, Rf_3. Eighteen homozygous wx-linked reciprocal translocation stocks, involving chromosome 9 with each of the other chromosomes, were involved in these tests. Pollen analysis of 16 of the 18 F_1 heterozygotes had blue:red ratios not significantly deviant from 1:1. Significant deviations from a 1:1 ratio, in favor of the blue-staining class, were encountered in two of three tested plants involving T3-9c, and in all six tested plants involving T2-9b. In the first case, the blue:red ratio was 5:4, in the latter it was 2:1. This preliminary evidence suggests that Rf_3 is located in chromosome 2 or in chromosome 3, probably the former.

We are continuing the effort to locate Rf_3. A number of different wx 2-9 translocation F_1 plants are currently being analyzed and progeny of crosses of these F_1 plants with S male-sterile individuals are now being grown. In addition, F_1 progeny involving crosses of the newly-arisen restorer strains with plants in the wx translocation series are being analyzed in our winter nursery. We anticipate that pollen analysis of these F_1 progenies will, with minimal effort, yield useful information on the linkage characteristics of the new restorers.

S. J. Gabay
J. R. Laughnan

ILLINOIS STATE UNIVERSITY
Normal, Illinois
Department of Biological Sciences

1. Comparison of fatty acid percentages of diploid and monosomic Zea mays embryos.*

Zea mays monosomics were employed to screen for genetic factors exhibiting dosage effects that alter maize embryo fatty acids. By comparing a given monosomic with its diploid siblings one compares simultaneously one versus two copies of every gene on that monosomic chromosome. We have successfully used this approach to detect genetic factors controlling total embryo lipids (Plewa and Weber, 1973a). The purpose of this study was to compare fatty acid profiles from specific monosomic types with those in their diploid siblings to determine if an alteration of gene dosage has an effect on fatty acid biosynthesis.

The maize monosomics were generated by the r-X1 deficiency, an X-ray-induced deficiency including the R locus in chromosome 10. The deficiency induces a high rate of chromosomal nondisjunction during the megagametophyte divisions. The r-X1 deficiency in inbred W22 was generously provided by Kante Satyanarayana.

R/r-X1 plants used as females were crossed by inbred Mangelsdorf's multiple chromosome tester that was also r/r. Scutellum samples were taken from each kernel of the r/r-X1 population and stored separately. The sampled kernels were planted and presumptive monosomics were detected by genetic markers. All presumptive monosomic plants were confirmed by chromosome counts. Since the r/r-X1 population was an F_1 produced by crossing two highly inbred lines, the r/r-X1 population was highly isogenic except for aneuploidy. In this study we compared monosomics 2, 7, 8, and 10 with diploid sibling controls.

The lipids were solvent extracted from scutellum samples and the fatty acids were esterified and analyzed by gas-liquid chromatography according to the methods of Plewa and Weber (1973b).

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The results are presented in Tables 1 and 2. The data from Table 1 indicate that embryo fatty acid profiles of monosomic 7 or 10 subpopulations do not greatly differ from the diploid control. There is a lower stearic acid percentage in monosomic 8 embryos than in the diploid control. However, the values for palmitic acid, oleic acid, and linoleic acid do not significantly differ from the control values. The monosomic 2 subpopulation has significantly increased oleic acid and decreased linoleic acid percentages as compared to the control subpopulation. There was also an increase in the palmitic acid percentage in the monosomic 2 subpopulation.

The control subpopulation and monosomic 7, 8, and 10 subpopulations are relatively homogeneous for the percentages for each fatty acid. However, the monosomic 2 subpopulation was surprisingly heterogeneous for the percentages of each fatty acid (Table 2). The only embryos analyzed exhibiting increased percentages of oleic acid and decreased percentages of linoleic acid were monosomic 2 embryos. As indicated in Table 2, certain fatty acid profiles from monosomic 2 embryos were similar to the control profiles.

The fact that monosomic 7 and 10 subpopulations were not significantly different from the control subpopulation indicates that monosomy <u>per se</u> does not significantly alter the relative proportions of the various fatty acids in maize embryos. Since monosomic 8 embryos were consistently lower in stearic acid, genetic factors located in chromosome 8 may determine the stearic acid content in maize embryos. Monosomic 2 embryos were highly variable, and the fact that some embryos had fatty acid profiles similar to control profiles has not been resolved. However, most of the embryos had significantly increased oleic acid and decreased linoleic acid percentages. These data support the hypothesis that oleic acid is the precursor of linoleic acid in higher plants (Mazliak, 1973). It is interesting to note that monosomic 2 embryos with decreased linoleic acid percentages had higher palmitic acid percentages. This suggests that preventing the desaturation of oleic acid to linoleic acid may cause an increase in fatty acid precursors of oleic acid. Thus, genetic factors are located in chromosome 2 that are involved in the conversion of oleic acid to linoleic acid.

Table 1

Comparison of mean fatty acid percentages in diploid and monosomic embryos

Chromosome constitution	Number analyzed	Fatty acids: mean per cent*			
		Palmitic	Stearic	Oleic	Linoleic
Diploid control	42	16.12±0.44	2.80±0.01	34.33±0.46	46.76±0.53
Monosomic 2	11	23.14±1.99	3.41±0.48	40.48±2.15	32.93±3.37
Monosomic 7	9	20.12±1.91	2.31±0.43	32.31±1.10	45.33±1.82
Monosomic 8	22	18.84±3.55	1.04±0.22	33.69±0.64	44.00±0.75
Monosomic 10	7	16.83±1.83	3.23±0.18	35.75±1.20	44.06±2.02

* ±1 standard error of the mean

Table 2

Fatty acid profiles of monosomic 2 embryo lipids

Sample number	Fatty acid percentages			
	Palmitic	Stearic	Oleic	Linoleic
MP34-37	14.67	3.01	39.45	42.07
*MP34-43	39.68	7.43	44.04	8.96
MP77-08	16.38	4.85	58.00	20.78
MP77-35	27.53	2.64	37.66	32.18
**MP77-49	23.16	1.95	38.56	36.36
MP81-81	22.90	1.78	35.37	39.95
MP81-110	18.72	2.54	38.16	40.59
MP83-73	24.66	2.69	34.53	38.12
MP84-62	18.56	3.28	35.68	42.51
MP84-89	20.76	2.60	33.71	42.95
MP85-98	27.47	4.69	50.08	17.76
Mean	23.14	3.41	40.48	32.93

* Double monosomic, 2-8
** Double monosomic, 2-7

Considering the altered stearic acid percentages in monosomic 8 embryos and the great differences exhibited by the monosomic 2 fatty acid profiles, it is clear that altering the gene dosage in certain chromosomes has profound effects on fatty acid levels. Thus, monosomic analysis has proven to be a valuable system in locating genes that express dosage effects controlling a biochemical pathway.

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Michael J. Plewa
David F. Weber

2. A monosomic mapping method.*

Recently, massive numbers of chemically induced maize mutants have been isolated. For this reason, it is desirable that the most efficient possible method be utilized in mapping these mutants. To this end, I would like to propose a new method of mapping certain classes of mutants to given chromosomes utilizing monosomics of Zea mays generated by the r-X1 deficiency. This method requires only one tester strain and two crosses.

The r-X1 deficiency is an X-ray induced deficiency of the r locus which was originally obtained by L. J. Stadler. Satyanarayana (unpublished) noted that gametes carrying the r-X1 deficiency included large numbers of monosomics and trisomics. He generously provided the deficiency to our laboratory where it has been studied for several years. Some of the characteristics of the system are:

1. The deficiency is only female transmissible.
2. We have isolated three or more cytologically and genetically confirmed cases of monosomy for nine of the ten maize

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chromosomes. One plant of the remaining type (monosomic 5) was found which by genetic analysis was monosomic for chromosome 5, but no cytological confirmation was possible. Thus, monosomics are produced for most, possibly all of the ten maize chromosomes.

3. Most of the monosomic types are sufficiently vigorous that successful crosses can be made. Monosomics 4, 6, 7, and 10 shed abundant pollen, and monosomics 3 and 8 shed sufficient pollen that crosses can be made with some difficulty. Monosomics 2, 3, 4, 6, 7, 8, and 10 can readily be utilized in crosses as female parents. Monosomics 1 and 9 can usually not be used in crosses but the one presumptive monosomic 5 plant was a good male and female parent. However, monosomic 9 plants can be readily recognized by a distinctive narrow leaf and conical plant shape. Thus, monosomics for most of the ten maize chromosomes can be used in crosses or recognized by distinctive phenotypes.
4. The nondisjunctive event is post-meiotic; thus there is noncorrespondence between embryo and endosperm markers.
5. Most monosomic types are generated with a frequency greater than 1% in the r-X1-carrying gametes.

If r-X1-carrying plants are crossed as females by plants with an unplaced recessive mutation that is expressed in the sporophyte, monosomics for the chromosome carrying the mutation would express the recessive sporophyte mutation. This is because the chromosome carrying the recessive was contributed by the male parent and no homolog was contributed by the female. If one identifies the monosomic chromosome, one identifies the chromosome on which that gene is located. However, identification of a monosomic chromosome at pachytene is difficult because the univalent chromosome usually folds back and pairs with itself, obscuring its morphological characteristics. Identification of chromosomes in root-tip preparations is also extremely difficult. Thus, cytological identification of the monosomic chromosome is impractical in most cases.

However, if the r-X1-carrying female parent in the above cross also carries a known recessive mutation on each of the ten maize chromosomes and the male parent carries the corresponding dominant alleles, the monosomic chromosome can easily be identified genetically. All diploid plants produced by this cross would be heterozygous for the ten recessives carried by the female parent and would give a 1:1 ratio for all genes in a backcross to the female parent. However, in a monosomic plant, the monosomic chromosome is contributed by the male parent and carries only the dominant allele. Since the only viable gametes produced by a monosomic are haploid, in a backcross one would obtain only dominants for the gene on the monosomic chromosome and a 1:1 ratio for genes on the other chromosomes.

We are developing a line for use in the genetic approach outlined above. For this purpose, we are introducing the r-X1 deficiency into Mangelsdorf's Multiple Chromosome Tester ($\frac{bm_2}{}$; $\frac{lg_1}{}$; $\frac{a_1}{}$; $\frac{su_1}{}$; $\frac{pr}{}$; $\frac{y_1}{}$; $\frac{gl_1}{}$; $\frac{j_1}{}$; $\frac{wx}{}$; $\frac{g_1}{}$).

To illustrate the use of this system, let us assume that a new recessive mutation (m) that is expressed in the sporophyte is found, and this mutation is located on chromosome 7. The cross to be made and the expected progeny are shown below (for the sake of simplicity, the r-X1 deficiency on chromosome 10 is not illustrated):

Chromosome number	Female parent	Male parent	2N progeny	Progeny expressing m phenotype (monosomic 7)
1	bm/bm	Bm/Bm	Bm/bm	Bm/bm
2	lg/lg	Lg/Lg	Lg/lg	Lg/lg
3	a/a	A/A	A/a	A/a
4	su/su	Su/Su	Su/su	Su/su
5	pr/pr	Pr/Pr	Pr/pr	Pr/pr
6	y/y	Y/Y	Y/y	Y/y
7	gl M/gl M	Gl m/Gl m	Gl m/gl M	Gl m/--
8	j/j	J/J	J/j	J/j
9	wx/wx	Wx/Wx	Wx/wx	Wx/wx
10	g/g	G/G	G/g	G/g

The only plants expressing the mutant phenotype (m) in the F_1 would be plants monosomic for the chromosome carrying the gene, m. When this plant is backcrossed to Mangelsdorf's Multiple Chromosome Tester, all genes in Mangelsdorf's tester would be segregating 1:1 except gl, and all progeny would be Gl. Thus, the unplaced gene is located on chromosome 7.

Although monosomic 9 plants cannot be used in crosses, the gene use to mark monosomic 9 plants (wx) is expressed in the pollen itself; thus if a pollen sample were collected from the plant and stained with IKI, monosomic 9 plants could be readily recognized because all pollen would stain darkly.

The advantages of this system over others currently in use are:

1. All genes on an entire chromosome are uncovered simultaneously in a monosomic plant.
2. A single tester strain is needed to make the analysis; thus a single cross and a single testcross would be needed to locate a gene to a specific chromosome.

Disadvantages of this system are:

1. Only sporophyte-expressed, recessive mutations can be mapped in this way.
2. Two generations are necessary, whereas a single generation is sufficient with TB translocations.
3. Monosomics are produced with a relatively low frequency; thus relatively large populations would be necessary from the initial cross.

I believe that the proposed system will be extremely useful in mapping certain classes of mutations.

David F. Weber

INDIANA UNIVERSITY
Bloomington, Indiana
Department of Plant Sciences

1. On the quaternary structure of the temperature-sensitive mutant Adh_1^{S-1108}.

Plants homozygous for the EMS induced, temperature-sensitive, alcohol dehydrogenase allele, Adh_1^{S-1108}, show a reduced level of enzyme activity, equal to about ten percent of that in sib plants which are homozygous for a fully active, normal allele. Recent studies have revealed

a striking difference in the quaternary structure of the temperature-sensitive dimer. In the normal enzyme the two subunits in the dimer are held together by hydrogen and disulfide bonds. Cleavage of the hydrogen bonds alone, by freezing in high salt, will not dissociate the dimer into monomers. Dissociation requires the simultaneous cleavage of disulfide bonds by a high concentration of B-mercaptoethanol (0.1 M BME in the dissociation medium). On the other hand, BME is not required for the monomerization of the temperature-sensitive dimer and complete dissociation occurs when the BME is omitted from the dissociation medium. This finding points to an absence of disulfide bonds between the subunits in the temperature-sensitive homodimer. The temperature-sensitive subunits which dissociated in the absence of BME are stable and readily reassociate with other ADH subunits.

Tests with heterodimers composed of one temperature-sensitive and one normal subunit have indicated that it is <u>not</u> the absence of the disulfide bond which is responsible for the temperature-sensitivity of the dimer. The temperature-sensitive subunit is stabilized in the heterodimer by the presence of the normal subunit. This subunit retains full activity in the heterodimer at temperatures which completely inactivate the ts homodimers. However, the heterodimer resembles the ts homodimer in that the subunits are not interconnected by disulfide bonds. The heterodimer dissociates completely when BME is omitted from the dissociation medium. Temperature-sensitive heterozygotes form normal homodimers in addition to the heterodimer and temperature-sensitive homodimer. Only the latter two enzymes dissociate when heterozygous extracts are frozen in BME-less dissociation medium. In all of this work, dissociation is determined by the elimination of ADH activity and ADH isozyme bands in starch gels after freezing, followed by the recovery of activity and formation of the appropriate heterodimers when the dissociated extracts are subjected to reassociation conditions.

Drew Schwartz

2. Preliminary genetic evidence that the $Adh_1^{FC^m}$ duplication produces a single transcript.

Schwartz (1973) has suggested that the $Adh_1^{FC^m}$ duplication (Schwartz and Endo, 1966) produces a single transcript. To investigate this possibility further, an ethyl methanesulfonate (EMS) mutagenesis study of the duplication was initiated. Seeds homozygous for the duplication were treated with .08 M EMS for ten hours at room temperature and then washed and dried by the method of Briggs, et al. (1965). The 4500 treated seeds were planted in the field and pollinated by one of three Adh_1 alleles: 1) the naturally occurring S allele, 2) a fully active EMS-derived mutant, W, specifying an enzyme which remains near the origin in pH 7.5 starch gel electrophoresis, and 3) an EMS-derived mutant of S that forms a reduced amount of monomers capable of dimerization. Mutant sectors were detected by electrophoretically testing ten kernels from each ear. Mutants in one or the other or both components of the duplication could be detected by a departure from the expected zymogram pattern. Of the 363 ears screened, fourteen had an electrophoretically detectable mutation involving one or both components of the duplication. Five classes of mutants were found.

Class 1: Change in the electrophoretic mobility of C^m; no change in F.

Two mutants were found in which the protomers specified by the C^m component were changed to faster migrating forms. A third mutant may involve a change in C^m producing protomers that migrate as F. This mutant was crossed by the low-dimerizing mutant of S and thus could also be interpreted as an elimination of the C^m subunit with the retention of the F. These two alternatives can be distinguished on the basis of activity ratios when the mutant is crossed by a fully active, fully dimerizing S allele.

If only F monomers are produced in the third mutant, the zymogram of scutellar extracts will show a 1 FF: 2 FS: 1 SS ratio of activity, when crossed to the normal, fully dimerizing S. If the C^m component were mutated so that the protomers produced migrate as F, then the activity ratio would be 2 FF: 3 FS: 1 SS, since the mutant C^m homodimer would migrate to the F position. The S plus mutant heterodimer would migrate to the FS position, and the F plus mutant heterodimer would migrate to the

FF position. This is the expected activity ratio, if there is random association within a population of 2 F: 1S monomers, with half of the F monomers being virtually inactive, as is the case with C^m.

Class 2: Change in the electrophoretic mobility of F; no change in C^m.

Two mutants were recovered in which the F component produced a form migrating to the position of the naturally occurring S.

Class 3: Elimination of the F subunit; no change in C^m.

Four mutants were found which produced homo- and heterodimer enzymes containing subunits specified only by the C^m component and the allele introduced by the pollen.

Class 4: Elimination of both the F and C^m subunits.

Four mutants were found in which only the isozyme specified by the allele introduced by the pollen parent was observed in the zymograms. These could be interpreted as a change in electrophoretic mobility of the protomers specified by one component of the duplication to that of the pollen allele and concomitant elimination of the other. However, it is more likely that these mutants represent a loss of both types of subunits specified by the FC^m duplication. This loss could be interpreted in two ways. First, the loss of both might be due to a deletion of the duplication. Secondly, a nonsense mutation in the first component could prevent the translation of the second component of the duplication as well. Since there is no unequivocal case of the elimination of the C^m subunit only, the latter is favored and the class four mutants are thought to represent mutations in the C^m component which block the translation of the remainder of the message, including the F segment. The various interpretations will be tested by checking the mutant homozygotes immunologically for cross-reacting material.

Class 5: Polarity type mutation.

One mutant was found in which only approximately 1/2 the normal number of monomers of C^m was produced and only 1/8 the normal number of F monomers was formed. The particular lesion in this mutant is a matter of speculation; yet it is clear that a single mutation has affected both components of the duplication.

In conclusion, the preliminary data indicate that the FC^m duplication produces a single message, which is translated in the direction of

$\underline{C}^m$ to $\underline{F}$. Additional mutants are being sought and a genetic and immunological analysis of those mutants described herein is underway.

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James Birchler

3. Further studies on the cause of variation in the frequency of chromatin loss induced by B chromosomes.

In the 1972 Maize News Letter we reported different rates of chromatin loss induced by B chromosomes when two plants of the high-loss strain were used as the pollen parent in crosses with two different genetic lines. When plant 30785-24 with 7 B's, homozygous for the $\underline{A_1}$ allele and the 3L knob, was used as the pollen parent onto $\underline{d\ lg_2\ a}$ silks, 12.7% of the F_1 kernels had colorless aleurone. Pollen from the same plant gave 21.7% colorless kernels when crossed to $\underline{a\ B\ Pl}$ testers. A sibling plant 30785-23 with 8 B's yielded 8.9% colorless seeds when its pollen was placed on $\underline{d\ lg\ a}$ silks and 19.5% colorless kernels on the $\underline{a\ B\ Pl}$ tester. These data are explicable if in the $\underline{d\ lg_2\ a}$ crosses there is preferential fertilization of the egg by the deficient sperm, while in the $\underline{a\ B\ Pl}$ crosses the deficient sperm preferentially unites with the polar nuclei to form the endosperm. Preferential fertilization, however, proved to be not responsible for the dissimilar rates since for both pollen parents, the high rate of endosperm loss in the $\underline{a\ B\ Pl}$ crosses was not associated with low embryo loss; there was no compensating increase in embryo loss if endosperm loss was low and vice versa.

Two alternative explanations were considered. One of these ascribed the dissimilar rates of loss to environmental differences of an unknown nature. Circumstantial evidence in support of this hypothesis is the fact that for both plants 23 and 24 the pollen used in crosses with

the a B Pl strain came from the main stalk while pollen from tillers was used in the d lg a crosses. Since the second microspore division in the tillers took place at a later time and under different environmental conditions than it did in the main stalks, and if the mechanism responsible for chromatin loss is sensitive to climatic or edaphic factors, a difference in rate of loss in the tiller and main tassel would not be unexpected.

The second alternative was based on a hypothetical difference in genetic constitution of the main stalk and tiller. Elimination of several B chromosomes from the tiller at the time it arose from the main stalk could reduce the number of B chromosomes in the tiller to a level which would modify the rate of A chromatin loss. In order to account for the genetic data, the 7 B's in the main stalk of plant 24 would be reduced to 3 B's in the tiller and in plant 23 the change would be from 8 in the main stalk to 3 in the tiller. This specific number of B's eliminated is necessitated because plants with 3 B's have approximately half the rate of loss as do individuals with 4 or more B's. Plants with two or fewer B's have little or no chromatin loss in their microspores.

Lending some credence to this hypothesis was the finding by Puteyevsky and Zohary (1970) that in Dactylis variation in number of B's occurred during tiller differentiation. A similar situation might hold for maize. The postulated variation in numbers of B's between tiller and main stalk is amenable to experimental test. In the summer of 1972, pollen mother cells were taken from the main stalk and tillers of 9 plants of the high-loss strain. In no case was a difference found in number of B's between the tiller and main stalk. Unfortunately, no adequate genetic data on loss rate were obtained from these 9 plants because adverse weather conditions inhibited pollen shedding. The cytological data showing no intra-plant variation in number of B's lend no support to a genetic difference in main stalk and tiller as the cause of low and high rates of loss. It is conceivable, though unlikely in our opinion, that in plants 23 and 24 a reduction in numbers of B's did occur during tiller differentiation. If so, it must be a sporadic and unpredictable happening.

The hypotheses of preferential fertilization and of differing genetic constitution of tiller and main stalk are not consonant with the experimental data. At the present we are in the unsatisfactory position of ascribing differences in rate of loss found when two female testers were employed in the high-loss crosses to unknown and undefined environmental conditions.

M. M. Rhoades
Ellen Dempsey

INSTITUTE OF CYTOLOGY AND GENETICS
Novosibirsk, U.S.S.R.

1. Polymorphism of isozyme pattern of catalase in the endosperm of diploid maize strains.

Catalase in the endosperm of maize is controlled by two loci $\underline{Ct_1}$ and $\underline{Ct_2}$ (1). Occasional aggregates of different subunits result in 2-5 isozymes. However, there are strains in which only one locus ($\underline{Ct_1}$) functions in the endosperm and only one isozyme is displayed.

In 1973 we investigated the isozyme pattern of catalase in 10 strains in the endosperm of self-pollinated maize plants grown from seeds analyzed in 1972. The isozyme pattern of 5 strains did not change, the quantity of isozymes in 3 strains increased from 1 to 4-5, the quantity of isozymes in one strain increased from 3 to 5 and the quantity of isozymes in another strain decreased from 5 to 3.

In various strains having 5 isozymes, the nature of the isozyme pattern may be different. Three possible types of pattern are indicated in Figure 1. The isozyme pattern may be symmetrical to isozyme 3 (Fig. 1b) and also may be asymmetrical (Fig. 1a,c). In the latter, the intensity of the isozyme stain may be weakened either in the direction from isozyme 2 to 5 or on the contrary from 5 to 2. However, it should be noted that brightness of isozyme 1, consisting of only subunits of $\underline{Ct_1}$ type, is not varied. In all three cases it remains the brightest.

Sometimes variations of the isozyme pattern are observed in various plants of the same strain. Three possible types of pattern in 5-catalase strains are presented in Figure 2.

Thus, the locus $\underline{Ct_1}$ may be considered as the main one, as its products are always found in the endosperm. Different alleles of the locus $\underline{Ct_1}$ provide the presence of various electrophoretic variants of catalase in the endosperm (2). The products of the $\underline{Ct_2}$ locus are not always displayed in the endosperm. On the basis of the described polymorphism, it may be assumed that there are different alleles at the $\underline{Ct_2}$ locus controlling the synthesis of the catalase subunits differing in their physical-chemical characteristics. The relative frequency of the two types of subunits provides both the variation of the isozyme catalase pattern in

different strains and the variation within one strain depending on the environment.

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E. V. Poliakova
S. I. Maletzky

2. Analysis of internode peroxidase in genetically determined dwarf forms of maize.

At present, evidence indicates that peroxidase and growth regulators interact (1,2,3,4).

Herein is presented an attempt to differentiate peroxidase isozymes by the degree of their involvement in the development and growth of maize internodes. This study was carried out on three maize lines carrying the mutation $\underline{br}_2$ or the normal analogue, as well as dwarf $\underline{d}_1/\underline{d}_1$, $\underline{d}_2/\underline{d}_2$ plants and their normal sibs. Because $\underline{d}_1/\underline{d}_1$ and $\underline{d}_2/\underline{d}_2$ plants have no pollen, dwarf plants were obtained by self-pollinating heterozygous $\underline{D}_1/\underline{d}_1$ and $\underline{D}_2/\underline{d}_2$ plants and normal plants by self-pollinating homozygous $\underline{D}_1\underline{D}_1$ and $\underline{D}_2/\underline{D}_2$ plants. The scheme used for the identification of $\underline{d}_1/\underline{d}_1$ and $\underline{D}_1/\underline{D}_1$ homozygous plants is shown in Fig. 1.

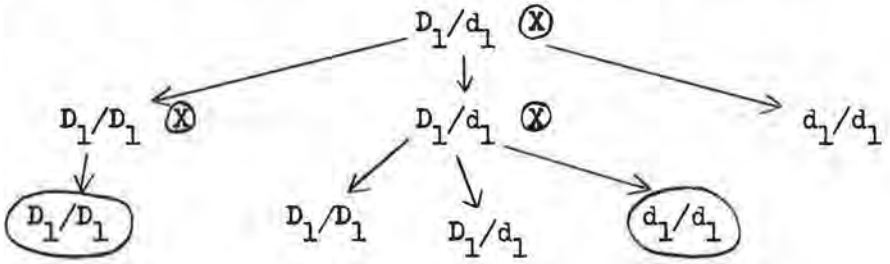


Figure 1.

Plants encircled in this scheme were analyzed. Peroxidase activity and isozyme patterns of peroxidases were investigated in small underdeveloped, growing and mature internodes. The plants were studied at the stage of the formation of the third internode, when the growing internode was about half as long as the internode which has developed before it. The staining

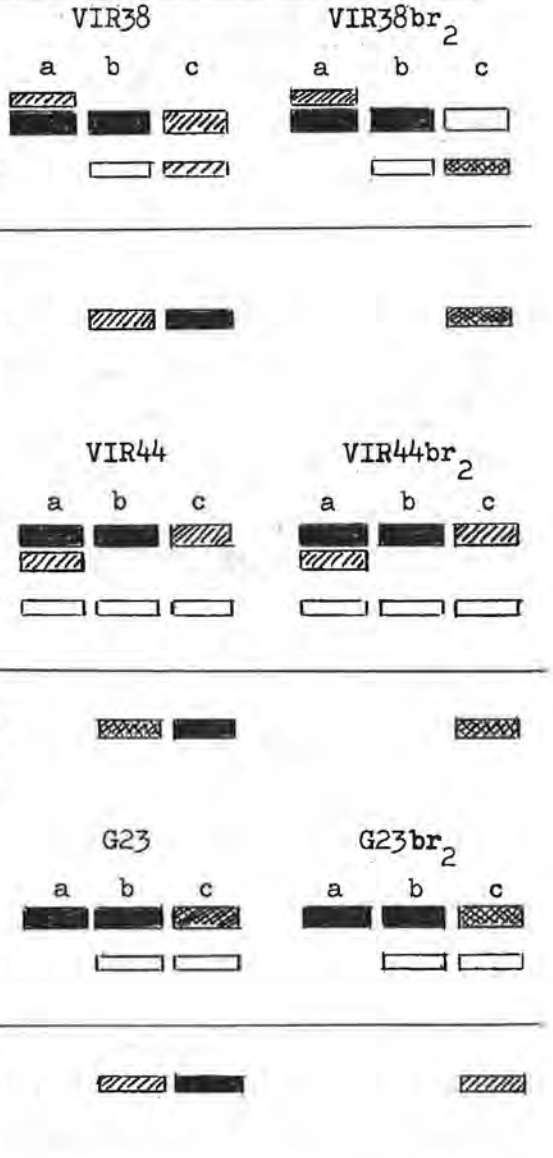
Table 1

The change in peroxidase activity and patterns of peroxidase isozymes in the course of internode development in semidwarf and normal plants

a-small underdeveloped internode; b-growing internode; c-mature internode.

(Activity=$\Delta D_{550-660mu}$/sec/ug protein)

Number of plant	a	c	Patterns of peroxidase isozymes
VIR 38			
1	56.6±1.5	73.2±1.6	
2	51.7±2.0	96.5±2.6	
VIR 38 br_2			
3	37.0±0.6	72.0±1.8	
4	45.8±1.8	73.5±2.9	
5	36.3±0.8	64.4±1.4	
VIR 44			
6	15.7±1.3	21.4±1.3	
7	18.3±0.9	52.4±1.5	
8	16.8±0.6	29.4±2.5	
VIR 44 br_2			
9	25.8±1.2	111.7±5.1	
10	22.6±0.9	107.6±4.0	
11	18.2±0.5	109.2±5.4	
G 23			
12	8.8±0.3	25.8±0.8	
13	10.6±0.9	37.1±1.7	
G 23 br_2			
14	17.9±0.7	29.7±1.2	
15	12.7±0.3	36.7±1.0	
16	14.8±0.5	25.0±0.9	



of peroxidase isozymes after starch gel electrophoresis was carried out with benzidine. Enzyme activity was tested by following the rate of benzidine oxidation at 550-660 mu (yellow light filter). The results were calculated as the change of optical density/sec/ug protein.

Data on enzyme activity and patterns of peroxidase isoforms are given in Tables 1 and 2. As a rule, internode growth in normal, semidwarf and dwarf plants is associated with increased peroxidase activity (Tables 1 and 2). In the mature internode peroxidase activity is maximal and is determined by plant genotype. The influence of the mutation $\underline{br_2}$ on peroxidase activity in the mature internode also depends on plant genotype. The mutation $\underline{d_1}$ increases enzyme activity in the mature internode by more than two times. The mutation $\underline{d_2}$ suppresses changes in peroxidase activity in the course of internode development.

15 zones are observed on the electrophoregrams of maize internodes. The schemes depict only those zones which change in the process of internode growth (Tables 1 and 2). Zones A-3 and A-4 correspond to two alleles at one locus not linked with $\underline{d_2}$ (our unpublished data). These zones change quite similarly during growth. In all normal plants, internode growth is associated with decreasing staining intensity of zones C-5 and C-6, on the one hand, and increasing staining of zones C-3, A-3, and A-4 in growing and mature internodes, on the other hand. In all the semidwarf plants, zones A-3 and A-4 are observed only in the mature internode. The influence of this mutation on the expression of zones C-3, C-5, and C-6 depends on plant genotype. In $\underline{d_1}/\underline{d_1}$ mutants, zone A-3 again appears only in the mature internode, while zone C-5 does not change during development. In $\underline{d_2}/\underline{d_2}$ mutants, isozyme patterns do not change during the growth of the internode (Table 2).

Thus semidwarf and dwarf plants differ sharply from normal plants in the regulation of the pattern of peroxidase isozymes in the course of internode development.

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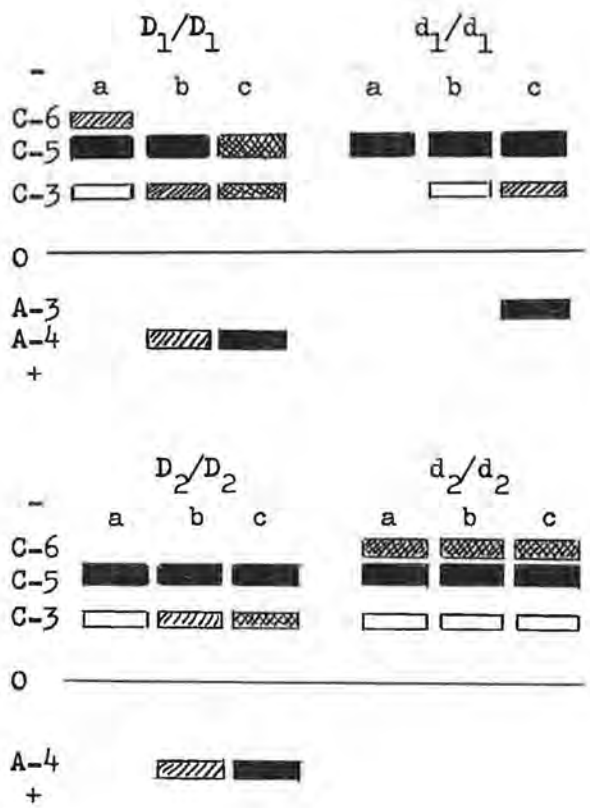
Table 2

The change in peroxidase activity and patterns of peroxidase isozymes in the course of internode development in dwarf $\underline{d_1}/\underline{d_1}$ and $\underline{d_2}/\underline{d_2}$ plants and their normal sibs

a-small underdeveloped internode; b-growing internode; c-mature internode.
(Activity=$\Delta D_{550-660mu}$/sec/ug protein)

Number of plant	a	c	Patterns of peroxidase isozymes
D_1/D_1			
1	19.7±0.5	50.6±2.3	
2	33.9±1.6	41.3±1.5	
3	28.2±0.8	52.6±2.5	
d_1/d_1			
4	17.6±0.6	75.0±5.1	
5	31.4±1.6	68.4±3.0	
6	26.2±0.8	71.4±1.6	
D_2/D_2			
7	21.0±0.4	84.0±3.2	
8	13.4±0.7	74.3±1.5	
9	13.8±0.7	140.0±6.4	
d_2/d_2			
10	28.6±0.8	26.5±1.4	
11	22.4±0.6	18.4±0.5	
12	30.6±0.9	27.8±0.9	



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E. V. Levites
S. G. Veprev
S. I. Maletzky

3. Study on ADH activity in the scutellum of diploid and tetraploid maize.

Diploid lines of maize, W64, Sg-25, and the W64 x Sg-25 hybrid, and a tetraploid obtained by colchicine treatment of the diploid hybrid (W64 x Sg-25) were used in this study. The tetraploid was reproduced for 5 generations by self- and cross-pollination within the strain. All these lines and the hybrids were homozygous for the gene $\underline{Adh_1^F}$ (our unpublished data).

Alcohol dehydrogenase (ADH) activity was tested by following the rate of NAD reduction at 340 mu. The results were calculated as nmoles of NADH/min/ug protein.

The two diploid lines showed differences in their ADH activity in the scutellum. The diploid hybrid has intermediate enzyme activity (Table 1). The activity of the tetraploid form was similar to that in the diploid hybrid (Table 1). The gene $\underline{Adh_1}$ was found to have no dosage effect.

Table 1

Alcohol dehydrogenase activity in the scutellum of diploid and tetraploid maize

	W64	Sg-25	diploid hybrid	tetraploid hybrid
ADH activity (nmoles NADH/ min/ug protein)	589	845	635	639

E. V. Levites
S. I. Maletzky

IOWA STATE UNIVERSITY
Ames, Iowa
Department of Agronomy

1. A nutrient medium for in vitro culture of maize and sorghum embryos.*

Recently, as part of a project investigating the possibility of obtaining a hybrid between maize and sorghum, we grew a large number of immature embryos in vitro. This was done because we felt that an endosperm-embryo incompatibility may have been responsible for the lack of success in previous attempts to obtain the intergeneric hybrid. To determine the optimum conditions for immature embryo development in vitro we tested the effects of a number of different levels of hormones, vitamins, and other supplements. Our intent was to develop a medium that would ensure the survival and subsequent normal development of as many potentially hybrid progeny as possible. The medium we finally adopted allowed us to grow routinely 10- to 12-day-old embryos, as small as 0.5 mm, and occasionally 6- to 7-day-old embryos, as small as 0.1 mm (Table 1). Embryos smaller than 0.1 mm could not consistently be excised without damage. Similar results were obtained with embryos from sorghum ♀ x maize ♂ pollinations.

The medium, modified from that of Murashige and Skoog (1962), contained the following components (concentrations in mg/l): NH_4NO_3 (1650), KNO_3 (1900), $CaCl_2 \cdot 2H_2O$ (440), $MgSO_4 \cdot 7H_2O$ (370), KH_2PO_4 (170), H_3BO_3 (6.2), $MnSO_4 \cdot 4H_2O$ (22.3), $ZnSO_4 \cdot 7H_2O$ (8.6), KI (0.83), $NaMoO_4 \cdot 2H_2O$ (0.125), $CuSO_4 \cdot 5H_2O$ (0.025), $CoCl_2 \cdot 6H_2O$ (0.025), thiamine · HCl (0.5), sucrose (60,000), Difco agar (10,000). Iron was added as 5 ml/l of a stock solution containing $FeSO_4 \cdot 7H_2O$ (5.57 gm/l) and disodium EDTA (7.45 gm/l). All components were autoclaved together. Optimum pH for normal growth was 5.0, adjusted with KOH or HCl as needed. This mixture of inorganic components was highly superior to other mixtures which have been used for culture of various species of embryos (Dure, 1960; Norstog and Smith, 1963; Raghavan and Torrey, 1963; White, 1963). Most hormones and vitamins at the levels tested were either toxic or had no effect on growth. Only thiamine was essential. Of the amino nitrogen

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Table 1

Growth and development of embryos of various sizes recovered from maize ♀ x sorghum ♂ pollinations*
(From Mock and Loescher, 1973)

Embryo size (mm)	Number of embryos	Percentage of embryos displaying		
		No development	Abnormal** development	Normal development
<0.1	23	39	56	5
0.1 - 0.2	8	25	16	63
0.2 - 0.4	8	13	0	87
0.4 - 0.6	18	0	11	89
0.6 - 0.8	5	0	0	100
0.8 - 1.0	8	0	0	100
1.0 - 1.2	4	0	0	100
1.2 - 1.4	3	0	0	100
1.4 - 1.6	3	0	0	100
1.6 - 1.8	2	0	0	100

* All progeny grown to maturity possessed maize phenotypes.

** Embryos displaying this type of development displayed only very small roots and (or) shoots.

sources tested, glutamine and casein hydrolysate both appeared to enhance growth but not survival rate of very small embryos; glycine and asparagine were toxic. Addition of coconut milk did not significantly promote growth, even of very small embryos. Yeast and malt extracts appeared to retard growth.

Except for very large embryos which began root and shoot growth within 24 hr after excision, most in vitro embryo development consisted of scutellar enlargement followed by root and shoot development. Once roots were well-developed in vitro, the plants could be transferred without difficulty to sterile soil and a large number were grown to maturity.

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W. H. Loescher
J. J. Mock

2. Variegation associated with controlling element systems in tribal maize from Colombia.

The appearance of color variegated kernels in accessions of maize conjures up visions of r-mottling, B-B-F, and controlling element systems. Such variegation appeared in an accession of maize grown by the Cuna Indians from the remote village of Unguia in the western part of Colombia, S.A. Some preliminary findings will be reported on crosses with this Colombia line.

Hypothesis for r-allele-factor interaction: For purposes of adapting these lines to North American conditions, plants arising from variegated kernels were crossed to an early maturing A_1 et/A_1 et stock. The F_1 was pollinated by all the color testers (rr; cc; a_1a_1; a_2a_2). Plants resulting from the series of crosses to the r tester were subsequently backcrossed to r/r; five ear cultures were obtained and the kernels classified as variegated and non-variegated (Table 1). The non-variegated kernels contained both colorless and a subclass designated incomplete color.

It is hypothesized that variegation is dependent on a factor (temporarily designated, F-co*) segregating independently of the r locus, which acts on the Colombia line r allele that has been designated r-co. In the absence of the factor the r-co allele gives rise to the incomplete color subclass previously alluded to. This subclass contains kernels ranging from very faintly colored to possibly completely colorless.

A X^2 test was done for each of the crosses to test the hypothesis of the r-co, F-co interaction. None of the X^2 values was significant at the 0.05 level of probability. Since the X^2 test for heterogeneity was not significant, the data were pooled over all crosses and a non-significant X^2 value of 0.007 was obtained.

r-co + F-co = variegation r-co + f-co = colorless

Table 2

Number of kernels of each of five phenotypic classes arising from the cross:

$$\underline{Dt},\ \frac{\underline{a_1}^{m(r)}\ \underline{Sh_2}}{\underline{a_1}^{dt}\ \underline{sh_2}} \times \frac{\underline{a_1}^{m(r)}\ \underline{Sh_2}}{\underline{a_1}^{m-1}\ \underline{sh_2}}$$

Phenotype----Sectored------Pale

Cross	round ($\underline{Sh_2}$)			shrunken ($\underline{sh_2}$)	Total	X^{2}**
	Pale	Colorless	Dotted	Pale*		
3 0341-1x115	97	146	61	130	434	6.82 ns
3 0341-2x527	87	148	42	81	358	2.38 ns
3 0341-3x704	120	195	61	146	522	2.09 ns
3 0341-4x531	104	120	51	89	364	2.70 ns
3 0336-1x702	114	170	45	105	434	2.01 ns

* In this category are also included shrunken kernels with dots in a pale background.

** A X^2 test for each cross was made on the hypothesis that there was one $\underline{Dt}$ segregating independently of the $\underline{A_1}$ locus and effecting the sectoring on the $\underline{a_1}^{dt}$ allele.

Table 1

Aleurone color segregation in crosses of ($\frac{r\text{-}co}{r}$), F-co* x r r. (The r r tester was wxwx).

Cross	Non-variegated	Variegated	Total	X^2
3 0334-1 x 808	350	107	457	0.61 ns
3 0334-2 x 515	278	105	383	1.19 ns
3 0334-3 x 516	282	78	360	2.13 ns
3 0334-4 x 511	204	80	284	1.52 ns
3 0334-5 x 921	277	93	370	0.00 ns

Test for relation to the En system: In another series of crosses, variegated kernels of the Colombia line were crossed to $a_1^{m(r)}/a_1^{m-1}sh_2$, an En tester stock (Peterson, 1965, Amer. Nat. 99:391). The resulting F_1 was testcrossed by a_1^{dt} sh_2/a_1^{dt} sh_2 (a_1^{dt}, an allele that responds to Dt producing colored dots on a colorless background; sh_2 is a recessive allele conditioning shrunken endosperm and is very closely linked to the A_1 locus). Kernels with colored spots or sectors were obtained and the resulting plants were backcrossed to $a_1^{m(r)}/a_1^{m-1}sh_2$; the kernels on each of five ears obtained from the above cross were counted and grouped according to their phenotypic appearance (Table 2).

None of the X^2 values was significant at the .05 level of probability. Since the heterogeneity X^2 (14.61) was not significant, the data were pooled over all crosses and a non-significant X^2 value of 1.34 was obtained.

Tests to determine whether F-co is a Dt allele are presently in progress.

Jaime Gonella
Peter A. Peterson

3. T-cytoplasm mitochondrial membrane activities.

In view of the striking effect reported by Miller and Koeppe (1971) of Helminthosporium maydis race T toxin in causing the immediate uncoupling of oxidative phosphorylation and irreversible swelling in KCl

medium of T (Texas) but not of N (Normal) mitochondria, a study was initiated to investigate the various details of the pathways of electron transport and associated activities in N and T mitochondria. On the basis of the details of the pathways in Jerusalem artichoke mitochondria developed by Coleman and Palmer (1972, Eur. J. of Biochemistry 26:499), the effects of the race-T pathotoxin on various steps in this network of enzyme reactions was investigated. The race T pathotoxin causes an increase in the activity of cytochrome oxidase and succinate cytochromic reductase, possibly due to a disturbance of the mitochondrial membranes which allows increased substrate accessibility acting as an uncoupler.

The first ATP-coupled site of the electron transport chain, which includes the endogenous NADH dehydrogenase, was studied using malate as a substrate in the absence of exogenous NAD^+. In T mitochondria, the pathotoxin strongly inhibited the oxidation of malate by intact mitochondria. Malate oxidation via endogenous NADH dehydrogenase in N mitochondria was unaffected by similar concentrations of pathotoxin. Upon the addition of NAD^+, however, there is a marked stimulation of malate oxidation in intact T mitochondria. Thus, the presence of an intermembrane malate dehydrogenase activity coupled to NAD^+ reduction leads to an initial and immediate stimulation of malate oxidation via the exogenous NADH dehydrogenase. This confirms that the inhibition of malate and oxoglutarate oxidation in T mitochondria by pathotoxin is almost certainly at the endogenous NADH dehydrogenase complex of the inner membrane.

Peter A. Peterson
Richard B. Flavell*
D. H. P. Barratt*

*Plant Breeding Institute, Cambridge, England

4. Location of pg^m of the En system.

pg^m (Peterson, 1960 Genetics 45:115) has been found to be allelic with a pg^m isolated by Neuffer in mutagen treatments. This is uncovered by TB-3b, which places pg^m on chromosome 3S.

Peter A. Peterson

5. A sensitive assay for T mitochondria.

A manuscript on very sensitive assays for H. maydis race T toxin on T cytoplasm is now in press (Nature, Spring, 1974). These assays require little toxin and mitochondrial substrate, are easy and quick to perform, and do not require materials that are not readily unavailable (example, fertile pollen). They therefore should be of considerable use as assays of Texas cytoplasm and should facilitate identification, purification, and characterization of the pathotoxin.

Peter A. Peterson
Richard B. Flavell*
D. H. P. Barratt*

*Plant Breeding Institute, Cambridge, England

IOWA STATE UNIVERSITY
Ames, Iowa
Department of Genetics

1. Linkage data for yellow dwarf 2 on chromosome 3.

In 1963 Dr. Irwin Greenblatt sent me seed of a yellow dwarf seedling mutant. Since a yellow-dwarf mutant has been previously located on chromosome 6, this mutant was called yellow dwarf 2 (yd2). The seedlings of this mutant are a medium yellow and less than half the height of normal seedlings.

F_2 progeny of crosses between yd2 and wxT3-9c (3L.09, 9L.12) gave indication of linkage with wx. Two point linkage tests were made with T3-9c, T3-9 (8447) (3S.44, 9L.14), T3-9 (020-5) (3 ctr., 9 ctr.), and T3-9g (3L.40, 9L.14). The results of these tests are shown in Table 1. Closest linkage is shown with T3-9g which has a breakpoint at 3L.40. This would suggest that the gene was located in the long arm of chromosome 3.

Crosses with TB-3a (L.1) resulted in the segregation of yellow dwarf seedlings in the F_1, thus confirming the location of the gene in the long arm of chromosome 3.

Table 1

Linkage data involving crosses of yd2 with T3-9c, T3-9 (8447), T3-9 (020-5) and T3-9g

Translocation	Parental classes		Recombination classes		Total	% recombination
	T +	+ yd	T yd	+ +		
T3-9c	179	209	12	32	432	10.2
T3-9 (8447)	155	136	30	35	356	18.3
T3-9 (020-5)	124	135	32	29	320	19.1
T3-9g	127	99	1	0	227	0.4

The results of a three-point testcross involving ra2 and lg2 are given in Table 2. In this testcross ra2 lg2 plants were frequently weak and failed to set ears, which accounts for the low frequency of this class. The data indicate close linkage with lg2, which is known to be in the long arm of chromosome 3. However, because of the low crossing over with lg2, it is impossible to place yd2 to the left or right of this locus.

Table 2

The result of the testcross of $\frac{ra2\ lg2\ +}{+\ +\ yd2}$ plants

Parental classes ra lg +	+ + yd	c.o. region 1 ra + yd	+ lg +	c.o. region 2 ra lg yd	+++	c.o. region 1 & 2 ra + +	+ lg yd	Total
66	121	23	28	1	0	0	0	239
%		21.3		0.4				

If yd2 is close to lg2 it should show fairly loose linkage with a. Table 3 shows the result of a linkage test with this gene that confirms the expectations of loose linkage. Since a small population is involved

in this cross, the observed recombination value of 40.6% is probably not too reliable.

Table 3

Two point testcross linkage data involving a and yd2

Parental classes		recombinations		Total	% recombination
A yd	a +	A +	a yd		
53	42	29	36	160	40.6

In sum, the data indicate that yd2 is located on the long arm of chromosome 3 in the vicinity of the lg2 locus.

Donald S. Robertson

2. Placement of luteus*-1106 on the chromosome 4 linkage map.

In 1960 an apparently spontaneous luteus mutant occurred in one of my stocks. The phenotype of this mutant is somewhat variable. Seedlings are not intensely yellow but tend to be on the pale side and some develop a little chlorophyll.

F_2 data from a cross with wxT4-9g (4S.27, 9L.27) indicated linkage. The testcross results given in Table 1 involve the following translocations: T4-9g, T4-6 (4447) (4S.28, 6L.14), T4-6b (4S.80, 6L.16), and T4-6 (8380) (4S.47, 6L.18). Although translocation linkage data are unreliable, the results in Table 1 are in agreement with the location of l*-1106 in the short arm of chromosome 4 between the breakpoints of T4-6 (4447) and T4-6b in the close proximity of the breakpoint of T4-6 (8380) (4S.47).

Pollination of stocks heterozygous for l*-1106 by plants carrying TB-4a (4S.25) resulted in ears segregating for luteus seedlings, thus confirming the placement of the gene in the short arm of chromosome 4.

Three-point testcross data involving su and gl4 are given in Table 2. These data place l*-1106 in the short arm of chromosome 4 to the left of su about two crossover units.

Table 1

Testcross data for crosses of 1*-1106 with T4-9g, T4-6 (4447), T4-6b, and T4-6 (8380)

Translocation	Parental classes		Recombinations		Total	% recombination
	T +	+ 1	T 1	+ +		
T4-9g	133	133	6	4	276	3.6%
T4-6 (4447)	148	177	4	8	337	3.6%
T4-6b	55	120	1	8	184	4.9%
T4-6 (8380)	142	121	3	1	267	1.5%

Table 2

Testcross data for plants of the genotype $\frac{+ \quad su \quad gl4}{1^*\text{-}1106 \quad + \quad +}$

Parental classes		c.o. region 1		c.o. region 2		c.o. region 1 & 2		Total
+ su gl/1 + +		+ + +/1 su gl		+ su +/1 + gl		1 su +/ + + gl		
113	132	3	3	22	38	0	0	311
%		1.9		19.3				

Donald S. Robertson

3. Location of pale yellow*-PI177593 on chromosome 4.

A pale yellow seedling mutant was found segregating in a Plant Introduction accession from Turkey. Young seedlings of this mutant start out medium yellow and fade to a pale yellow with age.

Data from an F_2 linkage test with wxT4-9g (4S.27, 9L.27) indicated the gene was on chromosome 4. Testcross results involving py*-PI177593 and T4-9g, T4-6 (4447) (4S.28, 6L.14), and T4-6 (8380) (4S.47, 6L.18) are given in Table 1. The data from Table 1 are not

Table 1

Testcross data from crosses of py*-PI177593 and T4-9g, T4-6 (4447) and T4-6 (8380)

Translocation	Parental classes		Recombinations		Total	% recombination
	T +	+ pg	T pg	+ +		
T4-9g	118	100	2	0	220	0.9%
	T pg	+ +	T +	+ pg		
T4-9g	56	77	0	1	134	0.7%
Total	351		3		354	0.8%
	T +	+ pg	T pg	+ +		
T4-6 (4447)	260	181	1	1	443	0.5%
	T +	+ pg	T pg	+ +		
T4-6 (8380)	193	168	2	2	365	1.1%

very helpful in placing the gene. They do suggest, however, that it is located in about the proximal one quarter of the short arm. This was confirmed when crosses with TB-4a (4S.25) failed to segregate for py*-PI177593 seedlings.

The results of linkage tests of this mutant with su are given in Table 2. Since py*-PI177593 is not uncovered by TB-4a and su is, the data from Table 2 would indicate that py*-PI177593 is 9.5 crossover units proximal to su.

Table 2

Testcross data from the cross su su x $\frac{+}{su}$ $\frac{py^*\text{-}PI177593}{+}$

Parental classes		Recombinations		Total	% recombination
+py	su+	++	su py		
120	128	21	5	274	9.5%

Donald S. Robertson

4. Linkage data for five pigment deficient mutants.

Over the years I have accumulated linkage data on the following mutants:

w*-5625 - This is an off-white albino mutant that is on the long arm of chromosome 1, 11.3 (n = 159) crossover units from the breakpoint of T1-9a (1S.13). Placement in the long arm was indicated by the segregation of the mutant in crosses with TB-1a (1L.2).

w*-PI168013 - This is an off-white albino mutant isolated from a Plant Introduction accession from Turkey. Since this mutant shows 0.6% (n = 171) recombination with T1-9c (1S.48) and is uncovered by TB-1b (1S.05), it is located in the short arm of chromosome 1.

17 - This is a seedling mutant that varies from yellow-green to pale green in color. It is uncovered by TB-9b (9S.4), thus indicating it is on the short arm of chromosome 9. Linkage tests with wx and c indicate 21.6% (n = 282) recombination with the former and 16.3% (n = 270) recombination with the latter gene. Since the c - wx distance is approximately 33 crossover units, 17 must be located between c and wx.

nec*-PI1217486 - This is a yellow necrotic mutant that was isolated in a Plant Introduction accession of Dakota Flint. Allele tests have shown this mutant to be identical to sienna-7748, nec*-6697, and 1*-Blandy2. The following linkage data with three translocations involving chromosome 8 indicate it is located on the long arm of this chromosome: T8-9d (8L.09) - 14.0% c.o. (n = 358); T6-8 (6873) (8L.29) - 4.3% c.o. (n = 299); and T6-8 (6187) (8L.51) - 2.5% c.o. (n = 321).

w*-PI1228183 - This is an off-white albino that was isolated from a Plant Introduction accession from Russia. Linkage tests with T3-9c (3L.09) indicate 34.7% crossing over (n = 251). No linkage is indicated with waxy,

hence this gene probably is on chromosome 3. This gene is not uncovered by TB-3a (3L.1); thus it is in the short arm or proximal 10% of the long arm.

Donald S. Robertson

UNIVERSITY OF IOWA
Iowa City, Iowa
Department of Botany

1. A mutant of chromosomal behavior in mitosis and meiosis.

The isolation of a B^9 isochromosome from the TB-9b translocation has been described (Carlson, 1970) as well as the isolation of misdivision products from the B^9 isochromosome (Carlson, 1973). Following conversion of the B^9 chromosome to an isochromosome, with apparent loss of a minute short arm, the ability of the chromosome to undergo nondisjunction at the second pollen mitosis was not lost and possibly not even impaired. However, among six telocentric derivatives of the B^9 isochromosome, four were found to be virtually incapable of nondisjunction. The other two derivatives carried out nondisjunction at the second pollen mitosis at a high rate. Differences between misdivision products of the isochromosome may be related to the extent of damage done to the centromere during misdivision. In any case, only the chromosomes which do not undergo nondisjunction will be discussed. These B^9 chromosomes will be referred to as mutants of nondisjunction. Experiments were carried out to analyze what component of nondisjunction is missing from the mutant chromosomes. In one experiment, the possibility that B chromosomes could restore nondisjunction to the mutant chromosomes was tested. One of the derivative chromosomes (mul-1) was combined with two isogenic Black Mexican stocks which vary only in the presence or absence of B chromosomes. Plants of $9^{\underline{Bz}}9^{B}$ (mul-1) $B^{9^{\underline{Bz}}}$ constitution, with and without B chromosomes, were crossed as male parents onto a bz tester. If B chromosomes restore nondisjunction to the mutant chromosome, generating bronze kernels in the testcross, it may be assumed that a gene(s) which

acts in a trans manner is missing from the mutant chromosome. However, as seen in Table 1, B chromosomes do not restore the normal non-mutant condition in which the frequency of bz kernels ranges from about 20% to 30%. In the four plants lacking B chromosomes the rate of nondisjunction is quite low and, while it rises somewhat in the presence of B's, it does not attain anywhere near normal levels. The percent of kernels with fractional or mosaic (Bz/bz) endosperm phenotypes is also given in Table 1. In all crosses the unstable kernel types are more frequent than the bz cases of nondisjunction, further demonstrating the inability of B chromosomes to restore "normal" behavior to the mutant B^9. In fact, the B chromosomes appear to destabilize the mutant chromosome, and one may wonder whether the apparent increase in nondisjunction in the presence of B's may not also result from a destabilization of the chromosome rather than true nondisjunction. At the present time, fractional (single event) and mosaic (multiple event) kernels have not been separated nor has the process of accepting or rejecting small fractional events been standardized. Thus, these data should be taken with some caution. However, the rise in Bz/bz kernels with the addition of B chromosomes is unmistakable.

One interpretation of these findings is as follows. The centromere has lost a function which is vital to nondisjunction. The addition of B chromosomes destabilizes the mutant chromosome at the second pollen mitosis, but does not generally induce nondisjunction. Let us assume, as suggested by the results of Rhoades, Dempsey and Ghidoni (1967), that heterochromatic regions adjacent to the B centromere become "sticky" at the second pollen mitosis and initiate nondisjunction. This process may occur in the mutant B^9, but nondisjunction fails because the centromere is incapable of unipolar orientation. The behavior of the mutant chromosome may be likened to that of a univalent chromosome in meiosis: it is undivided but not capable of orienting properly to one pole. The mutant B^9 undergoes a possibly delayed splitting and disjunction to opposite poles. The addition of extra B chromosomes contributes a gene activity which increases the stickiness of the centromeric heterochromatin, but does not allow for unipolar migration. The competition between nondisjunction and disjunction destabilizes the chromosome.

Table 1

Comparison of nondisjunction in the presence and absence of added B chromosomes for the centromeric mutant of nondisjunction, mul-1

Plant numbers are given for male parents which were $9\frac{Bz}{}9^{B}$ (mul-1) $B^{9\frac{Bz}{}}$. Female parent was a bronze tester. Since the appropriate $9^{B}B^{9}$ gamete is not always transmitted through the pollen, the data should be multiplied by two (approximately) to obtain actual frequencies.

Plants without B's

Male parent	Percent bz	Percent Bz/bz	Total seeds
2071 A	0.2% (3)	1.9% (29)	1499
2071 B	0.5% (7)	1.2% (17)	1422
2071 C	0.2% (4)	1.6% (25)	1590
2071 D	0.2% (2)	2.0% (26)	1315

Plants with B's

Male parent	Number of B's	Percent bz	Percent Bz/bz	Total seeds
2073 - 4	3	0.2% (3)	2.2% (33)	1467
2073 - 7	4-5	1.9% (22)	4.4% (51)	1152
2074 - 7	7	2.4% (50)	8.8% (181)	2071
2073 - 6	7-8	3.5% (47)	6.7% (97)	1435

Table 2

Testcross data of $9\frac{bzwx}{}9^{B\frac{Wx}{}}$ mutant $B^{9\frac{Bz}{}}$ ♀ x bzwx ♂

Female parent	Bz Wx	Bz wx	bz wx	bz Wx	Ratio $9^{B}B^{9}/9B^{9}$
mul-1	388	306	905	54	1.58/1.00
1849-13	337	204	761	58	2.24/1.00

The analogy to a meiotic chromosome leads to the question of whether genes that control meiosis also control mitotic nondisjunction. The answer may be yes, at least for the centromeric mutants of nondisjunction. The meiotic behavior of two misdivision products of the B^9 isochromosome (mul-1 and 1849-13) were followed by crossing $9^{\underline{bzwx}}9^{B^{\underline{Wx}}}$ (mul-1 or 1849-13) $B^{9^{\underline{Bz}}}$ plants as female parents to a bz wx tester. Formation of the major gametic classes ($9^{\underline{bzwx}}$; $9^{\underline{bzwx}}B^{9^{\underline{Bz}}}$; $9^{B^{\underline{Wx}}}B^{9^{\underline{Bz}}}$) can be determined genetically (Robertson, 1967). The raw data are given in Table 2. The calculated frequencies of the relevant classes show that the 9^BB^9 gamete exceeds the $9B^9$ class considerably. These two gametic classes were found in equal frequencies by Robertson (1967) and the author when normal B^9 chromosomes were present.

The above data suggest that a change in mitotic orientational behavior at the second pollen mitosis is accompanied by a change in meiotic orientation. The meiotic behavior of normal B^9's, as described by Robertson, is considered anomalous. Equivalence of the 9^BB^9 and $9B^9$ gametes means that in the 99^BB^9 trivalent the B^9 chromosome disjoins at random from its pairing partner, chromosome 9. Rhoades (1940) had earlier found that disjunction of a telocentric chromosome 5 from its partner in 5 5 telo 5 trivalents was regular. The mutant B^9's, discussed here appear to be reverting toward "normal" chromosome behavior. The finding can be rationalized if chromosomes normally require that tension be applied to the centromere for orientational stability (Nicklas and Koch, 1969). During the second pollen mitosis a chromosome undergoing nondisjunction is essentially a univalent and tension cannot develop on the centromere. The result is instability. Perhaps the B chromosome possesses a specialized centromere which does not depend on tension for orientational stability. Nondisjunction can then occur regularly, but during meiosis segregation from a trivalent is abnormal. The mutant B^9's, derived by misdivision of the isochromosome, may have modified centromeres which respond to tension during orientation. They are incapable of unipolar orientation at the second pollen mitosis and they tend to disjoin from chromosome 9 during meiosis. Although the mutant B^9 does not always disjoin from its pairing partner in a trivalent, as

the telocentric 5 does, Maguire has found (1970) trivalent situations not involving B chromosomes in which disjunction is also not completely regular. In Rhoades' work with the telocentric 5, proximal exchanges between 5 and telo 5 were studied. This may have added another inducement to disjunction which is unrelated to tension. Nicklas discusses the tendency for centromeres to face in opposite directions (1967), and this tendency may be more prevalent in closely associated centromeres.

The findings given here are tentative but two conclusions may result. First, nondisjunction consists of at least two events: stickiness of the centromeric heterochromatin followed by unipolar orientation and migration. Second, genes involved in meiotic and mitotic chromosome segregation may in some cases be identical.

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Wayne Carlson

2. B chromosomes induce nondisjunction in $9B^9$ pollen.

Roman (1949) found that the B^4 chromosome of TB-4a was incapable of nondisjunction at the second pollen mitosis when present in $44B^4$ plants. The $4B^4$ pollen, while unbalanced, functions at a fairly high rate and inheritance of the B^4 is regular, in contrast to the frequent nondisjunction found in $4^B B^4$ pollen. Since the 4^B chromosome is missing in $4B^4$ pollen, the distal part of the B (on 4^B) may carry a gene(s) required for nondisjunction. Alternately, the duplication of genes on chromosome 4 may for some reason prevent nondisjunction. Following Roman's work, similar results were obtained for several other A-B translocations, i.e., AB^A pollen does not support nondisjunction of the B^A. Since it seems unlikely that the duplication of several different groups of genes would each inhibit nondisjunction, it is generally assumed that distal regions of the B chromosome carry a gene(s) involved in nondisjunction. Ward's recent (1973) localization of a factor in the distal tip of the B which is needed for nondisjunction rests on this assumption. However, one might argue that genetic imbalance of any kind is detrimental to the fitness of pollen, and nondisjunction may in some way be linked to pollen fitness and therefore to balanced pollen. The data presented below demonstrate that B chromosomes do indeed carry a distally located factor(s) required for nondisjunction. Utilizing TB-9b, it is shown that the B^9 chromosome of $9B^9$ pollen can be induced to undergo nondisjunction in the presence of added B chromosomes. Hyperploid $9^{\underline{bz}\ \underline{wx}}$ $9^{B^{\underline{Wx}}}$ $B^{9^{\underline{Bz}}}$ $B^{9^{\underline{Bz}}}$ plants were constructed that differed in the presence or absence of B's. The two groups of plants (with and without B's) were related but not isogenic. The plants were crossed to a sh bz wx B Pl tester and Bz wx kernels were selected in the progeny. The Bz wx kernels are expected to derive mainly from $9^{\underline{wx}}$ $B^{9^{\underline{Bz}}}$ pollen. The very low rate of crossing over (< 0.5%) between wx and the translocation breakpoint virtually eliminates the 9^B chromosome from this group. In Table 1 are given the seedling classifications for plants grown from Bz wx kernels.

Table 1

Seedling classification of kernels with Bz wx endosperm phenotype.

Kernels were progeny of the cross sh bz wx B Pl x $9^{\frac{bzwx}{}}$ $9^{B\frac{Wx}{}}$ $B^{9\frac{Bz}{}}$ $B^{9\frac{Bz}{}}$ ± B's.

Male parent	Number of B's in male parent	Number of Bz wx kernels planted	Seedling classification of Bz wx kernels		
			Bz	bz	Green (not classifiable)
1763 - 1	0	21	20	0	1
1768 - 1	0	135	131	0	3
1770 - 1	0	126	121	0	1
1771 - 2	0	134	132	1	0
1772 - 1	0	62	57	1	0
		478	461	2	5
1764 - 2	2	23	7	10	4
1765 - 2	7	63	18	41	3
1766 - 1	1-2	32	14	18	0
1767 - 2	2	61	25	33	2
1769 - 2	3-4	34	10	22	1
		213	74	124	10

Germination was uniformly high and classification was relatively good, although some plants failed to develop color. Plants without B chromosomes show almost no nondisjunction and it is likely that the two bronze plants in the seedling classification resulted from hetero-fertilization rather than nondisjunction. Among plants with B chromosomes, the rate of nondisjunction is very high since approximately two-thirds of the seedlings were bronze. The reciprocal class of nondisjunction, in which a bronze kernel and purple seedling are present, was also found at high frequencies in plants with B chromosomes. The data

are not given here because of problems in achieving high germination frequencies for the bronze endosperm class, which also has a defective (sh) endosperm phenotype.

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Wayne Carlson

KRASNODAR RESEARCH INSTITUTE OF AGRICULTURE
Krasnodar-12, USSR

1. Combining ability of high protein opaque-2 maize lines for protein content in diallele crosses.

Twelve opaque-2 high protein lines were included in this study (Table 1).

Lines 1 to 9 were related by their high protein source, IHP, which in the local environment of Krasnodar Region shows 24-28% protein; lines 10, 11 and 12 were not related either to each other or to lines 1-9. All lines except 1 and 8 have a common opaque-2 source, Synthetic A o_2 (S Ao_2); line 1 had the source B 37 o_2, and line 8 had the genetic stock $o_2 ra_1 gl_1$ as a source of o_2. The lines from 9 to 12 were heterozygous for o_2.

Two-directional diallel crosses were made in 1972 in 3-5 female ears. A pollen mixture from 5-7 plants was used. The kernel protein content of the absolute dry matter was determined in separate ears, sibbed and female as well. The lack of outcrossing for protein made it possible to utilize the results of analyses for evaluation of the lines for protein. The morphological traits of lines were relatively uniform and corresponded to S_2, S_3 generations.

In 1973 all F_1 crosses and parental lines were sown in a randomized block design (plots of 10 sq.m.) using four replications. The grain

Table 1

Utilized lines

Line number	Pedigree	Generation	1972						1973
			Sibs			Crosses			
			No. of analyzed ears	Protein means (%)	Lysine (g)	No. of analyzed ears	Protein means (%)	Protein variation (%) min.-max.	Protein (%)
1.	[(Rustler x $B370_2$) x IHP]	S_3	1	16.8	4.3	39	17.8	16.2-19.9	18.9
2.	[(BC_2WF_9 x SAO_2) x IHP]	S_3	2	17.7	3.5	40	17.0	14.7-19.4	17.6
3.	[(MT305 x SAO_2) x IHP]	S_3	6	16.9	3.3	38	17.4	14.8-18.9	16.8
4.	[(MT324 x SAO_2) x IHP]	S_3	7	17.3	4.1	49	17.3	15.3-20.1	17.6
5.	[(D6 x SAO_2) x IHP]	S_3	4	19.3	4.0	48	17.1	15.8-20.1	17.2
6.	[(Grushevskaya x SAO_2) x IHP]	S_3	5	20.0	3.6	41	19.9	16.2-26.4	17.5
7.	[(A344 x IHP) x (BC_2A344 x O_2ra_1)]	S_3	3	17.9	4.3	41	16.9	15.2-19.1	15.9
8.	[(BC_1Cg25 x SAO_2) x IHP]	S_3	3	16.8	3.3	46	17.6	15.0-19.6	16.5
9.	BC_2(IHP x SAO_2)	S_3	4	22.9	3.0	49	24.1	20.7-27.9	23.4
10.	BC_3(Grushevskaya x SAO_2)	BC_3	5	13.8	4.0	46	13.8	12.3-15.5	12.7
11.	BC_1(Odessa 10 x SAO_2)	BC_1	3	15.1	-	43	15.5	13.2-17.8	16.0
12.	[BC_2(A96 x SAO_2) x IHP]	BC_2	5	15.0	4.0	55	14.7	13.2-16.5	14.3

Table 2

Means of opaque-2 hybrid yields** (q/ha) and protein content (%) of diallel crosses (reciprocal means)

Line	1	2	3	4	5	6	7	8	9	10	11	12
1	18.9	17.9	17.2	16.7	15.8	16.8	16.0	16.6	18.7	14.2	15.6	14.9
2	33.9	17.6	18.6	17.6	17.1	17.2	15.9	17.3	19.2	14.0	15.6	14.4
3	37.3	40.0	16.8	16.7	16.7	17.3	16.6	17.4	18.9	14.2	16.2	14.3
4	37.8	43.8	44.3	17.6	16.7	17.3	16.5	16.5	17.5	14.2	16.2	14.6
5	40.7	42.1	42.8	43.8	17.2	16.7	14.7	16.7	18.3	14.0	15.4	14.5
6	43.8	36.5	37.5	42.6	36.4	17.5	16.3	17.0	17.6	14.3	15.0	14.2
7	39.4	48.2	46.5	40.4	52.0	40.2	15.9	15.8	15.7	13.4	14.2	12.6
8	35.4	38.9	44.6	35.6	35.4	36.1	42.2	16.5	20.0	14.2	16.6	15.0
9	44.3*	35.8*	46.9	44.2	42.6*	37.4	40.2	22.5	23.4	15.2	18.8	16.9
10	44.2	45.5	50.9	53.6	46.0	42.3	45.4	39.2	48.7	12.7	13.0	12.0
11	51.3	54.1	40.7	60.0*	49.2	46.8	49.7	33.7	48.2*	48.2	16.0	13.9
12	57.1	52.6	57.3	56.7	48.4	48.5	58.2	43.5	74.0*	52.0	83.2*	14.3

* kernel yield for hybrids with +/+ and o_2/+ genotypes.

** kernel yield to be read below underlined values.

yield was evaluated in Q/ha at 14% moisture level. The protein content was evaluated separately in replicates 1 and 3 of each cross.

The same test included as standards some opaque-2 hybrids yielding approximately at the same level as the commercial dent hybrids. The protein level of the standards was about 10-11%. The data on yield and protein content are listed in Table 2 as means of the reciprocal crosses.

Hereafter, only results of the combining ability analysis for protein content are discussed.

The dispersion analysis showed a highly significant genotypical difference among crosses (for protein content: $F(165,495) = 24.41$). This allowed us to proceed with the combining ability analysis using Method 1 by Griffing (Model 1).

All variability sources significantly influenced the changes in test results (Table 3). The General Combining Ability (GCA) is the most significant in determining the protein level. The GCA variance was about 40 times more than the Specific Combining Ability (SCA). The variation of reciprocal differences was much less pronounced; the mean square of reciprocal effects was three times less than the SCA variance. Thus, we may conclude that the combining ability for protein content is mainly influenced by additive genes.

Table 3

Dispersion analysis of combining ability for protein content

Source	DF	Sum of squares	Mean squares	F	
				Estimate	Reference P 0.01
G C A	11	406.40	36.95	266.31	2.24
S C A	66	61.60	0.94	6.72	1.44
Reciprocal effects	66	18.95	0.29	2.01	1.44
Errors	495	--	0.14	--	--

The lines when compared individually showed important differences in general combining ability: the GCA effects varied from -2.3% to +2.3% (Table 4). Lines 2, 3 and 9 showed a high combining ability (they were related to IHP). Lines 5 and 7, also related to IHP, showed an intermediate combining ability, and lines 1, 4, 6 and 8 had a somewhat lower GCA than the previous lines. The lowest GCA effects were shown by lines 10, 11 and 12, which were unrelated to IHP.

In analyzing the SCA constants, it should be noted that for most of the hybrids non-additive genes do not affect the protein level; their constants do not significantly differ from 0. However, a relatively high specific interaction may occur in some cases (crosses 2x3, 4x9, 7x9, 8x9, etc.). The variances of SCA constants show a non-additive type of gene action; they may be a property not only of some crosses, but of the lines themselves. Thus, lines 7 and 9 showed SCA variances of 0.81 and 0.36, respectively, which are 2-8 times more than in other lines.

The effect of "negative heterosis" for protein level is confirmed by the SCA constants of parental lines, which are, as a rule, higher than in their crosses. None of the crosses of lines 1, 4, 5, 6, 7, 9, 10, 11 and 12 had SCA constants significantly higher than the parental ones. However, in crosses of lines 2, 3 and 8, some combinations showed constants significantly higher than the parentals; this gives us some hope that the negative heterosis for protein level in some combinations may be not only reduced, but excluded as well.

Though the reciprocal differences were significant in the whole experiment, the individual effects in most cases had moderate values: from 66 reciprocal combinations, only 12 differed significantly from 0 in both directions. The reciprocal effects variances were very low.

To evaluate the influence of the procedure on the results obtained, we analyzed separately the experimental data from nine lines related to IHP, using Methods 1 and 3 after Griffing (Model I). We obtained similar results, though numerical values differed slightly, and the order of lines in GCA and SCA was the same. The difference was due to the exclusion of unrelated lines, which reduced the variation in GCA and SCA.

The comparison of yield of high protein opaque-2 hybrids (Table 2) with that of the standard hybrid Krasnodarsky 82 o_2 [which in nine

Table 4

Analysis of combining ability of lines for protein content (Griffing, 1956, procedure, Model 1)

Line	SCA constants (sij) and reciprocal effects (rij)*												GCA effects (gi)	Variances	
	1	2	3	4	5	6	7	8	9	10	11	12		GCA	SCA
1	1.9	0.6	-0.0	-0.2	-0.8	-0.1	0.2	-0.5	-0.2	-0.1	-0.5	-0.4	0.4	0.16	0.43
2	-0.1	0.0	1.1	0.3	-0.1	0.6	-0.7	-0.1	0.1	-0.6	-0.7	-0.6	0.7	0.48	0.28
3	0.4	-0.5	-0.6	-0.5	-0.1	0.1	0.7	0.1	-0.2	-0.2	0.2	-0.5	0.7	0.48	0.19
4	-0.3	-0.4	-0.1	0.7	0.2	0.1	0.9	-0.5	-1.2	0.0	0.3	-0.1	0.4	0.16	0.16
5	-0.2	0.7	-0.1	0.1	1.1	0.2	-0.6	0.0	-0.1	0.2	-0.2	0.2	0.0	0.00	0.16
6	0.5	-0.6	0.1	0.4	0.0	0.5	0.6	-0.1	-0.5	0.1	-0.9	-0.5	0.4	0.16	0.16
7	0.0	-0.2	1.2	0.5	-0.1	0.2	1.5	0.0	-1.9	0.5	-0.5	-0.8	-0.1	0.00	0.81
8	0.0	0.1	-0.2	-0.3	-0.3	0.1	-0.4	-0.7	1.1	-0.7	0.5	0.2	0.5	0.24	0.22
9	-0.1	-0.2	-0.5	0.2	-0.1	-0.2	-1.3	0.4	2.7	-0.9	0.9	0.3	2.3	5.28	1.36
10	-0.2	-0.2	-0.2	0.1	0.0	0.0	-0.1	0.0	0.0	1.2	-0.2	0.1	-2.3	5.28	0.26
11	0.4	0.2	-0.2	0.0	0.4	0.2	-0.8	-0.5	-0.6	0.4	1.0	0.2	-0.5	0.24	0.31
12	-0.3	0.0	-0.2	-0.2	-0.1	0.4	-0.4	0.1	0.5	0.4	0.3	1.9	-1.8	3.24	0.44
RC variances	0.03	0.09	0.16	0.03	0.03	0.04	0.39	0.01	0.22	0.00	0.14	0.04			

Standard errors:

$\hat{g}_i - \hat{g}_j = 0.10$ $\quad \hat{s}_{ii} - \hat{s}_{ij} = 0.60$ $\quad \hat{s}_{ij} - skl = 0.30$

$\hat{s}_{ii} - \hat{s}_{jj} = 0.50$ $\quad \hat{s}_{ij} - \hat{s}_{ik} = 0.40$ $\quad \hat{r}_{ij} - rkl = 0.40$

*Reciprocal effects are below underlined means.

tests in this experiment yielded 61.0 Q/ha (P 0.05; LSD=5.9 Q/ha) at a protein level of 10.7%], showed that most of the experimental hybrids yielded much less than the standard. However, some crosses of lines 10, 11 and 12 with lines unrelated to the protein source yielded very close to the standard Krasnodarsky 82 $\underline{o}_2$, even when a negative correction of 10 - 12% was made in the yield of dent hybrids.

The results of the study of inheritance of protein level in F_1 crosses lead us to expect a relatively high protein level (16 - 17%) in hybrids.

The fact that the lines are related both to the high protein source and to the allele $\underline{o}_2$ source as well prevents us from making a conclusion about the possible level of heterosis attainable if totally unrelated high protein lines, pre-selected for combining ability, had been used.

The results of this work emphasize the necessity of creating high protein lines of different origin, totally unrelated to IHP. This would be essential for a breeding program of high protein $\underline{o}_2$ hybrids.

M. I. Hadjinov
K. I. Zima
A. A. Normov
V. Z. Pakudyn

2. <u>A simplified procedure of backcrossing for transferring the recessive trait to the recurrent parent</u>.

The routine procedure of developing counterparts differing from the recurrent parents in only one trait, determined by the recessive allele of a gene, may be further simplified with regard to the backcrossing and controlling the allele transfer (AT). The resulting reduction of the work needed will be about two times less.

The AT presence in BCn plants is commonly monitored by selfing or by crossing with a form homozygous for the transferred trait (TT).

We propose a procedure by which the backcrossing and control of AT may be achieved on the same ear of the plant selected for backcrossing. A tester is needed, possessing in homozygous condition, the AT and a dominant trait for kernel color. Such a tester can easily be developed in three generations by crossing the AT source with a genetic marker of the <u>ACR</u> type, for example, the Purple Embryo Marker.

With such a tester available, the BCn plants are pollinated with a pollen mixture derived from the parent and the tester. The ear, pollinated with such a pollen mixture, gives rise to two groups of kernels which are easily separated: 1) normal kernels, resulting from pollination by the recurrent parent (the new backcross) and 2) purple kernels, resulting from the analyzing cross. The latter class reveals whether or not the selected BCn plants provided the AT. If AT is present in the BCn plant, it must be heterozygous and 50% of the colored kernels should be homozygous for AT, that is, when the TT is expressed in the kernel stage ($\underline{o}_2$, $\underline{su}$, $\underline{ae}$, $\underline{du}$, etc.), about 50% of the kernels must show the TT pattern. Their sibs derived from the backcross are sown for the following BC.

When the TT pattern is expressed in the sporophyte ($\underline{bm}$, $\underline{lg}$, $\underline{br}$, etc.), it is necessary to initiate two plots from one ear: 1) kernels from the testcross and 2) BC-kernels. Plants for the following BC are taken from the preceding BC, whose sibs derived from testcrossing segregated for TT.

This method allows a reduction in the number of pollinations by two, the crossing procedure is simplified, and the results achieved are just the same. The mixture of pollen from the recurrent parent and the tester is prepared only once for backcrossing all the plants of one line. It is not necessary to label individually and harvest separately the ears of one line. There is no need to select the paired ears from testcrossing and backcrossing.

This procedure cannot be applied to some flints because of the presence of inhibitors of genes coloring the aleurone layer.

M. I. Hadjinov
M. V. Tshumak

UNIVERSITY OF MARYLAND
College Park, Maryland
Department of Botany

1. The ws_3 lg_1 gl_2 region.

For a number of years, $\underline{Ws_3}$ $\underline{Lg_1}$ $\underline{Gl_2}$ / $\underline{ws_3}$ $\underline{lg_1}$ $\underline{gl_2}$ x $\underline{ws_3}$ $\underline{lg_1}$ $\underline{gl_2}$ has been used to demonstrate linkage and crossing over to students in cytogenetics. This testcross has several advantages for instruction. Ample seedling populations are obtained in two weeks or less following planting in greenhouse benches. The phenotypes are of interest to the students and permit quite rapid and accurate classification. A light spraying with water aids scoring of $\underline{Gl_2}$ $\underline{gl_2}$ and $\underline{gl_2}$ $\underline{gl_2}$, since droplets adhere to the homozygous recessive seedlings and not to the heterozygotes. The other categories are obvious to the students.

Each student shells his own kernels directly from an ear and is not informed of the genes concerned until actual classification. Approximately 1200 seedlings are scored each year. Each classification by a student is verified by another; the few questionable seedlings are brought to the attention of the instructor. This procedure thus assures confidence in the pooled data. The distribution of the eight phenotypic classes discloses to the students that linkage and crossing over rather than independent assortment occurred. The order of the genes and recombination percentages are then calculated. Reference to McClintock's (1931) positioning of $\underline{lg_1}$ within the terminal four chromomeres of the short arm of chromosome 2 and the study of the linkage map (Neuffer et al., 1968) complete the exercise.

Accuracy of scoring is evident from the consistency of data from year to year. Accordingly, extensive good data have been accumulated for this region and are presented in Table 1.

The map in Neuffer et al. (1968) shows $\underset{0\ \ 4\ \ 11\ \ 30}{ws_3\ \ al\ \ lg_1\ \ gl_2}$ with the position of $\underline{al}$ uncertain. Recombination was less than indicated by the map. Coincidence was calculated from the data in Table 1, according to the method in Serra (1965), and a value of $0.28 \pm .04$ was obtained disclosing considerable interference.

Table 1

Results from testcrosses of $\underline{Ws_3}\ \underline{Lg_1}\ \underline{Gl_2}$ / $\underline{ws_3}\ \underline{lg_1}\ \underline{gl_2}$ x $\underline{ws_3}\ \underline{lg_1}\ \underline{gl_2}$

	Non-cross-overs	Single cross-overs region 1 ($\underline{ws_3}$-$\underline{lg_1}$)	Single cross-overs region 2 ($\underline{lg_1}$-$\underline{gl_2}$)	Double cross-overs	Total
No.	12,534	1,341	2,788	68	16,731
%		8.0	16.7	0.4	

The occurrence of double crossovers among the progeny from different ears was highly variable. We have recently found that the occurrence of doubles is not fortuitous but is dependent upon the frequency of single crossing over and the degree of interference for different female parents (Bard and Morgan, 1973).

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D. T. Morgan, Jr.

UNIVERSITY OF MASSACHUSETTS
Waltham, Massachusetts
Suburban Experiment Station

and

HARVARD UNIVERSITY
Cambridge, Massachusetts
Bussey Institution

1. A congruous background for the tr and pd genes.

The mutant genes in maize for a two-ranked spike (tr) and for single female spikelets (pd) are usually unstable in expression, especially in the heterozygous condition and in a genetic background of typical modern maize. A sporadic proliferation to many ranks and to paired spikelets appears to be promoted by the extensive vascularization of a thick modern cob. When the tr and pd genes are transferred to a background of the string cob (Sg) trait in combination with the teosinte chromosome 4 complex, the axis is vascularized more like that of teosinte and these mutant genes acquire a stable phenotype and are fully fertile in the homozygous compound (tr tr, pd pd).

Both the tr and pd reduce vascular development in the cob. The additive effects of such reductions in the compound tr tr, pd pd may result in either female sterility through lack of style development or the expression of one of the genes may exclude that of the other with the phenotype fluctuating back and forth on a single cob. But when teosinte chromosome 4 is introduced a harmonious balance is reached which allows fertile expression of the double mutant combination. This teosinte segment, discovered and extracted by Mangelsdorf, increases vascular development in the rind of the cob and this is tied into the spikelet supply trace by fusion in the glume cushion.

Walton C. Galinat

2. On the individual breeder's gene pool.

With the hazards of genetic erosion now common knowledge, the corn breeders, both commercial and public and almost without exception, have started their own isolated gene pools of variation into which they introduce all available exotic stocks of maize. The idea of their own

living bank is to have a ready source of variation to screen for any trait of emergency value. If the breeder wishes to maintain maximum variability comparable to that in a wild population of teosinte, only 1 or 2 seeds from each plant, regardless of ear size, should be saved to propagate the gene pool. In contrast, if all seed is bulked and a random sample planted, there will be automatic selection for certain traits such as increased ear size with resulting loss of variability. The least productive plants may, in some adverse circumstances, hold the greatest potential for corn improvement.

Walton C. Galinat

3. Chromosome morphology of white multiple tester and its hybrid with Burnham Spreader.

In view of the fact that the white multiple tester is used extensively in genetic studies, it was found desirable to study in detail the chromosome morphology to distinguish the individual chromosomes.

The ten chromosomes of these two stocks have been identified from preparations of cells showing all the ten chromosome pairs. The averages of ten such cells, with additional information on some of the chromosome pairs lying free of the rest either individually or in groups of varying numbers, have been tabulated. The rates of contraction of different chromosomes within cells and between cells tend to vary. Hence, a great number of cells was examined.

White multiple tester:

At pachytene the twenty chromosomes form 10 bivalents and one of them is the nucleolus organizing pair. The longest chromosome is 84.6 u with an arm ratio of 1.9. Chromosome 4 has a knob situated internally on the long arm. The fifth chromosome, which has a nearly median centromere, has a small chromomere in the longer arm. The nucleolus organizer chromosome has a small internal knob in the long arm and is similar in morphology to the organizer chromosome of other stocks; it occupies the 6th position by virtue of its length. The long arm of chromosome 8 bears an internal knob and adjacent to this knob towards the terminal end there is a small chromomere. Chromosome 9 is terminated by a knob in its short arm while chromosome 10 is distinct in having a short arm the greater part of which is heterochromatic (Table 1).

Table 1

Pachytene Chromosomes of White Multiple Tester

Chromosome	Long arm (microns)	Short arm (microns)	Total (microns)	Arm ratio	Knob position
1	54.0	28.8	84.6	1.9	-
2	41.4	28.8	72.0	1.4	-
3	41.4	18.0	61.2	2.3	-
4	36.0	18.0	55.8	2.0	IK/LA
5	28.8	27.0	57.6	1.1	
6	41.4	16.2	59.4	2.6	IK/LA N.O.
7	32.4	12.6	46.8	2.6	-
8	41.4	10.8	54.0	3.8	IK/LA
9	27.0	14.4	43.2	1.9	TK/SA
10	23.4	9.0	36.0	2.6	-

IK - Internal knob LA - Long arm N.O. - Nucleolus organizer
TK - Terminal knob SA - Short arm

White multiple tester X Burnham Spreader:

The white multiple tester was outcrossed with the Burnham Spreader stock and the resulting hybrid was studied in detail for the morphology of chromosomes at pachytene. In this stock the pachytene spread was found to be very good and all the individual chromosomes could be easily distinguished. By considering both the total lengths and arm ratios, as well as knob and chromomere patterns, all of the 10 chromosomes could be identified.

The mean absolute lengths of the longest and shortest chromosomes are 90.0 u and 45.4 u, respectively. Chromosome 2 has small chromomeres at the terminal region of the short arm, while 3 has a distinct chromomere adjacent to the centromere in its short arm. Chromosomes 4, 7 and 8 have internal knobs in their long arms. In addition, a subterminal chromomere is found in the short arm of chromosome 4. Chromosome 5, with a nearly median centromere, has a chromomere in one of its

arms. The nucleolus organizing chromosome, i.e., the 6th chromosome, has a conspicuous knob in its long arm. The 7th chromosome is heterozygous for the knob and has a heterochromatic region adjacent to the centromere in its long arm. Chromosome 9 has a terminal knob in its short arm, while 10 has a very distinct short arm which has a greater segment of its length heterochromatic (Table 2).

Table 2

Chromosomes at Pachytene of the WMT hybrid

Chromosome	Long arm (microns)	Short arm (microns)	Total length (microns)	Arm ratio	Knob position
1	53.6	36.0	90.0	1.4	-
2	48.9	34.2	84.9	1.3	-
3	53.3	25.9	80.6	2.1	
4	44.3	25.9	72.0	1.7	IK/LA
5	35.3	32.0	68.8	1.6	-
6	42.8	12.6	57.2	3.4	IK/LA
7	37.4	14.8	54.0	2.6	IK/LA
8	39.9	13.3	55.1	3.1	IK/LA
9	32.4	16.6	49.0	2.0	TK/SA
10	30.6	11.2	45.4	2.7	-

IK - Internal knob LA - Long arm N.O. - Nucleolus organizer
TK - Terminal knob SA - Short arm

P. Chandravadana
W. C. Galinat

4. More on affinity of Tripsacum chromosome 7 to maize and teosinte chromosome 4.

We reported previously that when the addition monosomic (20 + 1) for the Tr7 chromosome from Tripsacum marked by Su_1 was on a normal A158 maize background, it had a trivalent frequency of about 6.1 percent from associations with the maize bivalent for chromosome 4 (M4). When the maize background was changed by making the fourth chromosome bivalent

heterozygous for a teosinte segment (derived originally from either Florida or Nobogame teosintes in separate experiments), then the trivalent frequency shot up abruptly to a range of 60 to 70 percent (MNL 45 and 46).

In order to resolve the question as to whether the sudden increase in trivalency involving Tr7 was due to heterozygosity for the fourth chromosome bivalent or due solely to the introduction of the teosinte segment, a control addition monosomic for Tr7 was developed in which the teosinte segment was homozygous, again involving the fourth chromosome segment from Florida teosinte in one family and from Nobogame teosinte in another family.

At pachytene Tripsacum chromosome 7 was usually found as a univalent folding back on itself. It showed a very feeble association with the homozygous teosinte bivalent. More often it was found sticking to the terminal region of one or the other of the corn chromosomes. In a few instances it was in close proximity to the long arm of corn chromosome 4 although the common Su_1 locus is in the short arm, but it did not show any chiasmatic association. In one case the Tripsacum univalent was found sticking to the terminal knob of chromosome 9 of maize. The trivalent frequency scored at Diakinesis and Metaphase I was about 20 percent in comparison with the 60 to 70 percent rate seen in the triple heterozygote.

On the basis of the preceding cytological observations it appears that Tr7 has not shown sufficiently greater affinity to teosinte chromosome 4 than to maize chromosome 4 to account for the high rate in the triple heterozygote. Perhaps a slightly heteromorphic condition in the maize-teosinte heterozygote releases cryptic affinity for Tr7 that enhances the trivalent frequency, thereby explaining its high rate in the triple heterozygote.

P. Chandravadana
W. C. Galinat

5. Revision in a tentative identification of an extracted Tripsacum chromosome.

We had previously made a tentative (20 + 1) identification of an extracted Tripsacum chromosome (MNL 46:111) that had a partial ability to suppress expression of bm_2 as being Tr3, based on a length of 40.3 u

and an arm ratio of 3.1:1 in the monosomic condition. Since then, a change in the maize background and the derivation of the disomic condition for this Tripsacum chromosome requires a revision in this identification.

From the progenies derived by selfing the 20 + 1 plants we have now obtained three plants showing a 20 + 2 condition (72-445-5; 470-5, 471-8). From 31 observations on these three plants, the Tripsacum chromosome averaged 32.8 u with an arm ratio of 3.9:1 and, as previously evident, possessed a terminal knob in the long arm. This chromosome is more similar to Tr5 than to Tr3 in the idiogram of the original T. dactyloides prepared by Chandravadana et al. (1971). Because Tr5 had been assigned by us as the Tripsacum homeolog to maize chromosome 9, we have intercrossed this new Tr5 (?) to the old Tr5 as well as to the maize marker gene stock for M9.

In any case, it is clear that the new Tr5 (?) is not a partial homeolog to M1 as we had tentatively reported. Under the growing conditions of 1973, the expression of bm_2 in the presence of the extra Tripsacum chromosome was undeniable. Some plants carrying this extra Tr chromosome showed the multiple recessives, sr_1, br_1 and bm_2 loci marking almost the entire length of M1.

We shall check the synaptic behavior of the new Tr5 (?) with our original extraction of Tr5 in a hybrid made for this purpose. Also we shall check the capacity of new Tr5 (?) to cover the M9 markers known to be present on the old Tr5.

R. V. Tantravahi
P. Chandravadana
W. C. Galinat

6. On the relation of Tr9, a Tripsacum partial homeolog to M2, to maize M10.

Because Tr9, which carries the Ws_3, Lg_1, Gl_2, b, Sk, Fl_1 loci in common with the short arm of M2 occasionally associates with the short arm of M10 and may even transfer its terminal knob to M10 (MNL 44:117-119), this Tripsacum chromosome was tested for its capacity to cover the nl and g_1 markers on M10. For this purpose, these chromosome 10 recessives were added to the multiple recessive marker stock for chromosome 2. Within

the F_2 of an outcross of the M2 tester carrying Tr9 to the compound tester for M2 and M10, an equal number of Tr9 plants showed nl g_1 (16) as did not show nl g_1 (17 plants). We can only conclude that if Tr9 does have any true homeology to M10, it does not include the nl and g_1 loci. Because the nl and g_1 loci mark both arms of M10, the possibility of any true relationship to Tr9 seems slight.

Walton C. Galinat

UNIVERSITA DI MILANO
Milan, Italy
Istituto Di Genetica

1. Selection of lines in relation to the effect of the opaque-2 gene on kernel weight in maize.

In order to reduce the negative effect on yield associated with the conversion of normal strains into the opaque-2 endosperm type, it has been suggested that material be selected in which the expression of the opaque-2 gene on kernel weight would be modified (Alexander, 1966; Sreeramulu and Bauman, 1970). This suggestion is based on experimental results showing that the effects of opaque-2 on the physical traits of the endosperm vary with the genetical background. The phenomenon has been studied by comparing different hybrid combinations (Lambert et al., 1969; Salamini et al., 1970) and analyzing the variation within F_2 (Ottaviano and Cabulea, 1971) and synthetic varieties (Ottaviano, unpublished data).

This note gives a brief account of the results obtained by a selection experiment. The material used was derived from F_2 plants of the previous study; it consisted of half-sib families open pollinated by homozygous (o_2o_2) plants of the same population. Selection was made taking into account kernel weight differences between normal and opaque-2 endosperm types. To evaluate these differences using segregating kernels of the same ear, only heterozygous (o_2+) plants were used. The same procedure was adopted in the successive generation in which homozygous (++) plants were discarded on the basis of progeny testing. In each

generation, plants were selfed and the kernels produced were used for the evaluation. Within each half-sib group a two-way selection was performed: one way to reduce and the other to enhance the differences between opaque and normal kernels. The lines produced will be referred to as (+)-lines and (-)-lines, respectively.

A very high positive association was found between the differences and the weight of normal kernels, indicating that most of the difference variability is due to a multiplicative effect of $\underline{o_2}$. Therefore, in each group the values were adjusted to the mean weight of normals. This criterion should make it possible to utilize the variability due to genes interacting with $\underline{o_2}$ rather than to select mainly for kernel weight. After two generations of selfing and selection 36 S_3 lines (18 for each way of selection), the parents of the population (P_1= Bianchi $\underline{o_2o_2}$, P_2= W64A$\underline{++}$) and the F_1 (P_1 x P_2) were crossed with two testers: WF9 $\underline{o_2o_2}$ and R109B $\underline{o_2o_2}$. As the selected lines were held in heterozygosis ($\underline{o_2+}$) it was possible to produce homozygous ($\underline{o_2o_2}$) and heterozygous ($\underline{o_2+}$) progeny from each cross, so the effect of selection was evaluated considering both genotypical combinations.

The hybrids produced were planted together in two replications to form two complete blocks of 39 plots. Each plot consisted of two 10-plant rows: one formed by the normal progeny ($\underline{+\ o_2}$) and the other by opaque-2 progeny ($\underline{o_2o_2}$). Homozygous plants and five heterozygotes were detasselled in order to ensure open pollination by only segregating plants.

Ears of five detasselled plants were harvested in each row. Opaque and normal kernels from these segregating ears were separated and 50 kernels of each were weighed. Statistical analysis was performed using mean values per row; however, since nonsignificant differences due to the plant genotype ($\underline{o_2o_2}$ vs. $\underline{++}$) within crosses were found, the results are given in terms of mean values per plot.

The most significant results concerning differences in weight between normal kernels and their opaque-2 counterparts are given in the table.

Differences in 50 kernel weight (g.) between normal and opaque-2.

	observed	adjusted	% of normal
(WF9o_2) x			
(+)-Lines mean	0.54	0.59	4.0
range	0.07 - 1.05	0.33 - 0.91	0.6 - 6.6
(-)-Lines mean	0.73	0.69	5.3
range	0.37 - 1.27	0.53 - 1.02	3.0 - 8.6
(-)-(+)	0.19*	0.10*	1.3*
P_1,F_1,P_2	0.27,0.62,1.08	0.32,0.64,1.32	1.9,4.6,8.8
(R109o_2) x			
(+)-Lines mean	1.01	1.05	7.1
range	0.51 - 1.59	0.83 - 1.36	4.1 - 10.3
(-)-Lines mean	1.25	1.20	8.5
range	0.46 - 1.83	0.74 - 1.57	3.5 - 10.9
(-)-(+)	0.24	0.15*	1.4*
P_1,F_1,P_2	0.92,0.92,0.69	0.56,0.91,0.78	6.4,6.5,4.9

*significant differences ($P < 0.05$). Ranges are evaluated considering hybrid means.

Differences were linearly associated with kernel weight. Therefore, in order to evaluate selection response, the values were adjusted to the overall mean of normal kernels. Significant differences were found according to both criteria of comparison (original and adjusted means) and these results were the same with both testers. The values of the hybrids involving parental lines fell within the range of values of hybrids obtained crossing testers with selected lines; this shows that a recombination of genes interacting with o_2 introduced by both parents of the original population took place. Mean differences between the two groups of lines obtained by two generations of selection, even though significant, were not very great. This fits in with the prediction

made on the basis of genetical analysis of the population; in fact, the variability in the reduction of kernel weight due to the effect of opaque-2 was found to be under genetical control ($h^2 = 0.52$), but its amount was not very large when the portion attributable to the multiplicative effect of the gene was removed. However, it must be remembered that the hybrids compared differed in only one parent. Therefore, the differences would probably have been larger if crosses were made between lines selected in the same direction. Moreover, since the pollen has effects on endosperm development, the differences between hybrid values might have been underestimated because each combination was not pollinated by its own pollen.

In many cases it was observed that the reduction of differences between normal and opaque kernels was associated with modification of the endosperm phenotype, which shows visible translucent sectors. It remains to be seen whether this phenomenon changes the chemical composition of the endosperm.

E. Ottaviano
A. Camussi
M. Motto

2. Nucleolar patterns in microspore quartets of trisomic 6, in relation to trisomic inheritance.

Trisomic inheritance of the nucleolar chromosome (chromosome 6) was investigated. Microsporocytes collected from trisomic plants were examined in connection with the behavior of the three chromosome 6 units during meiosis. Root tips were collected, and their chromosomes counted, in a sample of individuals obtained from the cross: 2n+1 x 2n. Comparisons were made of the trisomic 6 frequency (38%) found among the progeny of this cross with the meiotic behavior of the chromosomes present in triplicate. The data indicated that post-meiotic losses of n+1 spores and/or of 2n+1 zygotes or embryos may take place, in addition to meiotic losses, and may partially account for the failure of trisomic types to reach the theoretical frequency of 50% in the progeny.

The finding of post-meiotic losses in this trisomic 6 material contradicts Einset's statement (Genetics 28:349-364, 1943) that failure of the extra chromosome to be transmitted to 50% of the progeny through the egg apparently was due only to its elimination as a univalent during

the meiotic divisions. After examining eight primary trisomes, Einset found the n+1 spore frequency close to the 2n+1 seedling frequency following the same type of cross as described above. However, a re-examination of Einset's pooled data shows an excess of n+1 spores over 2n+1 seedlings with a χ^2 value of 3.6, which corresponds to a P value of 0.06 (very close to the significance level of 0.05). The outcome of the present investigation supports the hypothesis that, after meiosis is completed, some mechanisms may limit trisomic transmission.

A great majority of microsporocytes (86.5%) examined at anaphase I showed a 2-1 segregation of chromosomes 6 to opposite poles. The remaining fraction was about equally distributed for events of univalent pre-division (equational division) and univalent lagging followed by loss.

The analysis of the second meiotic division revealed that most chromatids from pre-divided univalents were included in telophase II nuclei (only 2% of telophase II cells showed a laggard, while in nearly 6% of the anaphase I cells the unpaired chromosome was observed to undergo an equational division). The same conclusion is reached after analyzing the nucleolar patterns in microspore quartets:

Expected frequencies	Type 1	Type 2	Type 3	Type 4	Total
					
(A)	100	0	0	0	100
(B)	86.0	1.8	7.2	5.0	100
(C)	(17.8)	49.1	32.1	(1.0)	100
Observed frequencies					
(D)	20.3	32.3	46.4	1.0	100

Where:

(A) = Theoretical frequency with 100% 2-1 disjunction at anaphase I (no meiotic losses).

(B) = Approximate expected frequency of nucleolar organizer distribution based on meiotic observations.

(C) = Expected frequency of nucleolar distribution, calculated from the expansion of $(m+d)^2$ (type 4 and type 1 were arbitrarily separated).

(D) = The observed frequencies based on a population of 3,492 quartets.

m = frequency of mononucleolate dual organizer spores, calculated as .5662

d = frequency of dinucleolate dual organizer spores, calculated as .4338

If (D) is compared with (B), type 2 and type 3 are found in excess, at the expense of types 1 and 4. This discrepancy is explained by the tendency of two N.O.'s to develop a single nucleolus, or by the tendency of two nucleoli to become fused into a single structure, due to the proximity of the related chromosome segments.

A comparison of (D) with (C) revealed an excess of type 3 at the expense of type 2. This may be related to trivalent 6 formation, which would be responsible for the proximity of N.O.'s following a 2-1 chromosome 6 segregation at anaphase I. This proximity would be maintained through the second division in most cases.

A study of nucleolar patterns in microspore quartets of tetraploid maize was made by G. Doyle (MNL 44:155-157, 1970) who argued that the mirror image spatial relationship of the chromosomes in the two telophase I nuclei is often lost in the formation of metaphase II plates, which are rotated 90° with respect to the metaphase I plate.

Other less frequent types of microspore quartets with relation to nucleolar distribution were found, but will not be discussed here.

Type 4 quartets resulted, presumably, from pre-divided univalents which were both included in telophase II nuclei, after migrating in opposite directions. This diagonal configuration is peculiar, with regard to the behavior of univalents, to trisomic 6 individuals and, with some modifications, to monosomic 6 individuals (D. F. Weber, personal communication).

A. Ghidoni

3. A dominant "blotch leaf" factor, homozygous lethal, located in chromosome 9.

A heritable blotch leaf character, present in a stock obtained from K. V. Rinehart at Indiana University in 1967 and in an accession from E. B. Doerschug, was investigated. Both were sugary (su_1) and the two samples proved to have the same mutant. This material was apparently isolated from commercial sweet corn. Affected plants develop necrotic leaf spots starting from the two-leaf stage. Various degrees of expression are often found especially in plants belonging to families with different backgrounds. Necrotic blotches, normally 1-2.5 mm in diameter, often develop first on the distal part of the leaf blade, and then spread to most of the leaf blade and, with lower intensity, to leaf sheaths and other green parts of the plant. The necrotic spots are often surrounded by a yellowish halo and may, in extreme cases, merge to destroy most of the green surface. The character is better expressed at maturity. The pollen appears normal, but defective seeds are frequently produced upon selfing blotch leaf plants.

This character appears to be controlled by at least one dominant gene: in crosses of blotch x non blotch plants the blotch character is expressed in less than 50% of the F_1; after selfing blotch plants the character is expressed by a fraction of the progeny with a ratio close to 27:37. These preliminary observations indicate that three independent dominant genes may be involved in the expression of the blotch character. If the hypothesis is correct, the ratio found in the F_2 may be the result of selfing plants which were heterozygous for all three factors. To explain the ratios found in outcrosses of blotch plants, one must assume that they were possibly heterozygous for all three factors, while the non blotch parent may have been homozygous recessive for one or two of them, i.e. it carried at least one dominant factor.

Plants homozygous for the blotch character apparently did not occur, suggesting that at least one of these factors is homozygous lethal, or the homozygous combination of two or three genes may cause lethality.

After crossing blotch plants to a set of wx marked translocations, F_1 blotch plants were used in tests for linkage between the

blotch character and the regions marked by the translocations. Linkage was observed between the bloch character and wx with all of the 17 translocations used. This indicates that at least one of the factors is probably located in chromosome 9. An average of 22% recombination was obtained from pooled data involving all of the translocations. The translocations may have caused some pseudo-linkage in regions proximal to the breakpoints. Moreover, the location of wx with regard to the breakpoint differs from translocation to translocation. Finally, the independent segregation of two more factors makes it difficult to estimate the actual linkage. Therefore, other tests are underway to place the factor in chromosome 9 more accurately. The tentative symbol for this factor is Bl_4.

Similar characters, controlled by single factors, were described by various authors. A blotch leaf factor was first described by Emerson (1923) as a recessive (bl), occasionally behaving as a dominant. Another factor (bl_2) was investigated by R. C. Wiggans (unpublished, cited by J. Weijer, 1952). The bl_3 factor was described by N. W. Simmonds (1950) and assigned to chromosome 10, but was later placed in chromosome 2 by E. M. Clark. Other factors controlling similar characters include: a recessive factor reported by J. W. Cameron (1964); a necrotic leaf spot factor, recessive, allelic to zn_1 (A. R. Hornbrook and C. O. Gardner, 1970); and a leaf fleck factor (lf_1), recessive (J. L. Brewbaker, 1970). Recently M. G. Neuffer (1973, and personal communication) described two mutants induced by EMS treatment, producing necrotic leaf spots of different size; both factors apparently are single dominant genes, segregating 1:1 in outcrosses, and possibly characterized by lethality in the homozygous condition.

A. Ghidoni

4. Constitution of nonparental strands isolated from R intralocus recombinants.

The data to be presented have been obtained in an effort to explain a quite unexpected result we observed while studying recombination within the R region. Our previous work (Gavazzi and Calati, 1972) indicated that it is possible to isolate, in the progeny of testcrosses

of $\underline{R}^{st}\ \underline{r}^{r}$ and $\underline{R}^{sk}\underline{r}^{r}$ heterozygotes with an $\underline{r}^{g}\ \underline{r}^{g}$ tester, exceptional seedlings carrying properties of both parental markers, i.e., variegated (stippled or smoky) aleurone and red seedling or colorless aleurone and green seedling.

The parental genotype expressed in terms of the P and S components of the R locus is symbolized $\underline{p}\ \underline{S}^{var}/\ \underline{P}\ \underline{s}$, where $\underline{S}^{var}$ stands for either stippled or smoky seed component, while the lower case letters p and s, as used in this context, do not distinguish between presence of a recessive allele and absence of the gene component. The genetic analysis of the exceptional progeny derived from $\underline{p}\ \underline{S}^{var}/\ \underline{P}\ \underline{s}$ parents indicated that they carry nonparental $\underline{P}\ \underline{S}^{var}$ and $\underline{p}\ \underline{s}$ strands. The event leading to nonparental $\underline{P}\ \underline{S}^{var}$ strands is quite uncommon (2.69×10^{-4}) and it is confined, judging from our own experience, to those parental genotypes where one of the two chromosomes 10 carried the large knob (K 10) in the distal portion of the long arm.

On the other hand, when $\underline{P}\ \underline{S}^{var}/\ \underline{p}\ \underline{s}$ plants are mated to $\underline{p}\ \underline{s}$ males and progeny kernels germinated, seedlings with a recombinant phenotype are observed with a frequency as much as 200 times greater than the frequency of the original event producing the $\underline{P}\ \underline{S}^{var}$ strand.

This is true for all four of the $\underline{P}\ \underline{S}^{var}$ independent isolates so far tested. One of the four isolates, referred to as "case 2," was studied extensively with the intent of elucidating the genetic basis of the dramatic increase in recombination. This strand was originally isolated as a $\underline{g}\ \underline{P}\ \underline{S}^{sk}$ recombinant from a heterozygous $\underline{G}\ \underline{P}\ \underline{s}\ \underline{K}\ /$ $\underline{g}\ \underline{p}\ \underline{S}^{sk}$ female. The appropriate genotypes for studying recombination were obtained following the mating: $\underline{G}\ \underline{p}\ \underline{S}^{sc}/\ \underline{G}\ \underline{p}\ \underline{s}\ \times\ \underline{g}\ \underline{P}\ \underline{S}^{sk}/$ $\underline{g}\ \underline{p}\ \underline{S}^{sk}$ (where $\underline{p}\ \underline{S}^{sc}$ designates a self-colored derivative of $\underline{R}^{st}$). Progeny kernels with either genotype $\underline{G}\ \underline{p}\ \underline{s}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (A) or $\underline{G}\ \underline{p}\ \underline{S}^{sc}/$ $\underline{g}\ \underline{P}\ \underline{S}^{sk}$ (B) were grown in the field and the resulting (A) females were pollinated with $\underline{G}\ \underline{p}\ \underline{s}$ and the (B) females were pollinated with $\underline{g}\ \underline{p}\ \underline{s}$ males.

Kernels produced from these matings were germinated and the seedlings scored for pigment production. Individuals with nonparental phenotype (i.e., red seedling and colorless aleurone or green seedling and smoky aleurone from A parents; red seedling and self colored

aleurone or green seedling and smoky aleurone from B parents) appeared (see Table 1) with a frequency of 4.1% (192/4672) and 5.9% (282/4736) in the progeny of (A) and (B) parents, respectively. A sample of the exceptional plants was progeny-tested to ascertain the validity of the screening procedure.

As can be seen from the results in Table 2, the procedure is quite effective for the identification of nonparental strands. Only a very few seedlings of (B) parentage appear misclassified. They can be accounted for by one of the following two events:

1. the lack of pigment development in a $\underline{P}\ \underline{S}^{sk}/\ \underline{p}\ \underline{s}$ seedling or
2. the reversion of smoky to self-colored occurring during embryo sac development and giving rise to a kernel with colored endosperm and smoky embryo. The former affects the estimate of $\underline{p}\ \underline{S}^{sk}$, the latter of $\underline{P}\ \underline{S}^{sc}$ nonparental strands.

A third factor interfering with a correct estimate of the P-S recombination in the "case 2" strand is the reversion of smoky to self-colored occurring during meiosis of (B) parental females leading to a $\underline{P}\ \underline{S}^{sc}$ strand indistinguishable from a recombinant. The rate of meiotic reversion has been estimated in the progeny of (A) females crossed with $\underline{p}\ \underline{s}$ males and it amounts to 0.96×10^{-3}, a frequency almost negligible when compared to the total yield of $\underline{P}\ \underline{S}^{sc}$ strands from (B) parents.

The frequency of recombination between P and $\underline{S}^{sk}$, as estimated from the data of Table 1 after correction for misclassification (see progeny test), is 4.1% and 5.7% in A and B genotypes, respectively. Such a high frequency suggests that one of the two R components has been dislocated from its original position so that P and $\underline{S}^{sk}$ are now 4-5 map units apart.

Proximal marker constitution of recombinant strands

Determination of the proximal marker (g) constitution of nonparental strands isolated from testcrosses of $\underline{G}\ \underline{p}\ \underline{s}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (A) plants with $\underline{G}\ \underline{p}\ \underline{s}$ males required a further generation of selfing while in testcrosses of $\underline{G}\ \underline{p}\ \underline{S}^{sc}/\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (B) heterozygotes with $\underline{g}\ \underline{p}\ \underline{s}$ males the constitution was established directly by classifying green vs golden plant color in the progeny seedlings (in the latter case the classification

Table 1

Frequency of exceptional seedlings with a presumed recombinant strand in the progeny of testcrosses of $\underline{G}\ \underline{p}\ \underline{s}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (A) and $\underline{G}\ \underline{p}\ \underline{S}^{sc}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (B) heterozygotes

R constitution of parental genotype	Gametes tested	Presumed strand constitution			
		(1)	(2)	(3)	(4)
$\frac{\underline{p}\ \underline{s}}{\underline{P}\ \underline{S}^{sk}}$ (A)	4672	2299	2181	95	97
$\frac{\underline{p}\ \underline{S}^{sc}}{\underline{P}\ \underline{S}^{sk}}$ (B)	4736	2269	2185	140	142

(1) $\underline{p}\ \underline{s}$ or $\underline{p}\ \underline{S}^{sc}$; (2) $\underline{P}\ \underline{S}^{sk}$; (3) $\underline{p}\ \underline{S}^{sk}$; (4) $\underline{P}\ \underline{s}$ or $\underline{P}\ \underline{S}^{sc}$

Table 2

Progeny test of the exceptional seedlings isolated as presumed recombinants in the progeny of testcrosses of plants with (A) and (B) genotype

R constitution of parental genotype	Presumed recombinant strands	Presumed recombinants			
		Isolated	Tested	Conf.	Nonconf.
(A) $\underline{p}\ \underline{s}\ /\ \underline{P}\ \underline{S}^{sk}$	$\underline{p}\ \underline{S}^{sk}$	95	26	26	-
"	$\underline{P}\ \underline{s}$	97	71	71	-
(B) $\underline{p}\ \underline{S}^{sc}\ /\ \underline{P}\ \underline{S}^{sk}$	$\underline{p}\ \underline{S}^{sk}$	140	53	51	2 ($\underline{P}\ \underline{S}^{sk}$)
"	$\underline{P}\ \underline{S}^{sc}$	142	69	65	4 ($\underline{P}\ \underline{S}^{sk}$)

for golden was extended to parental seedlings.) Classification for the g marker of nonparental strands of (A) parentage is given below:

P s	p S^{sk}
G / g	G / g
60 / 4	1 / 18

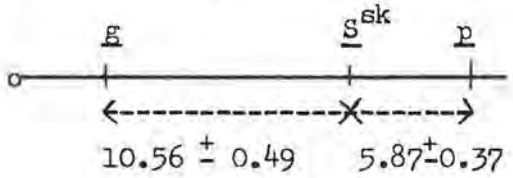
If one assumes that P is the proximal and S the distal R component (Stadler and Neuffer, 1953), the data would indicate that the majority of the recombinants (78/83) are associated with a second exchange in the region proximal to R. As to the three-point testcross data, involving G p S^{sc} / g P S^{sk} heterozygotes, recombinant strands with an additional exchange in the proximal region comprise 207 out of 232.

The results of this testcross are shown below:

P S^{sk}		p S^{sc}		p S^{sk}		P S^{sc}		total
g	G	g	G	g	G	g	G	
1616	191	201	1708	108	11	14	99	3948

The double exchange interpretation is at variance with the recombinational values for the long arm of chromosome 10 reported in the literature. A survey of published results clearly shows that in this chromosomal segment there is positive interference.

As an alternative one could interpret the results by assuming that the order of the P and S components of the "case 2" strand is inverted. According to this interpretation, the strand constitution together with the map length of each crossover region, as obtained from the three-point testcross data, would be represented as follows:



Distal marker constitution of nonparental strands isolated from (A) parents

The smoky derivative of stippled carries M^{st} (stippled modifier) as a distal marker. The constitution with regard to M^{st} can be ascertained only on nonparental strands of (A) but not (B) parentage, since only the former are heterozygous for M^{st}.

Since $\underline{M}^{st}$ affects specifically the expression of the $\underline{R}^{st}$ allele, detection of its presence or absence in nonparental strands required the matings outlined below:

1. $\underline{P}\ \underline{s}$ strands

   $\underline{P}\ \underline{s}\ /\ \underline{p}\ \underline{s}$ seedlings isolated in the progeny of testcross (A) were grown and selfed. Kernels from the self pollination were grown in the field and plants with red anthers (genotypically $\underline{P}\ \underline{s}\ /\ \underline{P}\ \underline{s}$ and $\underline{P}\ \underline{s}\ /\ \underline{p}\ \underline{s}$) were pollinated with a $\underline{g}\ \underline{R}^{st}\ \underline{m}^{st}$ male. Production of ears segregating for dark and light stippled or uniformly dark stippled indicated presence of $\underline{M}^{st}$ in the $\underline{P}\ \underline{s}$ strand, while production of ears uniformly light stippled indicated its absence.

2. $\underline{p}\ \underline{S}^{sk}$ strands

   $\underline{p}\ \underline{S}^{sk}\ /\ \underline{p}\ \underline{s}$ seedlings in the progeny of testcross (A) were grown and selfed; heterozygous $\underline{p}\ \underline{S}^{sk}\ /\ \underline{p}\ \underline{s}$ individuals from the selfed ears were grown in the field and crossed with $\underline{g}\ \underline{R}^{st}\ \underline{m}^{st}$ males. The presence or absence of about 6% dark stippled seeds from such crosses indicates the respective presence or absence of $\underline{M}^{st}$ in the $\underline{p}\ \underline{S}^{sk}$ nonparental strand.

The outside marker constitution of nonparental strands, as determined in the way just outlined, is given below:

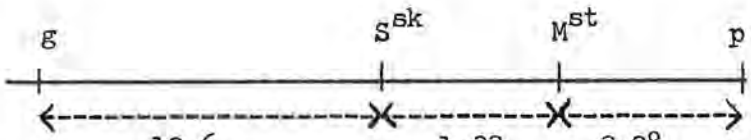
$\underline{P}\ \underline{s}$				$\underline{p}\ \underline{S}^{sk}$			
$\underline{G}$	$\underline{G}$	$\underline{g}$	$\underline{g}$	$\underline{G}$	$\underline{G}$	$\underline{g}$	$\underline{g}$
$\underline{M}^{st}$	$\underline{m}^{st}$	$\underline{M}^{st}$	$\underline{m}^{st}$	$\underline{M}^{st}$	$\underline{m}^{st}$	$\underline{M}^{st}$	$\underline{m}^{st}$
14	30	1	1	2	0	14	5

It can be seen, from these data, that among the strands selected as $\underline{P}$ - $\underline{S}$ crossovers, 47 had the exchange distally and 20 proximally to $\underline{M}^{st}$.

Since the $\underline{P}$ - $\underline{S}$ frequency of exchange in this mating (see Table 1) is 4.1%, $\underline{M}^{st}$ must then lie between $\underline{S}^{sk}$ and $\underline{P}$, 1.2 map units distal to $\underline{S}^{sk}$ (20/67 of 4.11).

By combining these data with the previous information the arrangement of the four markers on the "case 2" strand can be envisaged as shown below:

Linkage map:



Recombination values: 10.6 1.22 2.98

The main results of this analysis can be summarized as follows:

1. When P S^{var} / p s females, carrying a nonparental chromosome ("case 2" strand) derived from a p S^{var} / P s parent, are mated to p s males, P - S recombinants are observed in the progeny with a frequency as much as 200 times greater than the frequency of the original event producing the P S^{var} nonparental strand.

2. Linkage relations of the P component of the "case 2" strand have been determined in testcrosses involving the S component of R^{sk}, M^{st} and G_1. The recombination values obtained are best interpreted by assuming that the P component of the "case 2" strand is removed from its standard position and is relocated at a new position about 5 recombination units to the right of the R locus.

3. The strand with P in its new position shows a decrease of recombination in the g - S^{sk} and S^{sk} - M^{st} intervals. There is some indication that the dislocation of P is associated with physiological and morphogenetic effects, to be reported.

An interpretation of similar effects as well as a tentative hypothesis on the origin of the "case 2" strand is postponed to the time when the analysis of the other three strands will be completed. This analysis is presently underway.

G. Gavazzi
G. Galli

5. A new mutant affecting amino acid metabolism.

The problem of isolating auxotrophic mutants in eucaryotic organisms to use as experimental tools in dissecting the metabolic control processes has been faced by geneticists for some time (Nelson, 1967). Nevertheless, the success of their isolation in higher plants has been very limited when compared to the many results obtained in procaryotes, algae and fungi. Different hypotheses have been offered to explain this failure (Li and Rédei, 1969; Neuffer, 1974). However,

more experiments are needed to evaluate if this failure is a consequence of the difficulties encountered in analyzing complex organisms such as green plants. We have been working on this problem for some time. Most of the mutants we tested are not auxotrophic but temperature sensitive (Gavazzi, et al., 1973). Recently, we found a mutant that affects amino acid metabolism. The mutant is of spontaneous origin. It first appeared in our W22 stocks and it behaves as a monogenic recessive endosperm mutant, exhibiting a reduced and irregular growth of the endosperm. Germination is very poor. The coleoptile is regularly formed but the seedling becomes necrotic and dies before the emergence of the first leaf. Embryo cultures allow growth of the mutant up to about 50 days.

During this period, growth on either mineral or enriched medium (for media composition see Gavazzi, et al., 1973) proceeds slowly, in an irregular manner and with a very limited extent of shoot morphogenesis.

However, embryos cultured on enriched medium (CM) develop a far more abundant root apparatus than those grown on mineral medium (MM). The average fresh weight of the root apparatus together with the attached embryos of 48 day old mutants is 161 mg on MM, while on CM it is 304 mg. To identify the component of the enriched medium that promotes root growth in the mutant, excised roots were cultured on either basic Heller medium or on basic media with single additions of the organic components of the CM. Each medium contains 20 mg/l of sucrose. Root tips of the primary root, 5-10 mm long, were excised, one day after germination, and transferred aseptically to a 250 ml Erlenmeyer flask filled with 50 ml of liquid medium. The flasks, each with three root tips, were left on a shaker (80 rpm) for 12 days at 23 $\pm$ 2°C and 14 hrs/day of light. At the end of this period, root growth was measured as length of primary root, number of laterals, fresh and dry weight. For each weight determination, three roots were used. The average growth values $\pm$ standard error of wild type and mutant sibs are reported in Table 1. It is clear from the results that the only addition promoting growth of the mutant roots is casein hydrolysate; normal roots, on the other hand, are not stimulated in their growth by casein or the other additions but are inhibited by coconut milk. The effect of increasing doses of casein hydrolysate was then measured. It can be seen from the results, reported

Table 1

Effect of different supplements on growth of excised roots of the wild type and endosperm mutant.

Supplement	No. roots		LA		NL		FW		DW	
	+	m	+	m	+	m	+	m	+	m
Control (H)	12	9	89±4	15± 2	11±3	3±1	52±3	14±1	3.0±0.3	0.4±0.1
Casein hydr. (50 mg/l)	12	6	81±6	47±12	13±3	12±4	54±2	35±5	3.2±0.3	3.5±0.5
Yeast hydr. (10 mg/l)	6	9	71±3	12± 1	22±3	2±1	53±7	11±2	4.0±0.5	1.0±0.4
Yeast extract (10 mg/l)	3	3	78±7	13± 1	15±6	2±1	52	13	2.6	1.0
Coconut milk (2.5%)	9	9	20±1	10± 1	4±1	0	23±1	12±2	2.4±0.4	1.4±0.3

Root growth determined after 12 days of liquid culture.
LA = Length of primary root (mm/root)
NL = Number of lateral roots
FW = Fresh weight (mg/root)
DW = Dry weight (mg/root)

in Table 2, that the first two doses of 50 and 100 mg/l are equally effective in promoting growth and that the same is true for the two succeeding doses (200 and 400 mg/l) that account for a further increase in growth.

Table 2

Effect of casein hydrolysate at different concentrations on growth of excised roots of the endosperm mutant. For abbreviations see Table 1.

Supplement	No. roots	LA	NL	FW	DW
Basic medium (H)	3	12±0	4±0	10	0.7
H + cas. 50 mg/l	6	37±4	11±1	19±2	1.0±0.0
H + cas. 100 mg/l	5	35±3	9±2	14±1	0.9±0.3
H + cas. 200 mg/l	6	63±3	16±2	27±1	1.9±0.1
H + cas. 400 mg/l	6	77±7	15±2	29±1	2.1±0.1

Casein hydrolysate (acid) contains all the amino acids present in casein except tryptophan.

Excised roots of the mutant were then grown on Heller medium, either basic or supplemented with groups of amino acids (in the L form) except tryptophan.

The amino acids were grouped according to their biosynthetic relationship. Their concentration in the medium is that corresponding to an addition of 400 mg/l of casein hydrolysate. Of the four groups of amino acids tested (Table 3), the one containing ala, val, leu, ile, pro, arg, lys and glu is clearly the one with growth promoting effect. Further tests (Table 4) indicate that this effect is promoted by glutamic acid, proline and arginine. The experiment to establish whether the effect is due to a synergistic action of the three amino acids or to a single one of them has not yet been performed. However, since proline and arginine have glutamate as precursor, the latter is likely to be the component required for growth. Glutamate auxotrophy would be an interesting mutation for the study of nitrogen metabolism since the most

Table 3

Effect of groups of amino acids on growth of excised roots of the endosperm mutant. For abbreviations see Table 1.

Growth medium	No. roots	LA	NL	FW	DW
Basic (H)	12	14± 0	4±1	16± 1	1.2±0.2
H + (1)	6	68± 0	17±3	42±10	3.4±1.1
H + (2)	9	14± 0	4±1	15± 2	0.8±0.1
H + (3)	11	27±11	4±1	14± 1	0.8±0.1

(1): ala, val, leu, ile, pro, arg, lys, glu (for concentrations see text)

(2): phe, tyr, his

(3): met, thr, cys, gly, ser

Table 4

Effect of different amino acids on growth of excised roots of the endosperm mutant. For abbreviations see Table 1.

Growth medium	No. roots	LA	NL	FW	DW
Basic (H)	7	20±2	3±0	22±2	2.4±0.2
H + pro, arg, glu	14	98±6	36±3	57±6	6.0±0.7
H + val, leu, ile	8	18±1	2±1	22±1	2.5±0.1
H + lys	2	17	3	23	1.5

important reaction transforming ammonia into amino acids is a reductive amination that, under the catalytic influence of glutamic dehydrogenase, reduces d-ketoglutarate to L- glutamic acid.

Further experiments will elucidate the amino acid requirement of the excised roots as well as of the entire plant.

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G. Gavazzi
C. Piccardo

UNIVERSITY OF MISSOURI
and
U.S. DEPARTMENT OF AGRICULTURE
Columbia, Missouri

1. New mutants located with A-B translocations.

Continuing previous years' work (MNL 45:144, 46:131 and 47:148), we have located an additional 100 mutants. They are listed below by chromosome arm and can be added to those previously reported.

1S	1L	2S	2L	3S	3L	4S	4L
1 wl	1 wl	1 v	1 Yg	2 pg	1 wl	1 gl	1 v
1 yg	1 yg	1 wt	1 spotted	1 v	1 pg		2 nec
6 pg	1 pg sr	1 pg	1 pg	1 zb	1 sr		1 et
1 nec	1 ad	3 d	1 d	1 d	1 gl		1 mn
1 leth	1 rgd	1 nl	1 nl		1 d		
1 bleached	2 d	1 nec			1 bz		
1 et	1 bt	1 sr					
1 mn	1 wrinkled	1 ys					
		1 et					

5L	6L	7L	8L	9S	9L	10S	10L
1 spl lvs	2 w	1 wl	1 wl	4 w	1 v	1 wl	1 l
	3 wl	1 g	3 v	1 sh	1 d	2 zb	1 v
	1 v	1 gl	1 nec	1 zb	1 ad	1 sr	3 pg
	1 nec	1 nl	1 de			2 ad	1 ad
		1 et					1 nec
		2 de					
		2 mn					

We welcome interest by fellow workers willing to determine linkage and allelism of mutants already placed to chromosome arm and would be pleased to send seed of all the mutants of a particular arm to those interested. Mutants on the following arms have already been taken: 3L, 7L, 8L, 9S, 9L and 10L.

Since we are routinely crossing large numbers of mutants by the full set of A-B translocations, we are willing to include in our plantings a limited number of mutants from fellow workers who have something that they feel is urgent. We prefer F_1 seed. Send 20^+ kernels for each by May 1.

M. G. Neuffer
J. B. Beckett

2. Absence of auxotrophic mutants in corn and other eukaryotes.

The failure to obtain obligate auxotrophic mutants in corn and other eukaryotes has been a puzzle, especially in view of the remarkable success with fungi and bacteria. Numerous attempts by the author and others to grow various types of lethal and sub-lethal mutants on supplemental media have been mostly unsuccessful. Only a few mutants have been found in higher plants (Gavazzi, et al., MNL 47:114-121; Nelson and Burr, 1973, Annual Review of Plant Physiology 24:493-518; Li and Redei, 1969, Biochemical Genetics 3:163-170).

One possible explanation may lie in intercellular transfer of gene products. It is possible that the mutants that are commonly observed and studied are for genes whose product is not transferable (nondiffusible or cell limited or unstable). Failure of transfer is suggested by the fact that chimeras for most of the known recessive mutants in corn have distinct borders. Distinct borders are generally found for chimeras resulting from chromosome loss, from reversions

arising through the action of controlling elements and from spontaneous mutations.

If transfer is an important factor, what would be the phenotypic expression of various types of mutants with and without effective intercellular transfer of gene product? A series of predictions can be framed for the alternatives of transferable vs. nontransferable product and universally vs. stage-specific vital functions, such as chloroplast assembly or chlorophyll synthesis.

If one treats mature pollen with a mutagenic agent and produces in the pro-embryo nucleus a recessive mutant for a gene controlling a universally vital function involving a nontransferable product, one may expect the following consequences. The mutant will potentially be a cell lethal but will survive through the gametophyte generation because of the covering of the sperm by the tube nucleus and will form a viable zygote. The lethality of the mutant will not be tested until sporogenesis when, for the first time, cells will arise with only a mutant allele in the nucleus. These will lack the vital function and will abort. The phenotype will therefore be a normal appearing F_1 plant with 50% aborted pollen and ovules (semisterile pollen and ear). The mutant will not be transmitted to the next generation.

A mutant that controls a vital function for which the product can be transferred will survive through the F_1 as above, but the gametes produced will have the advantage of gene product from normal diploid cells of the supporting tissue. Thus, the gametes of the F_1 plant may survive to achieve fertilization. (This may or may not be true for the microspores, since the ♂ gamete does go through a short period of independent existence.) Assuming both ♂ and ♀ gametes succeed, the selfed F_1 will produce 1/4th homozygous mutant kernels. The mutant embryos may not be lethal at first, however, because the endosperm may carry stored gene product supplied by the ear parent. Lethality will occur when this endosperm supply is exhausted and the seedling must make its own product. The mutant would therefore be expressed in the F_2 as a normal seedling that dies when the endosperm nutrients are exhausted (about the 3-leaf stage). The necrotic lethals appear to be of this kind. If the ♂ gamete does not survive, then only normal pollen grains will effect fertilization, and the F_2

will include only normal plants. Half will be heterozygous, however. The mutant would escape detection unless special techniques such as differential transmission of linked markers are used to detect it.

A mutant that controls a stage-specific vital function such as chloroplast assembly and a product that is confined to the cell where it is produced should form viable mutant gametes because chloroplasts are not necessary for gametogenesis. The F_1 selfed will produce 1/4th homozygous mutant embryos that will grow into chlorophyll-less seedlings which will survive only as long as the endosperm nutrients last. This type of mutant would be expressed in the F_2 as white or yellow seedlings that die at endosperm depletion. The commonly occurring w, wl and l mutants would fit in this category.

A mutant that controls chlorophyll synthesis through a product that is transferable and stored may have F_2 seedlings that initially are normal or nearly normal green (depending on the efficiency of transfer) if the ear parent supplies the gene product and it is present in the embryos and endosperm until depleted by the seedling. At depletion, the green seedling should deteriorate in one of two ways. If intact chlorophyll can continue to function without the product, the first 2-3 leaves will be normal green and subsequent leaves will be more white or yellow. The plant will live for some time on the photosynthesis of the first leaves, but eventually will die. If, however, the manufactured chlorophyll in the first leaves required continued gene product to function, at depletion the existing leaves will begin to fade to white or yellow or to discolor, and the seedling will die rather abruptly. Two alternative phenotypes would appear for the F_2 of this type of mutant: (1) Initially green seedlings which produce newer leaves that are white or yellow. The mutants should survive to the 5th or 6th leaf stage. (2) Initially green seedlings which begin to fade rather abruptly to white or yellow and die. Mutants representing the first of these types have not been seen, while a number of the latter have been obtained as a result of treatment with EMS.

This sort of reasoning may lead to the recognition of a number of new classes of mutants.

M. G. Neuffer

3. Dominant mutants induced by EMS.

From a population of 3693 F_1 plants produced by treatment of pollen with EMS (method, MNL 45:146), five good dominant mutants were obtained. They included one striped virescent, one dwarf, one yellow striped and two which mimic disease lesions caused by Helminthosporium species. In addition to these five viable mutants, a larger number of dominant inviable cases also occurred, but these could not be propagated and were lost without confirmation. All five have been transmitted through the pollen for at least two generations.

The striped virescent mutant first appears as a nearly white to pale green seedling that gradually changes to a green seedling with white or pale green stripes much like v5, though the striping may be more extreme. Viability is good, though homozygotes may be too extreme to survive in some cases.

The dwarf mutant is very extreme, rarely growing more than four inches high. Plant parts are small, and internodes are shortened. Some plants produce a few normal anthers which have normal pollen. The mutant does not respond to gibberellic acid.

The yellow striped mutant is not expressed in the seedling, but first appears at the 6-8 leaf stage, when a yellowing of tissue between the veins of the terminal half of all leaves occurs. The appearance is like that of ys, but less extreme. As the plant matures, strong anthocyanin appears on the blade of affected leaves. Viability is good, though plants with extreme expression may be small and weak.

The disease lesion mutants are described under a separate heading below.

M. G. Neuffer

4. Dominant disease lesion mutants.

The first of the disease lesion mutants (designated Les) referred to above appears initially at the 3-leaf seedling stage. One to several irregular, elliptical shaped watery spots (1-3mm x 4-7 mm in size) appear scattered over the surface of the first leaf. In 24 hours these spots develop a necrotic appearance on the top surface of the leaf and sometimes a drop of dark brown exudate on the underneath surface.

Apparently there is a breakdown of cell membranes, allowing some cell fluids to escape and collect in a drop under the lesion. This is followed by an enlargement of the spot and a drying of the drop of exudate to form a lesion with a light center of dead cells surrounded by a dark ring of dried exudate which is in turn surrounded by a region of degenerating necrotic cells. This gives a halo type effect that is typical of lesions from fungal infection. Some lesions remain at this stage, while others continue to spread until the whole leaf becomes withered. This progression of events continues slowly up the plant as other leaves develop and mature. At flowering, a mutant plant may have 3 or 4 fairly sound leaves with each leaf from top to bottom being progressively more withered. The appearance is typical of and practically indistinguishable from the symptoms expressed by a susceptible plant infected with H. maydis, except that the ears produced do not have the hyphae and spore masses usually associated with fungal infections.

The second disease lesion mutant (designated Spt) is first expressed by tiny light spots on the first leaf of a two-leaf seedling. The cells in the spot die abruptly, leaving small, almost white necrotic spots (0.5 mm x 1-3 mm). The spots remain small, and new ones appear on successive leaves as the plant matures. The appearance is typical of the reaction of one type of resistant (hypersensitive) host to the fungus H. maydis. The mutant has very little effect on plant vitality.

Crosses between heterozygotes for the two mutants (+/Spt x +/Les) produced normal plants, plants with lesions, with spots and with both. The phenotype of the double mutant plants was modified, however. Lesions and spots appear on the same leaf, but the lesions were smaller, less deleterious and more frequent, while the spots were larger and less frequent. Subsequent crosses to determine allelism have not given conclusive results because of erratic expression in the progenies of the double heterozygote.

The question of whether these mutants are disease mimics or are cases of susceptibility to some commonly occurring disease-producing organism has been considered. All of the cultures were grown either in fields that were fairly free of Helminthosporium species or in a greenhouse in winter. Segregating sibs and all other cultures were free of

the effect. Crosses were made on several unrelated lines using pollen from mutant plants. In all cases the offspring segregated for mutant and normal types.

To exclude the possibility of a common external infection, kernels of a cross of normal by mutant (for both cases) were washed in 2.63% sodium hypochlorite, soaked in water overnight, and the embryos excised. The embryos were washed again and grown on a sterilized agar medium in testtubes. The mutant phenotype appeared on a number of the cultured seedlings, though not in numbers that would confirm a 1:1 ratio. Efforts to obtain fungal cultures from affected leaves have been unsuccessful.

Based on evidence so far obtained, one would conclude that the mutants are dominant mimics of two states of susceptibility to the fungus H. maydis. However, the striking resemblance of the mutant phenotypes to actual disease lesions raises some doubt.

A number of mutants resembling the large lesion type have been reported. Emerson (Cornell Memoir 70:3-16, 1923) describes a recessive mutant called blotched leaf (bl) which is expressed just before flowering. Simmonds (MNL 24:26-27, 1950) and Hornbrook and Gardner (Radiation Botany 10:113-117, 1971) report similar cases. Ghidoni (Academia Nazionale Dei Lincei, 1973) reports a necrotic lesion mutant linked to wx.

M. G. Neuffer

5. Interactions of the brown-plant anthocyanin factors.

The loci A, A2, Bz, Bz2, and C2 have recessive expressions characterized by partial or complete replacement of anthocyanins by brown pigments in husks, sheaths, cobs, and other plant tissues. Strains homozygous for B and Pl, in intensely pigmented, uniform background were developed. The comparative phenotypic expressions of a, a2, bz, bz2, and c2 now can be described with confidence from several years of tests and observations, along with the effects of these factors in combinations, two at a time. For the combinations, F2 families segregating for two factors were graded and defined without knowledge of genotype. Genotypes were identified individually from testcross results following harvest (the technical assistance of M. D. Murray in the conduct of these tests

is appreciated). The phenotypes of each of the combinations, recessive for one factor and homozygous dominant, heterozygous, or homozygous recessive for the second are given in Table 1, arranged repetitively to simplify systematic comparisons.

The effects of a, a2, and bz in combinations have been reported previously from limited observations in progenies segregating for b, pl, and other factors (Laughnan, Genetics 36: 559, 1951; Coe, Am. Naturalist 91: 381, 1957). Little can be added, apart from comparative descriptions, to the observations reported by Laughnan for these three factors, namely that a is epistatic to a2 and bz, and that a2 and bz together display a new phenotype. Combinations involving bz2 and c2 show some new information and unique effects. In all combinations a is epistatic: all display the gold phenotype. In all combinations c2 results in substantial weakening of color, similar to its effects singly (i.e., purple vs. mocha), and shows a dosage effect for intensity without alteration of the brown color type of the factor with which it is in combination. The combination a2 bz2, unlike a2 bz, is not different from a2 Bz2; consequently these plant color interactions agree with complementation tests in the aleurone tissue (Reddy and Coe, Science 138: 149, 1962) in signifying that the action of Bz2 follows the action of A2. The combination bz bz2 has a new phenotype, similar to that of a2 bz, providing no information on the order of action of Bz and Bz2 but suggesting that Bz function (glucosyl transferase) is diverse, and that it affects the formation of brown pigments specifically. In fact, it can be specified that the actions of A, Bz, and C2 are each essential to the formation of the darker brown pigmentations (ochre and mahogany), as are B and Pl, but A2 and Bz2 are not essential to their formation. The sensitivity of bz2 and of c2 tissues to dosage for A2, in which heterozygosity appears to heighten the intensity of brown pigmentation, is curious but apparently real. The dosage effect of A in bz2 plants, reducing necrosis when A is heterozygous, suggests that A may be limiting in the development of the necrotic phenotype.

The following action sequence is supported by the plant color interactions:

A - A2 - Bz2

Table 1

Expressions* of the factors controlling anthocyanins vs. brown pigments in plant tissues, singly and in combinations, with B and Pl.

	a a	a2 a2	bz bz	bz2 bz2	c2 c2
+ +		ochre-nec.	mahog.-nec.	mahog.-nec.	mocha
+ a	gold	ochre-nec.	mahog.-nec.	mahog.	mocha
a a		gold	gold	gold	wk. gold
+ +	gold		mahog.-nec.	mahog.-nec.	mocha
+ a2	gold	ochre-nec.	mahog.-nec.	dk. mahog.-nec.	dk. mocha
a2 a2	gold		caramel	ochre-nec.	wk. ochre
+ +	gold	ochre-nec.		mahog.-nec.	mocha
+ bz	gold	ochre-nec.	mahog.-nec.	mahog.-nec.	mocha
bz bz	gold	caramel		caramel	wk. mahog.
+ +	gold	ochre-nec.	mahog.-nec.		mocha
+ bz2	gold	ochre-nec.	mahog.-nec.	mahog.-nec.	mocha
bz2 bz2	gold	ochre-nec.	caramel		wk. mahog.
+ +	gold	ochre-nec.	mahog.-nec.	mahog.-nec.	
+ c2	lt. gold	lt. ochre	mahog.-nec.	mahog.	mocha
c2 c2	wk. gold	wk. ochre	wk. mahog.	wk. mahog.	

*gold: medium intensity brown with rich gold cast
ochre: dark brown with some yellow cast
mahog. (mahogany): dark brown with brick-red cast
mocha: medium brown with soft reddish-gray cast
caramel: light brown with slight yellow to red cast
nec.: necrotic in sheaths and husks after flowering
dk.: darker than is typical for that color
lt.: lighter than is typical
wk.: lighter than lt. (weak color)

The action of Bz follows that of A; that of C2 cannot be specified.

The action sequence for these factors indicated by complementation in the aleurone tissue is not in disagreement with the above:

C2 - A - A2 - Bz - Bz2

E. H. Coe, Jr.

6. Nonrandom pairing in trisomics with two standard chromosomes 3 and one chromosome 3 treated with ethyl methane sulfonate or nitrosoguanidine.

Standard trisomic 3 plants were crossed with F_2 material derived from pollen treated with EMS or NG by Neuffer (MNL 45: 146). The trisomic hybrids had two standard chromosomes 3 marked with a_1 and the mutagen-treated chromosome 3 carrying A_1. These were crossed as male parents with a_1. Numbers of plants with various percentages of A gametes are given in Table 1. At the bottom of the table are given the numbers of plants having percentages of A gametes significantly lower and higher than 33%.

The results are similar to those found when the odd chromosome was irradiated (MNL 40: 109-114) in that there seems to be an equal number of cases in which the percentage of gametes is higher than 33%. Occasionally, cases were found in commercial Corn Belt inbred line-standard trisomic hybrids (MNL 43: 127-129) where this phenomenon also occurred.

In the past, no hypothesis could be advanced to explain this "negative preferential pairing."

Now it appears that there might be a very simple explanation. Nonrandom pairing of chromosomes in polyploids is caused by two factors: The first is differential affinity resulting from structural nonhomology. The second factor involves differences in the time of synaptic activation. The following assumptions are made:

1. Chromosomes are capable of synapsis only at zygonema.
2. Chromosomes undergo a secondary structural change at the beginning of zygonema. This change will be called synaptic activation.
3. Only two chromosomes which both have synaptically activated homologous segments can synapse.
4. The time of synaptic activation is under genetic control. Homologous or homoeologous chromosomes from different races of a species may have different times of synaptic activation.
5. Synaptic activation will not be synchronous in all sporocytes. The frequencies of sporocytes with synaptically activated chromosomes will be distributed (probably normally) about a point in time.

Table 1

Nonrandom pairing in trisomes 3 with two standard chromosomes 3 and a chromosome 3 treated with a chemical mutagen. Numbers of plants with various percentages of $\underline{A}_1$.

Percent $\underline{A}$ / Family No. →	1	2	3	4	5	6	7	8	9	10	11	12	13
10	1	-	-	-	-	-	-	-	-	-	-	-	-
13	1	-	-	-	-	-	-	-	-	-	-	-	-
16	1	-	-	-	-	-	-	-	-	-	-	-	-
17	3	-	-	-	-	-	-	-	-	-	-	-	-
18	1	-	-	-	-	-	-	-	-	-	-	-	-
19	1	-	-	-	-	-	-	-	-	-	-	-	-
20	1	1	-	-	-	-	-	-	-	-	-	-	-
21	2	-	-	-	-	-	-	-	-	-	-	-	-
26	-	1	-	-	-	-	-	-	-	-	-	-	-
27	1	1	-	-	-	-	-	-	-	-	-	-	-
28	-	2	2	-	-	1	-	-	-	-	-	-	-
29	-	-	-	-	1	1	1	-	-	-	-	-	-
30	-	-	-	-	1	-	1	-	-	1	1	2	2
31	-	-	-	-	2	-	1	-	-	-	1	2	-
32	-	-	2	-	3	1	1	-	-	-	1	1	-
33	-	-	1	4	3	1	3	2	-	1	-	1	2
34	-	-	1	1	-	-	-	-	1	5	2	-	-
35	-	-	1	2	1	-	-	1	1	2	-	-	-
36	-	-	-	1	1	-	1	1	2	1	-	-	3
37	-	-	5	1	-	-	-	2	1	1	-	-	-
38	-	-	-	1	-	-	-	2	-	-	-	-	1
39	-	-	-	-	-	-	-	-	-	-	-	-	-
40	-	-	-	1	-	-	-	-	1	-	-	-	-
Total	12	5	12	11	12	4	8	8	6	11	5	4	8
-	12	5	2	0	2	2	1	0	0	1	1	0	2
N	0	0	6	7	9	2	7	5	3	9	4	4	4
+	0	0	4	4	0	0	0	3	3	1	0	0	2

6. In the case of trisomes, if the odd chromosome has an earlier time of synaptic activation than the standard chromosomes, then the frequency of sporocytes with an odd chromosome and one of the standard chromosomes activated will be greater than the frequency of sporocytes with the two standard chromosomes activated. Consequently, more synaptic associations between an odd chromosome and a standard chromosome will be formed than would be expected on a random basis.

   If the time of synaptic activation of the odd chromosome is later than that of the standard chromosome, then there will be more standard-standard associations than at random.

   In tetraploids, precocity or retardation of synaptic activation would produce only homogenetic associations. The phenomenon of "negative preferential pairing" is peculiar to trisomes.

7. The control of the time of synaptic activation is very mutable. Irradiation and chemical mutagens can shift the time (backward or forward) of synaptic activation.

G. G. Doyle

7. Telocentric 6L trisomes and their possible use in the commercial production of hybrid corn.

A preliminary report on this project has been given (MNL 46: 142-146). Additional data have been collected and will be presented.

The telocentric 6L chromosome arose spontaneously in a culture of primary trisomic 6, probably by the transverse division of a univalent chromosome 6 at meiosis.

The telocentric is apparently stable; conversion into an isochromosome has not yet been observed, but only 59 plants have been examined cytologically. It is possible that isochromosomes are formed in chimeric (Y-y) kernels if the telocentric behaves like the B^9 chromosomes described by Carlson. Individuals from chimeric kernels have not yet been examined. However, some sort of change has been occurring in the telocentric chromosome because the trivalent frequency is quite variable and, perhaps related to this, the rates of male and female transmission are also variable.

A total of 1,024 P.M.C.'s from 11 plants was examined. The trivalent frequency ranged from 35% to 62% with a mean of 48%. The chi square for homogeneity gives a p value of less than .0005.

The disjunction patterns of the chromosomes may be determined by examining the quartet stage. Quartets with two anucleolate spores are formed when the two normal chromosomes 6 go to the same pole. The frequency of these quartets has been determined in 21 plants. Out of a total of 7,483 quartets, 3.62% had two anucleolate spores. A chi square test for homogeneity gave a p value between .10 and .05.

The genetic data are summarized in Table 2.

Table 2

Cross	No. of plants	No. of gametes	%Y	%y
t6Y/y/y x y/y	522	163,809	31.30	68.70
y/y x t6Y/y/y	202	113,381	1.39	98.61
t6Y/t6Y/y/y x y/y	1	341	68.03	31.97
y/y x t6Y/t6Y/y/y	2	662	42.60	57.40

The chi square for the cases where the telocentric trisome is the male is very high, with a probability of less than .0001. The female data are not homogeneous; the rate of transmission of the t6/6 gametes seems to be heritable. One family had transmission rates all around 40%; another had rates around 25%. There seems to be no clear pattern.

The telocentric tetrasome or ditelocentric has been isolated. From the limited data, it appears that there is a high rate of quadrivalent formation which leads to the production of t6/t6, t6/6, and 6/6 gametes. Also, the 3 to 1 disjunction of the quadrivalent leads to t6, t6/6/6, t6/t6/6, and 6 types of gametes. Gametes with only the t6 chromosome do not function. When the ditelocentric trisome is used as the male parent, most of the functioning gametes are the 6 type. This is not desirable if the breeding scheme outlined in MNL 46 is to work. Structural differences must be introduced so that only 6/6 and t6/t6

bivalents are formed in the ditelocentric. This should be 20 times less difficult than the project of allotetraploidizing corn in which all 20 arms must be structurally differentiated. The telocentric has been crossed with inversions and translocations and has been irradiated.

Another problem is the transmission of t6/6 gametes in the male sterile maintainer stock (the telocentric trisome). Progeny tests indicate that the frequency of crossing over of the Ms onto the normal chromosomes is very low and this problem can be disposed of by the structural modifications mentioned above. The results of progeny tests of plants from Y kernels are given in Table 3. Diploid plants with Y/y are the result of crossing over. A large number of plants from Y kernels were found to be y/y, particularly when the telocentric came through the pollen. The telocentric is apparently lost.

Table 3

Cross	t6Y/y/y and Y/y/y	Y/y	y/y	Total	% "cross-overs"	% cross-overs
[t6Y/y/y x y/y] x yy	570	5	7	582	0.86	0.54
[y/y x t6Y/y/y] x yy	264	39	44	347	11.24	0.30

Since only part of the population can be progeny-tested (the Y class), the figures in the % "crossovers" must be multiplied by the % Y and multiplied by 2 to estimate the true crossover frequency between Y and the centromere.

There are some grievous errors in the 1972 report. Among the progeny of the ms/ms x t6 Ms/ms/ms there would be two crossover types Ms/ms and Ms/ms/ms. While the trisomic would be in a lower frequency, it is probable that a few of the plants in the first column in Table 2 are of this type. If t6Y/y/y and Y/y/y plants are progeny-tested as the female they cannot be distinguished from each other.

Another error is the statement that the telocentric trisomic method of handling genic male sterility has an advantage over

Patterson's duplicate-deficient method because his method would lead to homogeneity of cytoplasms since the duplicate-deficient chromosome is not transmissible through the pollen. Obviously, the duplicate-deficient chromosome is not backcrossed into inbred lines, but the translocation is. The translocation is both male and female transmissible. The duplicate-deficient chromosome is extracted later.

G. G. Doyle

UNIVERSITY OF NEW HAMPSHIRE
Durham, New Hampshire

1. Relation of hydroxamic acid content (DIMBOA) to resistance to Helminthosporium turcicum.

In 1959, the cyclic hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) was first reported in maize and has since been directly implicated in resistance to several pathogens. DIMBOA occurs naturally in the glucosidic form and is converted to the fungitoxic aglucone through mycelial penetration or mechanical injury.

The objectives of this study were to determine the amount of DIMBOA in thirteen inbred lines of corn utilizing the colorimetric procedure of Hamilton and to correlate DIMBOA concentration with resistance to Helminthosporium turcicum (northern corn leaf blight). Eleven inbred lines of maize obtained from R. Hallauer, Ames, Iowa, plus the two genotypes BxBx and bxbx obtained from R. H. Hamilton at Penn State University were used in this study. The bx allele designates plants deficient in DIMBOA whereas Bx is the normal allele. All of the eleven inbred lines were assumed to be carrying the Bx allele. Plants were grown in the greenhouse to 60-76 cm extended leaf height (height of the youngest leaf fully extended) for the colorimetric analysis for DIMBOA. One to two gram samples of whorl tissue were collected and extracted in ethanol. The extracts were concentrated and chromatographed by method of thin layer chromatography. Areas of spots containing DIMBOA were removed, eluted in ethanol, centrifuged and decanted into a cuvette. Upon addition of $FeCl_3$, a blue color developed which was analyzed colorimetrically

and plotted against a standard curve (from purified DIMBOA). Concentrations were expressed as mg DIMBOA/g fresh weight. Seedlings were grown under the same greenhouse conditions to 60-76 cm height for inoculation with H. turcicum. Plants were placed in an inoculation chamber, inoculated and incubated for 18 hrs. at 68°F. and 100% humidity. The degree of infection was determined on the youngest expanding leaf of each plant five days after inoculation. Percent leaf infection was calculated with the use of a transparent grid that estimated area of leaf lesions relative to the total area of the leaf.

Results showed mean concentrations of DIMBOA to range from 0.66 mg to 0.06 mg with the exception of the bxbx genotype which contained a concentration of DIMBOA below the limits of detection by this method. Percent leaf infection varied from 8.24 to 19.04 percent. A significant negative correlation ($r = -0.61$) was obtained between these two traits indicating that inbred lines of maize with high concentrations of DIMBOA generally have improved resistance to H. turcicum.

We have recently developed a more rapid procedure for analysis of DIMBOA which should be useful in breeding programs.

B. J. Long
G. M. Dunn
D. G. Routley

NEW MEXICO INSTITUTE OF MINING AND TECHNOLOGY
Socorro, New Mexico
Department of Biology

1. Water-insoluble protein electrophoresis: a potential tool for maize gene product analysis.

Most of the work that has been reported on genetic variation at the protein level has involved water-soluble proteins, especially isoenzyme systems. There exists, however, in plant and animal cells alike, a large fraction of protein which is water-insoluble. Because of the problems inherent in handling this material and in finding a suitable solvent system that can be maintained throughout separation and analysis, e.g., in a gel electrophoresis system, little work has been done with

these proteins. However, several systems are available for such analysis, and of these I have found one using phenol, acetic acid, urea and water, both as the sample solvent as well as the running solvent in the gel, to be very reliable and repeatable.

Sodium dodecyl sulfate (SDS) has, along with other detergents, come to be widely used as a dissociating and solubilizing agent for water-insoluble proteins. However, applications of this method to plant materials have been quite limited (Hoober, 1970, J. Biol. Chem. 245: 4327-34). The use of phenol, acetic acid and water (2:1:1, w/v/v) as a hydrophobic protein solvent was first suggested by Takayama, MacLennan, Tzagoloff and Stoner (1964, Arch. Biochem. Biophys. 114: 223-30). While this is a good solvent, acrylamide will not polymerize in the presence of phenol, so Takayama, et al. prepared their gels with 5 M urea and 35 per cent acetic acid. Both the SDS and the Takayama systems have proven unreliable with the plant materials used in our laboratory. Cotman and Mahler (1967, Arch. Biochem. Biophys. 120: 384-96) overcame the problem of two different solvent systems in the gel electrophoresis of mammalian neuronal membrane proteins by adding the phenol solvent to the gels after polymerization. I have adapted their technique for the electrophoresis of hydrophobic proteins from several sources in maize: chloroplasts, seeds and pollen.

In each case the sample was prepared so as to remove the water- and lipid-soluble components. Chloroplasts were isolated and disrupted by sonication (Shortess, 1974, J. Exp. Bot., in press), seeds were homogenized in a Sorval Omnimixer, and pollen was ground with an equal weight of sand in a mortar with a pestle. The residue in each case was washed twice in water, once in cold 90% acetone, once in cold 100% acetone and once in diethyl ether. This was allowed to air dry at room temperature and could be stored indefinitely at room temperature without adverse effects. The hydrophobic protein was dissolved in phenol, acetic acid and water (2:1:1, w/v/v) in 3 M urea (PAW/U). Half molar sucrose was added to increase the viscosity, which facilitated sample application to the gel. The chloroplast extract was dissolved at the rate of 7.0 to 10.0 mg/ml, while the seed and pollen extracts were dissolved at the rate of 50 to 100 mg/ml. The latter two extracts contained a great deal more

extraneous material, starch in the case of the seeds and sand in the case of the pollen, which was removed by centrifugation after at least an hour of constant agitation in the solvent.

The gels as polymerized contained 12% acrylamide, 0.3% N,N'-methylenebisacrylamide and 35% acetic acid. The catalysts were ammonium persulfate, 0.064 gm/16 ml of solution and N,N,N',N',-tetramethylethylenediamine 0.08 ml/16 ml of solution. They were cast in glass tubes, 5 x 70 mm I.D. After polymerization under a fluorescent lamp for two hours, the gels were pre-electrophoresed for at least eight hours at from one to two mA/gel. The baths contained 10% acetic acid. Then the gels, still in the glass casting tubes, were placed in PAW/U and allowed to equilibrate by diffusion. The solvent was changed after two, 24 and 48 hours, and the gels were ready for use after 72 hours from the start of the equilibration, being constantly agitated during the whole period. The equilibration was carried out at 20°C, since higher temperature causes bubbles to form between the gel and the tube.

From 50 to 100 ml of sample were placed on the gel. This was overlayered with PAW/U without sucrose. The gels were run at 3.0 mA/gel for 75 minutes, with 10% acetic acid in both baths. The upper electrode was the anode. After electrophoresis the gels were removed from their tubes and stained in 0.5% amido-Schwartz black stain, and destained electrophoretically. All gels were stored in 10% acetic acid with no detectable loss of banding pattern after a year.

All of the materials analyzed produced from ten to 20 separate, distinguishable bands. When seeds from 20 inbred maize lines were examined, a great deal of variation, presumed to be genetic, was observed among the water-insoluble proteins of the seeds (Shortess, 1973, Genetics 74: s253). Some lines showed a high degree of homogeneity while others revealed single band variants within the line. Variations have also been observed among the chloroplasts and pollen from various lines, but to a much lesser extent.

The exact function of these proteins is yet to be determined. I have some evidence to support the hypothesis that at least some of those hydrophobic proteins found in the seed, almost all of which are found in the embryo, simply serve a storage function, to be used by the seed

during germination. If this is the case, then this would account for the considerable variation among these proteins without any overt phenotypic variation. If they are merely storage molecules, they would serve only to provide amino acids for the seedling prior to the initiation of photosynthesis, a very nonspecific function. As far as the pollen and chloroplasts are concerned, one may also presume that at least some of these proteins are membrane components. There is no evidence to support any specific function.

David K. Shortess

NORTH CAROLINA STATE UNIVERSITY
Raleigh, North Carolina
Department of Genetics

1. Comparison of mitochondrial DNA from hybrids with normal and Texas cytoplasms.

Texas cytoplasm (cms T) contains factors for male sterility and susceptibility to two leaf diseases, southern leaf blight, Helminthosporium maydis and yellow leaf blight, Phyllosticta zeae. These traits are known to be inherited in an extra chromosomal fashion. A recent study (1) has found a difference in the response of mitochondria from corn with normal and sterile cytoplasm when challenged by a pathotoxin from H. maydis race T. Since this study indicated the involvement of mitochondria, mitochondrial DNA (mt DNA) must be given consideration as a possible site of factors responsible for the traits associated with cms T. Indeed, mt DNA from normal and T cytoplasms may be speculated to differ in base composition by virtue of significant alterations in the mt DNA of the Texas cytoplasm. In this connection, the mt DNA from normal and Texas cytoplasm has been isolated and characterized with respect to buoyant density and molar percent guanine and cytosine (molar % GC).

Mitochondrial DNA was isolated from two hybrids, NC232 x T204N (normal cytoplasm) and NC232 x T204 cms T. Roots and coleoptiles from 7-10 day old etiolated maize seedlings and leaves from 2-3 month old plants served as sources of mitochondria. Plant materials were ground in

a Waring blender with buffered sucrose and mitochondria were isolated by differential centifugation in a sucrose gradient. The mitochondrial fraction was suspended in Tris buffer containing 1% sodium lauryl sulfate, appropriate amounts of CsCl were added and it was stored overnight in a refrigerator. The mixture was then centrifuged at slow speed to remove the protein meniscus. Mitochondrial DNA was pelleted from the remaining clear fraction by centrifugation at 50,000 rpm for 18 hr. Preparative CsCl density gradient centrifugation (2) was used to further purify the mt DNA. Buoyant density determinations in CsCl were carried out according to the usual procedures (2) with a Beckman model E analytical ultracentrifuge.

The buoyant densities and molar % GC of the mt DNA's were identical for the two hybrids (Table 1). Furthermore, the mt DNA's from both hybrids were resolved as single components. Since no differences were found in the GC content and buoyant densities of mt DNA isolated from the two hybrids, it can be concluded that the cytoplasmic dissimilarities between the normal and the Texas cytoplasm cannot be accounted for by discernible alterations in the base composition of mt DNA. The technique used in this investigation has limited resolution; therefore, if the differences between the two cytoplasms were due to minute alterations (e.g., point mutations) in the mt DNA, they would remain undetected.

Table 1

Buoyant densities* and molar % GC of mt DNA from NC232 x T204N and NC232 x T204 cms T

Buoyant Density	NC232 x T204N	NC232 x T204 cms T
g/cm^3	1.706	1.706
% GC	46.9	46.9

*Two determinations were made for each DNA type.

Maize mt DNA has a mean buoyant density of 1.706 g/cm^3 and a molar GC content of 46.9%. This is in agreement with the conclusion that higher

plant mt DNA's have a buoyant density of 1.706 ± 0.001 g/cm^3 and a molar GC of 46.8% (e.g., 3). Furthermore, our results confirm the finding in higher plants that mt DNA forms a single band in CsCl with no satellites. From this and a previous study (2), we are now able to summarize the buoyant density and molar % GC data for the three types of maize DNA, nuclear, chloroplast and mitochondrion.

Table 2

Buoyant densities and molar % GC of nuclear, chloroplast and mitochondrial DNA of maize

	Buoyant density, g/cm^3	Molar % GC
nuclear DNA	1.702	42.9
chloroplast DNA	1.700	40.8
mitochondrial DNA	1.706	46.8

References:

1. Miller, R. J. and D. E. Koeppe. 1971. Science 173:67-69.
2. Shah, D. M. and C. S. Levings, III. 1973. Crop Sci. 13:709-713.
3. Wells, R. and J. Ingle. 1970. Plant Physiol. 46:178-179.

C. S. Levings, III
D. M. Shah

OSMANIA UNIVERSITY
Hyderabad, India
Department of Genetics

1. Confirmational studies of the accumulated flavonol in a_1 mutant aleurone tissue.

Extensive chemical analysis of a_1 mutant aleurone tissue showed the accumulation of quercetin in the hydrolysates. Average Rf values, Abs. Max. in UV, and data from infrared and mass spectral analyses confirmed its structure. Trace amounts of kaempferol (tetrahydroxy) and

quercetogetin (myrcetin?) were also present as revealed by mass spectral information. In addition, a fluorescent spot, probably phenolic in nature, was observed on chromatograms, with an Rf value of 0.18 in BAW (4:1:5). Its exact chemical nature is not yet known.

A. R. Reddy
G. M. Reddy

2. Differential action of intensifier gene.

Quantitative estimations of pigments from various double mutant combinations of the homozygous recessive intensifier gene, such as in bz_1; in bz_2; in a_1; in a_2; in Pr, from single mutants such as a_1; a_2; bz_1; bz_2, and from dominant A C R tissue have been made to determine whether the action of the homozygous recessive intensifier gene in enhancing pigment levels is uniform in all combinations. It was consistently found, through O.D. values, that recessive in increases the pigment levels in the descending order Pr, bz_1, bz_2 and a_2, whereas there is no statistically significant increase in in a_1 tissue. It seems that in increases the anthocyanins, d-deoxyanthocyanin and leucoanthocyanidin, which are known to be either the end products of the pathway or synthesized very late (leucoanthocyanidin) in the pathway. However, homozygous recessive intensifier does not boost the levels of the flavonol pigment.

A. R. Reddy
G. M. Reddy

3. Analysis of developing kernels.

A C R Pr plants were self fertilized and ears were harvested periodically 15-20 days after pollination. Colorless seeds from surface sterilized cobs were analyzed for suspected intermediates in anthocyanin synthesis. It was observed that 15 day old aleurone tissue extracts revealed the presence of quercetin and leucoanthocyanidin. No flavonoid pigment was detected in aleurone tissue of 10-14 day old kernels. Quantitative studies on the levels of various flavonoid pigments and their suspected precursors in the developing aleurone tissue are in progress.

A. R. Reddy
G. M. Reddy

4. Chemical analysis of certain mutant aleurone tissue.

The chemical nature of the mutants $\underline{C}^{I}$, $\underline{c}_1$, $\underline{c}_2$ and $\underline{r}$ was studied by using certain diagnostic chemical tests, chromatography and spectroscopy. Chromatograms of all these mutant tissues are colorless and no phenolic substance was detected. Various chemical tests, such as ferric coloration, sodium borohydride-HCl (for detecting flavonone), sodium borohydride-DDQ test (for detecting dihydrochalcones), Pachecko's test (also for detecting flavonone), HCl test (for detecting leucoanthocyanidins), Magnesium-hydrochloric acid test and Zinc-hydrochloric acid test (general), led to the conclusion that none of these mutant tissues accumulates any detectable flavonoid pigments. However, it was found that $\underline{c}_1$ extract (aqueous MeOH) responded positively to acid tests, i.e., effervescence with sodium bicarbonate and reduction of potassium permanganate. On paper chromatography with the solvent mixture ethylacetate: formic acid:water (10:2:3), it gave one colorless spot which fluoresced under UV (long range) with an Rf value of 0.63 and another faint fluorescent spot which runs along with the solvent front. When the chromatograms were sprayed with 1% sodium nitrite in 10% acetic acid and fumed with ammonia, the spot turned yellow. Thus, the preliminary analysis suggests that the accumulated substance in $\underline{c}_1$ mutant aleurone tissue may be a phenolic acid whose Rf values closely resemble those of chlorogenic acid. Further studies are in progress.

A. R. Reddy
G. M. Reddy

PURDUE UNIVERSITY
West Lafayette, Indiana
Department of Agronomy

1. Soft endosperm genes.

A group of endosperm mutants have been studied which have phenotypes similar to "opaque" but their expression is dependent on duplicate recessive factors. To distinguish them from the floury series (monogenic with dosage effect) and the opaque series (monogenic

recessive), the new genes have been designated soft endosperm. Expression of mutants 5586 and 4918 is dependent on sen, located on chromosome 3, and sen 2, located on chromosome 7. Mutant 4921 is dependent on sen 3, located on chromosome 1, and sen 4, not yet located. The expression of mutant 5595 is dependent on sen 5, located on chromosome 2, and sen 6, located on chromosome 5.

The amino acid profile of these mutants is approximately normal and the amylose-amylopectin ratio is normal.

T. R. Stierwalt
P. L. Crane

UNIVERSITY OF SOUTH CAROLINA
Columbia, South Carolina
Department of Biology

and

MSU/AEC PLANT RESEARCH LABORATORY
East Lansing, Michigan

1. Partial purification of the catalase-specific inhibitor in maize.

We previously reported evidence of a catalase inhibiting substance active in 24 hr. scutellar extracts that was apparently absent by the fourth day of germination (1). We have since initiated attempts at purification and characterization of the factor.

The inhibitor precipitates in 30-45% saturated ammonium sulfate solutions, as does approximately 80% of the catalase activity. Both activities are also retained by an Amicon XM-100 ultrafilter (100,000 MW exclusion). Gel filtration on Sephadex G-200 yields coincident peaks for inhibitor and catalase activity. In addition, a peak of inhibitor activity is seen in the void volume of the column suggesting an apparent molecular weight of several hundred thousand. This high apparent molecular weight and the copurification suggest the presence of a catalase-inhibitor complex. Attempts to dissociate this complex with urea, heat, high salt, and high and low pH have been unsuccessful. We have recently succeeded in preparing a catalase-sepharose affinity column, and expect it to be an invaluable aid in resolving this problem.

The catalase specificity of the inhibitor is demonstrated by the fact that, while it is fully active on beef liver catalase (1), it does not inhibit maize peroxidases, a group of catalytically related hemoproteins (Table 1).

Table 1

Effect of inhibitor on peroxidase. Scutellar extracts from days 1 & 4 were assayed for peroxidase and catalase activities, mixed in a 1:1 ratio, and assayed again for both enzymes.

	Peroxidase activity	Catalase activity
Day 1 Extract	12.6 u/ml	144 u/ml
Day 4 Extract	68.6	73
Expected Activity	40.6	109
Observed Activity	41.0	62
% Inhibitor	0	43

Work supported in part by AEC Contract AT (11-1)-1338.

Reference:

(1) Sorenson, J. C. and J. G. Scandalios. Isozyme Bulletin #6, 1972.

J. C. Sorenson
J. G. Scandalios

2. Purification of maize peptidases.

Leucine aminopeptidase isozymes in maize have been investigated by Scandalios (J. of Heredity 56:177, 1965) and by Beckman, Scandalios, and Brewbaker (Genetics 50:899, 1964). The aminopeptidases were shown to be controlled by four separate loci each exhibiting a pair of codominant alleles. Recently, a maize enzyme which cleaves the trypsin substrate, α-N-benzoyl-DL-arginine p-nitroanilide, was found in maize (Melville and Scandalios, Biochem. Genetics 7:15, 1972). A fast (more anodally migrating at pH 7.0) or slow variant is present in maize inbreds. Heterozygotes possess both isozymes with no hybrid enzyme band

formation. Genetic analysis showed the variants to be co-dominant alleles of a single locus designated Ep-1.

Quantitative assays for LAP and endopeptidase activity show the highest levels for both occur in the developing kernel and in the scutella and embryo of the germinating seedling.

We are presently attempting to co-purify the aminopeptidases and the endopeptidase from the inbred line W64A, which possesses the fast endopeptidase variant. One-day imbibed seed are used. Three LAP bands are present in the crude extract (LAP-A, LAP-B and LAP-C, with respect to decreasing anodal migration at pH 7.0). Both LAP and the endopeptidase precipitate at 40-55% saturation with ammonium sulfate and elute from a G-100 Sephadex column in the same volume. The enzymes bind to PE-52 Whatman cellulose at pH 7.5 and are eluted with a linear KCl gradient. An activity peak containing LAP-C elutes before a peak of activity containing LAP-A and LAP-B (as indicated from electrophoresis of the fractions). The endopeptidase peak is intermediate. Homogeneity has not been achieved yet as protein OD280 peaks do not correspond with enzymatic peaks. The endopeptidase shows activation upon addition of ammonium sulfate to 40% saturation. Activation results in activities of approximately 250% of the level of activity in the crude extract. LAP does not show activation.

Lila Ott
J. G. Scandalios

3. Diaphorase isozyme patterns in the immature endosperm of Zea mays kernels.

Diaphorase (E.C. 1.6.99.-) is a low molecular weight, flavin-containing enzyme which is involved in electron transport in the oxidation of NADH. As well as being found in the free enzyme, diaphorase is also associated with a number of substrate inducible, multisubunit enzymes, such as nitrate reductase and sulfite reductase. In these multisubunit enzymes, the diaphorase active site has been shown to be distinct from the other activities of the enzymes [Losada, M. et al. (1968) Prog. Photosyn. Res. Proc. Int. Congr. 3, 1504-9].

It is conceivable that the diaphorase gene also codes for the subunit which contains this activity in other enzymes. The zymogram

technique can be used to test this hypothesis. Genetically determined electrophoretic variants of diaphorase must be found. Alterations in the charge properties of this enzyme could also lead to alterations in the electrophoretic mobility of those multisubunit enzymes which utilize the diaphorase subunit in their function. If this hypothesis can be demonstrated, then diaphorase becomes an interesting enzyme to study in terms of the regulation of enzyme synthesis.

The endosperm of several inbred lines of corn was tested for diaphorase isozymes. Three phenotypes were observed (Figure 1). The F_1 hybrids between inbred lines with different phenotypes show a 3-banded pattern. Genetic analysis is now warranted to determine whether these phenotypes represent the expression of two genetic loci with codominant alleles at one locus, and a possible null allele at a second locus. It is also possible that the two diaphorase isozymes found in the inbred lines represent duplicated loci, and in one line of corn, this duplication has not taken place.

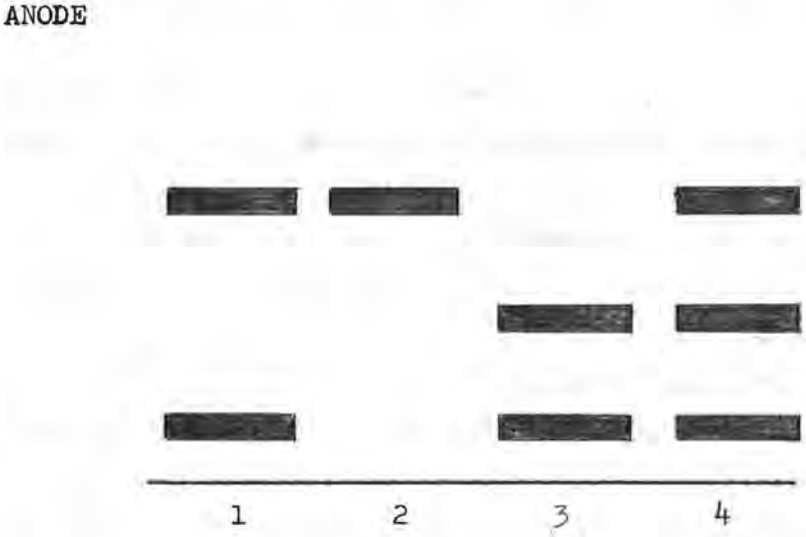


Figure 1. Diaphorase isozyme patterns found in the liquid endosperm of immature maize kernels.

1. Phenotype for inbred lines W64A, 67-2, 77, 67-75, T_4, 81, 66-37, V_4.
2. Phenotype for inbred line 38-11.
3. Phenotype for inbred lines T_{21}, 8, 78, T_{20}.
4. Isozyme pattern for the F_1 hybrid from the cross T_{21} X T_4.

Before conclusions can be drawn concerning the genetics of diaphorase in maize, it must be demonstrated that the activities measured here represent the single subunit enzyme, and that the alterations in electrophoretic mobility are not the result of association with multisubunit enzymes. This can be verified by determining the molecular weight of these isozymes from tissue extracts using standard techniques such as sucrose gradient centrifugation.

Edwin H. Liu
J. G. Scandalios

4. New alleles and chromosome localization of the locus for maize endopeptidase.

In a previous report (Melville and Scandalios, 1972) we described a single form of maize endopeptidase designated EP-1 with two variants A and B. The $\underline{Ep}_1$ locus coding for the EP-1 isozymes was found to be completely linked to a locus determining a yellow or white endosperm, probably the $\underline{Y}_1$ locus on chromosome 6. ($\underline{Y}_1$ = yellow; $\underline{y}_1$ = white). All kernels with yellow endosperm were found to be of type A or AB and all the white of type B.

Further work with maize trisomics for chromosome 6, containing the $\underline{Y}_1$ marker, confirms the close linkage between $\underline{Ep}_1$ and $\underline{Y}_1$, but the linkage is not complete. From a cross population of about 300 individuals, at least three kernels with white endosperm ($\underline{y}_1$) were found having the EP-1A component. In addition, one of the examined $\underline{y}_1$-marker samples was homozygous for the A type.

In the trisomic samples, three new EP-1 variants were found designated C, D and E in order of discovery (Fig. 1) and a non-expressed (null) variant designated O. Formal genetic analyses show that all variants are coded by alleles in the $\underline{Ep}_1$ locus. One of the samples examined was homozygous for the null-allele.

From samples containing kernels trisomic for chromosome 6, several plants were found giving three endopeptidase bands on the zymogram. All the three-banded plants were trisomics, indicating that three different alleles are present on the three replicates of chromosome 6. The phenotypes D$\underline{A}$ and $\underline{A}$C (Fig. 1), showing a gene-dosage effect in diploid tissues, are caused by two chromosomes with an A allele and one with a D or C allele, respectively.

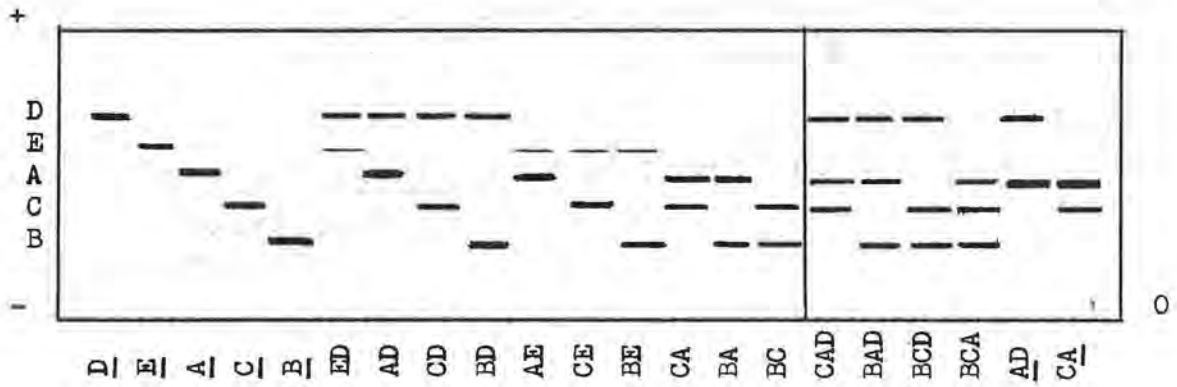


Fig. 1. Electrophoretic patterns of maize endopeptidase. The diagram shows the five diploid homozygotes, the ten corresponding diploid heterozygotes, the four three-banded trisomics and two of the two-banded trisomics with dosage effect. The E-type has not been incorporated in the trisomic samples. The relative strength of the bands is approximately according to the intensity on the zymogram.

Some of the Tri-6 plants were verified as trisomics by chromosome counts but showed two bands without dosage effects. Progeny from such plants pollinated with plants having two other alleles showed several cases of patterns without female parent bands, indicating a null allele on the third chromosome in the trisomic female parent. The genotypes for a typical cross and the progeny were the following:

D/B/O (♀) x A/C (♂) → D/A, D/C, A/B, C/B, A/O, C/O, D/A/B, D/C/B.

The progeny may also contain the genotypes D/A/O, D/C/O, A/B/O, and C/B/O, but chromosome counting is necessary to distinguish them from the respective diploids without the null allele as they are phenotypically identical. The progeny from one of the crosses contained kernels without endopeptidase activity. Both the parents were trisomics of the phenotypes AB or DB without dosage effect indicating the genotypes to be A/B/O and D/B/O, respectively. A small fraction of the progeny will then be of the genotype O/O (null).

Several crosses were made between various EP-1 types of the trisomic plants, and all the four three-banded trisomics shown in Figure 1 were found and several of the two-banded trisomics showing gene-dosage effects in the diploid tissues (scutellum or roots from 5-8 day seedlings). The E-allele is not present in any of the trisomic plants but is found in

homozygous condition as well as heterozygous with any of the other alleles including the null-allele.

Reference:

Melville, J. C. and J. G. Scandalios, 1972, Biochem. Genetics 7:15-31.

G. Nielsen
J. G. Scandalios

UNIVERSITY OF TENNESSEE
Knoxville, Tennessee
Department of Plant and Soil Science

1. Tcms restoring genes in open-pollinated varieties.

Transfer of Texas male-sterile cytoplasm to several varieties was begun several years ago for the dual purpose of obtaining satisfactory restoring inbreds and the possible development of equilibrium populations for use in areas where the use of hybrid seed was not practical. The intent was that cultivators of maize could produce their own seed by harvesting ears from pollen sterile plants that would have been pollinated by plants carrying restorer gene(s) and presumably producing some degree of vigor. For various reasons, the method did not prove to be practical.

The percent fertile plants, following the crosses to Tcms, ranged from 0 in T61Y Syn. to 40.5 in Jellicorse (MGCNL31). Varieties Jellicorse, Rockdale and Salisbury White were considered good potential sources for restoring genes. Ten backcrosses of each variety using bulk pollen were made on sterile plants of the variety before the study was terminated.

Fertile plants in Jellicorse, Neal Paymaster, Teko Yellow, and Potchefstroom Pearl were self pollinated to homozygosity for fertility restoration. Crosses were then made among plants within and between varieties, as well as with restoring inbred T115. Following selfing, all crosses produced only fertile plants and the restoring gene(s) in all varieties are considered to be identical with those in T115.

L. M. Josephson
H. C. Kincer

2. Conversion of inbreds T115 and T202 to non-restoration of Tcms.

T115 is a natural restoring inbred to Texas male-sterile cytoplasm, as well as to several other sterile cytoplasms with which it has been tested. It is an excellent seed parent and it would be desirable to have a non-restoring version. Inbred T202 has reacted as a partial restorer in all crosses.

Non-restoring versions were developed by first crossing to a non-restoring source. Plants in the segregating progeny were then crossed to a Tcms tester and backcrossed by the recurrent inbred. The following generation, backcrossed progeny corresponding to testcrosses with only sterile plants were selfed to obtain the succeeding segregating progeny. Nine generations of backcrossing were made for each inbred. Sufficient generations of selfing followed to obtain homozygosity for non-fertility restoring to Texas cytoplasm.

The non-restoring versions of T115 and T202 have not been tested on other male-sterile cytoplasms. Seed can be obtained from the Tennessee Agricultural Experiment Station.

L. M. Josephson
H. C. Kincer

3. Conversion of virus-resistant inbreds to various male-sterile cytoplasms.

Following the epiphytotic of southern corn leaf blight in 1970, interest developed in using male-sterile cytoplasms other than the Texas source. Eleven virus-resistant inbreds have been converted to cytoplasms C, R, RB, J and S. C and RB appear to be similar based on these 11 inbreds. Cytoplasms J and S react similarly but form a different group from the other three. Based on tests with inbreds T232 and Ga209, nine other cytoplasms are similar to J and S while eight form a separate group different from all the above cytoplasms. A male-sterile cytoplasm occurring in variety El Salvador is stable and appears to be in a different group from the above.

All of these cytoplasms are resistant to race T of Helminthosporium maydis, the causal organism of southern corn leaf blight.

L. M. Josephson
H. C. Kincer

4. Attempts to modify cell organelles of Tcms by irradiation.

The pathotoxin response of male-sterile cytoplasms susceptible to race T of Helminthosporium maydis, the causal organism of southern corn leaf blight, as well as cytoplasms resistant to race T has been investigated and reported by Krueger, Josephson and Hilty (Phytopathology, in press). Mitochondria were isolated by standard procedures and respiration determined polarographically with a Clark oxygen electrode.

Mitochondria from cytoplasms susceptible to H. maydis were susceptible to respiratory control ratio (RCR) decline in the presence of the pathotoxin produced by the fungus while those from resistant cytoplasms were resistant. This indicates that the mitochondria susceptible to the pathotoxin will be susceptible to H. maydis and vice versa.

These studies would indicate that it may be possible to modify the cell organelles of Tcms through irradiation and make it possible to continue using Tcms in production of seed. Plants of Tcms T220 were irradiated with 750R and 1500 R at megasporogenesis and the mature egg cell stages, as well as combinations of both, in 1972. Plants grown from this seed were artifically inoculated in 1973 with H. maydis. No plants showed complete resistance but there were some indications of restricted secondary spread of blight. Seed from these plants will be tested for reaction to blight in 1974. Additional irradiation of plants of Tcms T220 was continued in 1973. Plants were irradiated in the zygote stage as well as at megasporogenesis and the mature egg cell stages. Plants grown from this seed will be tested for reaction to leaf blight in 1974.

L. M. Josephson
M. J. Constantin

UNIVERSITY OF TEXAS
Austin, Texas

1. Apparent juxtaposition of homologues at premeiotic mitotic early anaphase.

Sporocyte samples (from KYS and Coop chromosome 2 tester stock) collected at early tassel development were examined in acetocarmine squash preparations with a Zeiss photomicroscope equipped with a bright field 63X oil immersion objective, N.A. = 1.4. Most of the sporocytes in these samples were at premeiotic interphase. Previously unnoticed details of metaphase and anaphase in the occasional cells found at these stages were observable with this optical system. At premeiotic metaphase all or most chromosomes gave the appearance of having been pressed into parallel alignment throughout their length with each other and with the metaphase plate. At early anaphase, separating sister chromatids formed configurations which superficially resembled bivalent configurations of metaphase I to early anaphase I of meiosis (generally considered to be held together by terminalizing chiasmata). The significance of sister chromatids apparently tending to resist separation at the premeiotic mitosis is not understood; the most appealing speculation may be that these cells tend to develop some of the attributes of meiosis prematurely, in this case presumably some sort of generalized adhesiveness of sister chromatids. Of special interest is the fact that very similar configurations of separating sister chromatids tended to lie closely adjacent to each other in pairs at the premeiotic mitotic early anaphase stage. These paired configurations probably represent the most convincing demonstration yet seen of homologous pairing at the premeiotic mitosis in maize.

M. Maguire

2. Experimentally produced meiotic abnormalities.

Reports of previous years have dealt with the induction of diverse meiotic abnormalities in maize microsporocytes when various irritants were introduced adjacent to tassels containing sporocytes at meiosis and the tassels were gently heated in the presence of these substances. Of particular interest was a tendency noticed in some cases for apparent

sister centromere separation at the first meiotic division followed by plate re-orientation of these centromeres, so that equational distribution at the first division and disjunctional distribution at the second division were possible. Results of a systematic study of defects found after application of the various irritants used show that this type abnormality seems to be associated only with treatment with ethylene glycol and related compounds. These include carbowax, a polyethylene glycol which is a common base for medicinal ointments.

M. Maguire

UNIVERSITY OF TORONTO
Toronto, Ontario
Department of Botany

1. Differential Giemsa staining in maize.

Direct application of mammalian Giemsa banding techniques to the somatic chromosomes of maize does not result in banded chromosomes. Further, techniques employed with other plants do not yield suitable banding in maize. This report describes a series of experiments designed to obtain reproducible banding patterns concomitant with the maintenance of chromosome morphology.

Slides were prepared according to Chen[1] with the exception that a 23 hour cold treatment (4°C) was used in lieu of the 8-hydroxyquinoline treatment. Cover slips were removed by the dry ice method and the slides were air dried. The dry slides were stored in a dessicator for up to one week. Dry slides were then "pretreated" with one of various reagents or a combination thereof (Table 1), stained in Giemsa solution, air dried and made permanent.

Giemsa stain is a complex mixture of dyes and as expected, different sources, e.g., Fisher Scientific Co., Gurr R66 and Curtin Scientific Co., produced variable results. That is, different dilutions and staining times were required to yield equivalent staining; Fisher brand is used currently. Reference to Table 1 shows the range of pH, concentration and temperatures used to stain the slides. Salient points include the

following: a) the Giemsa solution should be buffered within a pH range of 6.8 - 7.0. The molarity of the buffer is important; that is, a concentration greater than 0.1M inhibits staining. b) The magenta compound[2] was present consistently if the stain was preheated to 40°C. c) We found that lower stain concentrations (1-2%) with longer times yielded more reproducible results. Staining times have not been given because these varied with the concentration of the stain and the "pretreatment" applied. In addition, fresh slides (less than one day old) required longer staining than older slides. d) The intensity of staining varied from cell to cell on any one slide. e) Only chromosomes completely outside the cell displayed clear banding, since the cell wall obscured the banding pattern. f) Banding patterns were not always present in every chromosome in any one metaphase spread.

Table 1

Band Inducing Reagents (A) and Staining Methods (B)

A. Pretreatment	Reagent	Range of pH	Range of Conc.	Temp.
I Denaturant				
(i)	NaOH	12.9	0.1N	room temp.
(ii)	NaOH + NaCl	9-12	0.07N - 0.112N	room temp.
(iii)	$Ba(OH)_2$	12.9-13.5	0.1N	room temp.
Renaturant	SSC	7.0-8.2	2X	60° -65°C
II Proteolytic Enzyme	Trypsin	6.0-8.0	0.025-0.2%	4° -35°C
III Protein Denaturant	Urea	-	6M	room temp.
IV A.S.G.	SSC	7.0	2X	60° -65°C
B. Stain	Solvent			
Giemsa	Sörensen's Buffer (M/15)	6.7-7.0	1 - 20%	room temp.-50°C

C-banding

Centromeric banding was obtained by denaturing with $Ba(OH)_2$ (pH 13.5) and renaturing with 2X SSC, (pH 8.5) (Table 1) for two hours at 60°C or 65°C, and staining in 10% Giemsa (pH 6.8). These bands consistently appeared only on condensed chromosomes. Chromosome morphology was unsatisfactory due to a swollen, distorted appearance.

G-banding

No G-bands (chromosome cross banding revealed by Giemsa stain) were obtained using NaOH or NaOH-NaCl denaturants (Table 1). Depending on the length of these "pretreatments," chromosome morphology ranged from a distorted, ghost-like appearance to complete disintegration. Some large bands similar to those reported by Vosa[3] were obtained with $Ba(OH)_2$ (pH 12.9) plus 2X SSC (pH 7.0, 60°C). Again, chromosomes were distorted.

The acetic-saline-Giemsa (A.S.G.) technique produced faint G-bands after 2 hours incubation in 2X SSC at 60°C. No banding was apparent after longer incubation times (up to 24 hours).

The protein denaturant urea was used, following the technique of Döbel *et al.*[4] Moderate banding was obtained, but chromosome morphology was unsatisfactory. Further attempts with lower molarity urea solutions are in progress.

Reasonable G-banding has been obtained with trypsin (0.1%, 32°C, pH 7.5)[5], and staining with 2% Giemsa (M/15 Sörensen's buffer, pH 7.0) at 40°C. Chromosome morphology is still not totally acceptable.

References:

[1]Chen, C. C. 1969. Canad. J. Genet. Cytol. 11: 752.

[2]Sumner, A. T. and H. J. Evans. 1973. Exptl. Cell. Res. 81: 223.

[3]Vosa, C. G., Lopes, M. and R. de Aguiar. 1972. Maize Genet. Cooperation News Letter 46: 165.

[4]Döbel, P., Rieger, R., and A. Michaelis. 1973. Chromosoma 43: 409.

[5]Yamasaki, N. 1973. Chromosoma 41: 403.

W. Gary Filion

UNIVERSITY OF VICTORIA
Victoria, British Columbia
Department of Biology

1. The action of Pl in maize seedlings.

Recent reports on Pl have been primarily concerned with its action in association with the cherry allele of the R locus to produce pericarp pigmentation (MGNL 43:201). The effects of Pl on plant color have been summarized by Briggs (J. Heredity 57:35-42). Briggs reports that A B Pl plants produce a strong purple, sunlight independent pigment in the plant, whereas A B pl produces a lesser "sun-red" pigmentation which requires sunlight to elicit pigment formation. We have previously reported an apparent repression or retardation of pigment formation in young seedlings by Pl (MGNL 46:172), and we now report more detailed information on this effect as a function of the maturity of the tissue. We have compared anthocyanin concentrations of the first four leaf sheaths in W22 Pl and pl stocks of r^g B; r^g B^b; r^rB; and r^r B^b, as well as Ecuador 1172 R^r Lc and r^g Lc. Lc is the leaf color factor extracted from the Ecuador 1172 strain and found to be distal to the R locus at a distance of between 1-2 map units from R.

Optical density measurements of leaf sheath pigments were made on several plants every week for a period of seven weeks. The first sample of each leaf sheath was taken just as the internode appeared from the surrounding leaf sheath and the sampling was continued until the leaf had lost its chlorophyll and was beginning to drop off. The amount of pigment reduction or enhancement by Pl appeared to be directly proportional to the amount of pigment present. That is, the amount of reduction or enhancement of pigment as a percentage of the total amount of pigment present was approximately the same for all stocks tested. For this reason the results from all stocks were pooled and the general pattern of effect is shown for the first four leaf sheaths in Figure 1.

There are several points to be noted from these graphs.

1) Pl appears to repress pigment formation in the young tissues of each of the first four leaf sheaths (Fig. 1).
2) If we consider the point in time when the Pl leaf sheath first becomes more pigmented than the pl sheath, then by

Fig. 1. Representation of the pooled pigmentation data for the first (a), second (b), third (c), and fourth (d) leaf sheaths as a function of the age of the leaf. The first sample from each leaf was taken as the ligule emerged from the leaf sheath of the overlapping leaf and the last sample when the chlorophyll was gone and the leaf was falling off. The solid line represents the Pl stocks, and the dotted line the pl stocks.

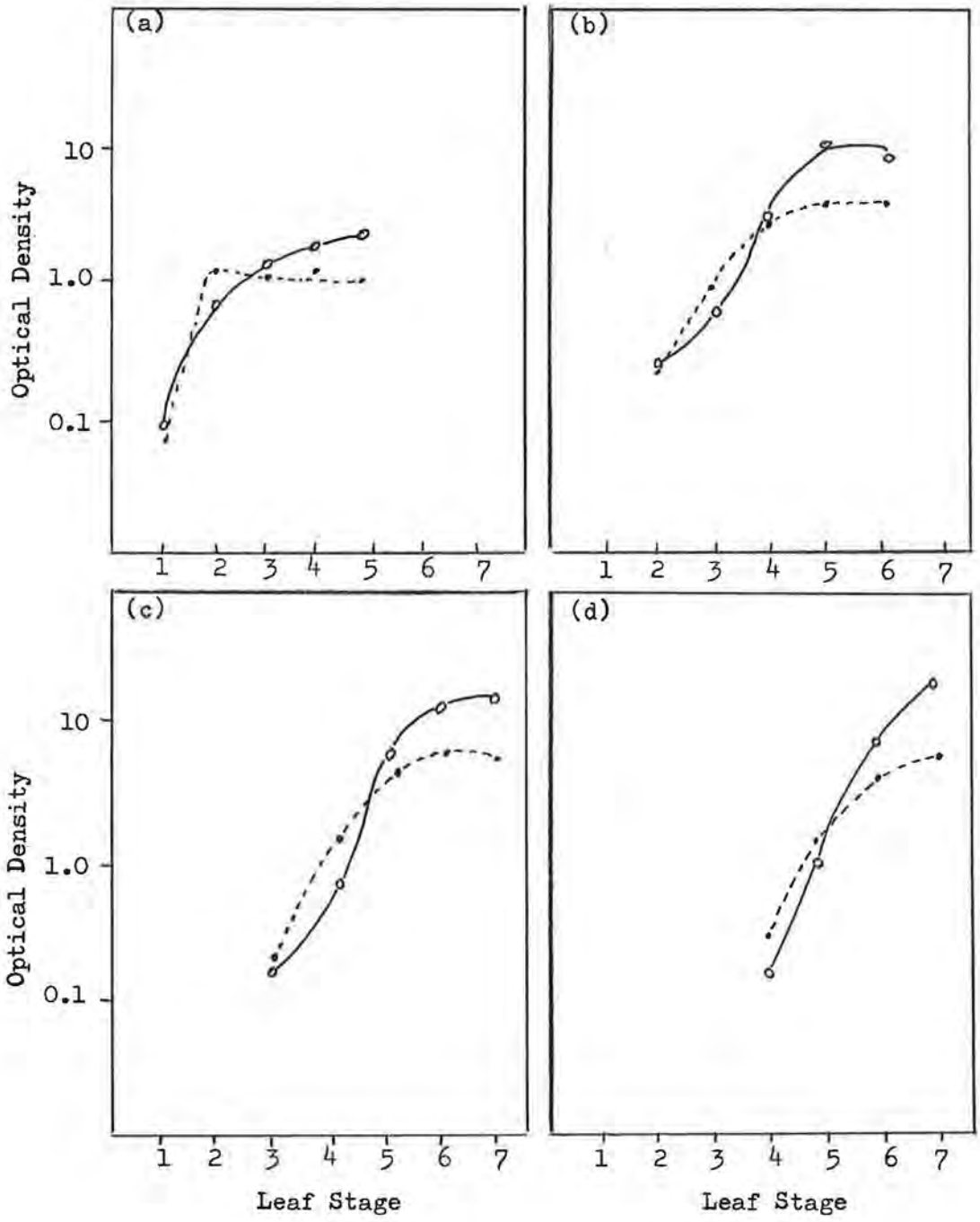


Fig. 2. Graph plotting length of time from germination to time when the Pl leaf sheath becomes more pigmented than the pl sheath, as a function of leaf tested.

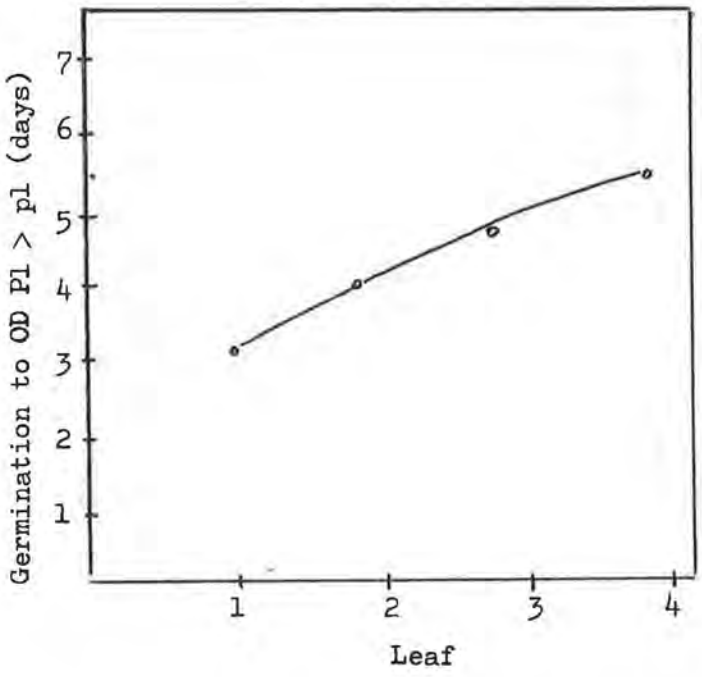
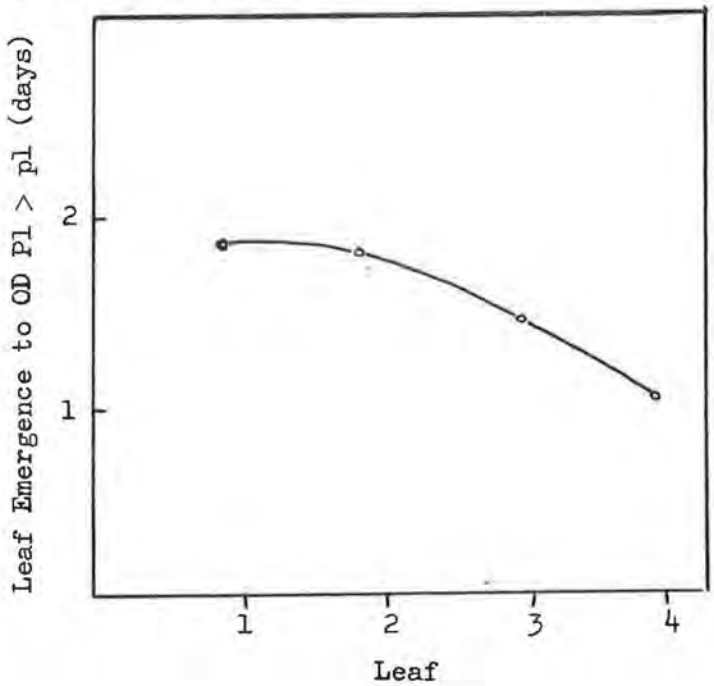


Fig. 3. Length of time from internode appearance to time when the Pl leaf sheath becomes more pigmented than the pl sheath, as a function of the leaf tested.



graphing the length of time to this point from germination as a function of the leaf tested, it can be seen (Fig. 2) that this time interval is not the same for each leaf. In fact, the curve shown in Figure 2 may be extrapolated to an asymptote at about the sixth leaf stage.

3) To emphasize the developmental aspects, Figure 3 plots the length of time from the internode appearance (youngest tissue sampled) to the point when the repression is reduced, as a function of the leaf tested. This plot demonstrates that in successive leaves, the reduction of the repression occurs in successively younger tissues. An extrapolation of this curve would predict reaching the X-axis at about the sixth leaf. In other words, in the sixth and subsequent leaf stages, no repression would be expected.

This early repression of pigment formation by Pl is most interesting in light of the fact that in both our stocks and those reviewed by Briggs, B Pl is more strongly pigmented than B pl in the mature plant. In addition, Briggs suggests that the B Pl pigment is light independent and B pl is light dependent. Sastry has also been able to show that pigment formation in the pericarp of R^{ch} stocks is light independent with Pl and light dependent with pl (MGNL 39:178). If this light interaction held true for all tissues, one would expect the pl plants to produce less pigment than Pl in all stages measured in this experiment, because the major part of these leaf sheaths are covered by overlapping leaf sheaths. This, however, is not the case and the decrease in the amount of pigment in the Pl stocks occurs in spite of any possible light interactions and not because of it.

Thus far, our data are not sufficient to propose a working model for the action of the Pl gene. It seems, however, that Pl action in the seedling stage is uniform for all genotypes tested, and it appears to act proportionately on any pigment being produced. Thus, any proposal for the mechanism of Pl action must explain the uniform action on all pigmenting genes, the repression of pigment formation in the seedlings, and the light independent action associated with it by Briggs. A more physiological approach is now being taken and it is hoped that subsequent investigations will reveal the mode of action of the Pl gene.

David Kyle
E. Derek Styles

2. Expression of pr in plant tissues.

For several years now we have been trying to develop an early inbred line of maize suitable for genetic studies. Two years ago, one family, derived initially from crosses between Gaspé plants and strains of the W22 inbred line, segregated $\underline{R}^r$ and $\underline{r}^r$, $\underline{P}^{wr}$ and $\underline{P}^{ww}$, and also what appeared from the kernel phenotypes to be Pr and pr. The $\underline{R}^r$ and $\underline{P}^{wr}$ factors were traced back to the W22 inbred strain, and the $\underline{r}^r$ and $\underline{P}^{ww}$ to the Gaspé plants. The origin of the pr factor has not been traced as yet, as the records indicate no previous segregation for red and purple seeds.

The accompanying table shows that this family has several unusual features, including:

1) A silk color factor requiring the $\underline{R}^r$ factor for the synthesis of 3-hydroxylated anthocyanins and the $\underline{P}^{wr}$ factor for synthesis of 3-deoxyanthocyanins.
2) The expression of the pr factor in such plant parts as cob, silks, and anthers as well as in the aleurone. This pr factor affects the anthocyanins, the 3-deoxyanthocyanins, the 3-deoxyleucoanthocyanidins, and the C-glycosylflavones. From the orange color of the $\underline{P}^{wr}$ pr cob, the pr factor appears to affect the P locus pigment also, probably by conditioning the formation of the precursor apiforol rather than the normal precursor luteoforol.

Oldriska Ceska
E. Derek Styles

3. Assay for the effects of chemical and natural substances on growth using empty endospermic sac injection.

Tonita (Proc. Int. Conf. Plant Growth Substances 1967 and 1970) has reported effects on flowering of substances injected into the empty endospermic cavity of young winter wheat seedlings. We have tested this method with maize and have used it to examine the effects of natural and chemical substances on three different stocks of maize: a) an early inbred line derived from crosses with Gaspé flint, b) an early maturing commercial hybrid, Polar Vee, and c) a strain of the comparatively late maturing inbred W_{22}.

Table 1 (Article 2)

Analysis of a family showing pr expression in the plant tissues

Cob color	red		orange		white		
Seed Color	purple	colorless	red	colorless	purple	red	colorless
Anther Color	red	red	pink	pink	red	pink	red or pink
Silk Color	red	green	orange	green	red	orange	green
Predominant flavonoids in silks							
3-hydroxylated anthocyanins	Cyanidin based	———	Pelargonidin based	———	Cyanidin based	Perlargonidin based	———
3-deoxyantho-cyanins	Luteolinidin based	Luteolinidin based	Apigeninidin based	Apigeninidin based	———	———	———
3-deoxyleuco-anthocyanidins	Luteoforol	Luteoforol	Apiforol	Apiforol	———	———	———
C-glycosyl flavones	Orientin type	Orientin type	Vitexin type	Vitexin type	———	———	———

Treatment of plants included gibberellic acid (10, 30, 100 ppm.), kinetin (10 ppm.), 3' and 5' mononucleotides (100 ppm.), and juices derived from the growing parts of the three stocks.

The procedure was as follows: Seeds were dusted with fungicide and sown individually in small plastic containers containing a mixture of soil, sand, peat moss and vermiculite. Ten days later the seedlings were taken from their containers and rinsed in tap water to remove the soil mixture from the seed. The empty seed-coat-bag stage was reached around the tenth day after planting; most of the seedlings retained the seed-coat-bag at this growth stage, and some of them still had milky endospermic liquid inside the bag. Seedlings with attached empty seed-coat-bags were selected for the investigation.

Natural and chemical substances were injected directly into the empty endospermic cavity of the seed by using a Hamilton Microlita syringe. In each case, 35.0 $\pm$ .5μl was injected into the bag. Injection was done by penetrating the empty endospermic cavity with the needle head and slowly injecting the solution.

Following injection of the chemicals and natural substances (10 plants per group), the plants were planted in a greenhouse. The plants were watered twice daily and a nutrient solution was occasionally supplied at the time of watering.

Measuring of plants was started on the 13th day after initial seeding. The experiment was terminated 60 days following seeding, at which time the Gaspé line and Polar Vee had flowered and the W_{22} was approximately half-way to maturity. Measurements were made to the closest $\pm$ .5 cm. of the newest node. In all cases two controls were included; one group was injected with 35.0 $\pm$ .5μl of distilled water and the other was grown naturally without injection.

Natural substances were prepared by squeezing out juices around the growing point of the 3 stocks to be investigated. Approximately 15 of these plants were individually homogenized in a Waring blender for one minute with 100 ml. of distilled water. The homogenized juice was filtered through a triple layer of cheesecloth three times, and then centrifuged for ten minutes at 3000 r.p.m. The supernatant was then stored at 4°C (maximum of 2 hours) until ready for use.

When this method was followed, Polar Vee responded to the gibberellic acid treatment showing increased final heights of 30%, 50% and 100% of controls with the concentrations of 10, 30, and 100 ppm, respectively. The Gaspé line showed an inverse response to concentrations, showing final heights of 100%, 60% and 6% with concentrations of 10, 30, and 100 ppm. No effects of the gibberellic acid treatment were observed with W_{22} at the time the experiment was terminated.

Kinetin enhanced the growth of Gaspé and Polar Vee but its effect was less obvious in the W_{22} variety.

All mononucleotides with the exception of cytidine 3' monophosphoric acid had some growth promoting effect. However, 5' nucleotides appeared to enhance growth more than the 3' nucleotides. Adenosine 5' monophosphoric acid and guanosine 5' monophosphoric acid had the greatest effect.

No conclusive growth differences were observed with any of the extract-injected plants.

Early indications suggest that none of these substances affects the flowering rate of maize; however, further studies are indicated.

Generally, the idea of empty endospermic sac injection seems a good one and may provide a good hormonal assay.

W. D. Binder
E. D. Styles

UNIVERSITY OF VIRGINIA
Charlottesville, Virginia

1. Induction of mutants in maize pollen.

Pollen of inbred B14 was irradiated with gamma rays from a large Co^{60} source stored in the pool of the reactor at the University. The corn was grown in 1972 at the Blandy Experimental Farm in Boyce, 90 miles away. Pollen was collected early in the morning, brought to Charlottesville for irradiation and returned to Blandy, where sib pollinations were made the same day. The pollen was irradiated in a container lowered into the water surrounding the Co^{60} source to a depth determined to give an exposure of 1300 r. This was the most common

dosage used in previous experiments with developing corn plants, and one known to produce losses of dominant markers.

However, in the present study we are interested in mutations for seedling characters. This investigation is similar to the one where mutations were induced by treating maize seeds with thermal neutrons (Singleton 1969). In that experiment, 3.8% of the progenies tested segregated for some seedling character (42/1096).

In 1973, the sib-pollinated seed was grown at Blandy and several hundred self-pollinations were made. A severe bird infestation destroyed many of the ears, even though they were covered by pollinating bags. Only 140 ears were harvested. These were tested for seedling mutants by sowing 30 seeds from each ear in the biology department greenhouse in Charlottesville. Of those tested, two segregated in a monogenic ratio for mutants: one an albino, the other a yellow-green that seems to be different phenotypically from $\underline{yg}_2$. Remnant seeds are available from this ear, No. 66. Also, 11 remnant seeds are available of progeny 103, the one segregating for albino seedlings.

The data are so limited that it is not possible to conclude that the mutants observed were really induced. However, this seems a more likely possibility than that the stock of B14 was segregating for these two mutants. Anyone wishing to study these may obtain what seed I have. I do not plan to grow any genetic corn this year.

Reference:

Singleton, W. R. 1969. Induced Mutations in Plants. International Atomic Energy Agency, Vienna, STI/PUB/231, p. 479-483.

W. Ralph Singleton*

*Permanent address: 1841 University Circle, Charlottesville, Virginia 22903.

Table 1

Types and frequency of chromosome aberrations induced by BUdR (100ug/ml) + FUdR (5ug/ml) at 25°C with concurrent exposure to UV (350mu; 51uW/Cm^2x100)

Fixation time (hr) after treatment	No. of metaphases analyzed	No. (or %) of abnormal metaphases		Aberrations (per 100 cells)							
				Fragment at metaphase	Fragment at anaphase	Centromere break	Chromatid break	Isochromatid break	Ring chromosomes	Despiralized chromosome	Endomitotic cell
5											
without UV	143	26(18.18%)	ns	12.57	0.70	-	-	1.4	0.70	0.70	2.10
with UV	102	27(26.47%)		20.5	0.98	0.98	-	-	1.96	0.98	0.98
10											
without UV	117	46(39.31%)	ns	26.49	0.85	-	1.71	2.56	-	0.85	6.83
with UV	159	78(49.06%)		40.25	3.77	1.26	2.50	-	-	-	1.26
15											
without UV	104	51(49.04%)	**	40.38	-	-	-	-	2.88	3.84	1.92
with UV	115	85(74.91%)		52.17	-	4.35	12.17	-	1.74	-	3.48
20											
without UV	150	94(62.67%)	**	58.00	-	2.00	-	-	-	-	2.67
with UV	179	149(83.24%)		62.56	-	0.56	6.14	1.67	2.23	1.12	8.94
25											
without UV	122	22(18.03%)	**	18.03	-	-	-	-	-	-	-
with UV	157	94(62.56%)		62.56	-	-	-	-	-	-	-
Control	150	-		-	-	-	-	-	-	-	-

ns Not significant ** Significant at 1%

UNIVERSITY OF WESTERN ONTARIO
London, Canada
Department of Plant Sciences

1. Chromosome aberrations produced by 5-bromodeoxyuridine (BUdR) and 5-fluorodeoxyuridine (FUdR) with concurrent exposure to U.V. in Zea mays L. root tips.

We studied the influence of bromodeoxyuridine (BUdR) replacement for thymidine on the pattern and level of chromosome damage in corn root tip cells. High wavelength ultraviolet light (350 nm) was employed to convert the bromodeoxyuridine in DNA to its photoproduct. BUdR was introduced during a single generation cycle (10 hr) and during that period the mitotic index was severely depressed but recovered to near control level within the next cycle following removal of the inhibitor. A short ultraviolet treatment following removal of BUdR from the medium had no markedly different influence on the mitotic index than did the use of the chemical alone. Nevertheless, the pattern of chromosome breakage was markedly different in the roots treated with BUdR and BUdR plus ultraviolet. Root tips treated with BUdR showed a high yield of chromosome breakage for two generation cycles following the replacement by BUdR of DNA thymine, but breakage was greatly reduced during the third post replacement replication.

In the nuclei treated with BUdR and then converted to the photoproduct with ultraviolet light, the level of chromosome breakage was not significantly different from the BUdR treated culture during the first post treatment generation, but during the second generation the U.V. effect was pronounced and during the third post treatment generation the U.V. enhancement of chromosome breakage had grown threefold greater than that observed in nuclei exposed to BUdR alone (Table 1). Root tips exposed to U.V. alone showed little or no chromosome damage during the period when the U.V. enhancement of BUdR induced breakage was most pronounced (Table 2).

We conclude from this experiment that BUdR treatment followed by high wavelength ultraviolet treatment provides a tool for

Table 2

Types and frequency of chromosome aberrations induced by UV (350mu; 5luW/Cm^2 x 100) alone at 25°C.

Fixation time (hrs) after treatment	No. of metaphases analyzed	No. (or %) of abnormal metaphases	Aberrations of each type per 100 cells scored					
			Fragment at meta.	Centro-mere break	Chroma-tid break	Ring Chromo-somes	Endo-mitotic cells	Bridge at anaphase
5	180	0 (0%)	-	-	-	-	-	-
10	169	19 (11.24%)	8.87	-	1.77	0.59	-	-
15	190	36 (18.94%)	14.74	0.53	1.58	-	1.53	1.58
20	188	8 (4.25%)	4.25	-	-	-	-	-
25	170	0 (0%)	-	-	-	-	-	-

specifically damaging newly replicated chromosome or chromosome regions.

Ram S. Verma*
J. E. Cummins
D. B. Walden

*Present address: Department of Pediatrics
Box 2741
University of Colorado
Medical Center
Denver, Colorado 80220
U.S.A.

2. Precise chromosome movements prior to somatic metaphase in maize.

Light and electron microscope observations of early somatic prophase have shown that the chromosomes of maize are attached to the nuclear membrane. The numerous attachment points are apparently randomly distributed along the chromosomes but include the telomeres and centromeres of the chromosomes observed.

The somatic chromosomes were clearly visible during early prophase where they were organized in a polarized bouquet arrangement reminiscent of their previously held anaphase configuration. The chromosome arms were projected toward one end of the nucleus and the centromeres were found located at the other pole of the nucleus.

The centromeres of the prophase chromosomes were observed to move in a coordinated fashion from the "centromere pole" of the nucleus, along the nuclear membrane, to occupy an equatorial position by late prophase. The chromosomes then moved inward toward one another along the equatorial axis of the nucleus to form the new metaphase plate. This chromosome movement established the plane of the new metaphase plate at right angles and equatorial to the long axis of polarization of the prophase nucleus. Consequently, the plane of cell division was established along the axis of the polarization of the nucleus.

John D. Horn

3. The absence of nonhomologous associations of somatic chromosomes in maize.

All possible distance combinations of the ten pairs of somatic chromosomes of maize were accumulated for four cold arrested stocks

(T_{1-4}) and three arrested with 8-hydroxyquinoline or monobromonaphthalene (T_{5-7}). See Horn and Walden (MGCNL, 1970, 1971) for statistical and procedural considerations. An analysis of variance was performed on the data (Table 1) and means were compared by Tuckeys hsd procedure (Table 2).

Table 1

Comparison of treatment means among homologous and nonhomologous chromosomes using Tuckeys hsd procedure.*

Homologues T_{1-4}	Nonhomologues T_{1-4}	Nonhomologues T_{5-7}	Homologues T_{5-7}
39.1	42.9	43.1	43.6

*means underscored by a contiguous line are not significantly different $p \leq 0.05$

Table 2

Source of variation	df	SS	MS	F
Subgroups	3	160.5		
r (T_{1-4} vs T_{5-7})	1	38.5	38.5	0.57
c (Homologues vs Nonhomologues)	1	54.4	54.4	0.81
r x c	1	67.6	67.6	32.1**
Error	106	229.3	2.1	
Total	109	389.8		

**significant at $p \leq 0.05$

The data clearly showed that the use of chemical antimitotic agents in stocks T_{5-7} increased the separation of homologues at metaphase to the same degree of separation observed for nonhomologues in the same (T_{5-7}) or cold arrested stocks (T_{1-4}). Homologous chromosomes were shown to be significantly more associated in the cold arrested stocks T_{1-4} than

were the homologues of the chemical arrested stocks T_{5-7} or the non-homologues of either stocks T_{1-4} or T_{5-7}.

When the distances between all possible nonhomologous chromosomes taken pairwise were compared to the expected distribution of distance for randomly associating chromosomes, only four of a possible 315 distance distributions were significantly different ($p \leq 0.05$). There was no evidence for the nonrandom association of nonhomologues in any of the treatments examined in this study.

J. D. Horn
D. B. Walden

4. Cycloheximide-affected metaphase morphology in maize.

In squash preparations of 3:1 alcohol-acetic acid fixed root-tips of maize, 8% of the metaphase figures were observed to be polar in configuration. Roots of intact, 4 day old seedlings were treated with a 15 minute pulse of 0.002% cycloheximide at 27°C followed by a 5 minute wash in 27°C running water. An immediate post-treatment increase in the frequency of polar metaphases was observed with a maximum of 61% of the metaphases being polar at 30 minutes after treatment. The increased number of polar metaphases arose as a result of a reduced number of cells leaving metaphase. The proportions of prophase and normal metaphase cells to the total number of cells observed remained at the control levels throughout.

The post-treatment polar metaphase configuration is unique and strongly resembles a polar view of normal anaphase. Electron microscope studies showed that the nuclear membrane was still intact in the cycloheximide treated polar metaphase cells.

A pattern of radiating arms was observed and this was characteristic and repeated from cell to cell. This pattern of chromosomes may be representative of the spatial organization of the chromosomes during the previous anaphase and consequently during interphase and prophase.

J. D. Horn

UNIVERSITY OF WINNIPEG
Winnipeg, Canada
Biology Department

1. K10 and crossing over in Tp9Tp9.

Past studies have revealed that B chromosomes and K10 will both increase recombination in particular regions of the genome. B chromosomes have affected crossing over in the centromere regions of chromosomes 5 and 9, whereas, abnormal 10 enhances crossing over in centromere regions of chromosomes 3 and 5. B chromosomes have very little effect on the centromere region of chromosome 3, and K10 has little effect on the same region of chromosome 9. Differences in regional responses have also been noted between male and female flowers. Although not yet tested for B chromosomes, more intimate synapsis and increased recombination in heteromorphic knob regions and aberrations has been observed with K10.

The presence of a single B in plants homozygous for the transposition in chromosome 9 (between C and Wx) elevates crossing over considerably. In order to determine if the response of Tp9Tp9 is specific to B chromosomes, the effect of K10 was studied.

Plants were synthesized as Tp9Tp9 and being either k10k10 or K10k10. Crossing over between C and Wx was determined. The data are given below.

Sporocytes	# plants	Used as	# progeny	% C.O.	Average ear size
k10k10	20	female	6409	22.8	320.5
	17	male	5050	29.5	
K10k10	17	female	4227	27.0	248.7
	14	male	3454	32.4	

The results of t tests on data from C Tp Wx/c Tp wx female parents indicated that the increase in recombination from 22.8% in k10k10 to 27.0% in K10k10 plants was significant ($P < .01$). However, no significant difference could be demonstrated for crossing over in the male. The difference between male and female recombination within the k10k10 and K10k10

groups were significant ($P < .01$), as was the drop in seed set in K10k10 plants as compared to k10k10 plants ($P < .01$).

Data obtained by Weber (MNL 42:56-59) are relevant to these observations. Little if any effect of K10 on the C-Sh-Wx region was found in plants heterozygous for Tp. Although a small increase in recombination may have occurred in female cells, no effect of K10 was found in male flowers.

The results indicate that the Tp9 region (when homozygous) is sensitive to the presence of both B chromosomes and K10. Increased recombination has been demonstrated, however, only when crossing over is determined through the female. Furthermore, the data from this sample suggest that K10 has an adverse effect on the reproductive capacity of the organism.

I wish to thank Dr. M. M. Rhoades and Miss Ellen Dempsey for their time and effort in making the final testcross in this experiment.

Edward J. Ward

UNIVERSITY OF WISCONSIN
Madison, Wisconsin

1. The mutations, de*-91 and de*-92.

Two recessive, defective seed mutants, de*-91 and de*-92 were sent to this laboratory by Francesco Salamini (Montanaso). Both are small-seeded papery pericarp mutants that are distinguishable from normal kernels on the same ear three weeks after pollination. They are not allelic in spite of their phenotypic similarity. Plants grown from mutant seeds are normal.

Neither mutant is allelic to bt, bt2, sh, sh2, sh4, mn, or o5.

In extracting seeds harvested 22 days post-pollination in order to test the activity of enzymes involved in starch synthesis, it was observed that the supernatant fraction following homogenization with an equal weight of buffer and centrifugation lacked the pronounced opalescence of such extracts from other mutants or from normal seeds. Assays of the protein content of such supernatants showed that the soluble protein content of de*-91 and de*-92 is abnormally low (Table 1).

Table 1

The soluble protein content* of mutant and non-mutant endosperms 22 days after pollination

Genotype	Mg Protein/g Fresh Wt.	Mg Protein/Endosperm
W64A	8.3	1.3
W64A o2	8.5	1.3
W64A X 182E	6.7	2.2
de*-91	3.5	0.6
de*-92	4.0	0.6
de*-95	7.8	1.5
de*-Ke	8.4	1.3
de*-Kg	8.0	1.5
de*-Kn	8.4	1.9
de*-7005	7.2	2.0

*Protein content measured by the Lowry method with BSA as a standard.

In view of the low content of soluble protein, we extended the observations to the storage proteins of the mature endosperm to ascertain if there is a coordinate reduction in the storage proteins. Accordingly, 4 g of dry, defatted powder prepared from mature endosperms of de*-91, de*-92, and 6004-3 (normal seeds from an ear segregating an opaque mutant) were extracted according to the method of Landry and Moureaux (Bull. Soc. Chim. Biol. 52:1021-1037, 1970). The results are given in Table 2. It should be noticed that many more endosperms of the mutant stocks comprise the 4 g of material analyzed. The protein content per endosperm for the mutant endosperms as compared to normal is similar for total protein and for each solubility fraction. Therefore, the constraints on protein synthesis apply to all the proteins being synthesized. The higher protein content of the endosperm powders from the mutants as compared to normal is interpreted as indicating that the constraints on protein synthesis noted here result in even more severe restrictions on starch synthesis.

Table 2

Protein fractions from mature endosperms of 6004-3 ⊗, de*-91, and de*92

Solvent		6004-3	de*-91	de*-92
Na Cl 0.5M	Mg. Protein*	40.5	50.3	46.0
	Mg. Protein/Endo.	3.1	1.1	1.0
	% Protein	12.0	10.6	9.0
Isopropanol 55%	Mg. Protein	134.5	192.1	236.9
	Mg. Protein/Endo.	10.4	4.4	5.3
	% Protein	40.0	40.5	46.5
Isopropanol plus 2-mercaptoethanol	Mg. Protein	21.1	25.4	32.3
	Mg. Protein/Endo.	1.6	0.6	0.7
	% Protein	6.3	5.4	6.3
Borate Buffer pH 10 plus 2-mercaptoethanol	Mg. Protein	30.2	19.9	23.7
	Mg. Protein/Endo.	2.3	0.5	0.5
	% Protein	8.6	4.2	4.7
SLS** 0.5 plus 2-mercaptoethanol	Mg. Protein	72.2	97.7	85.9
	Mg. Protein/Endo.	5.6	2.2	1.9
	% Protein	21.4	20.6	16.9
Unextracted Protein	Mg. Protein	37.6	88.4	84.7
	Mg. Protein/Endo.	2.9	2.0	1.9
	% Protein	11.1	18.7	16.6
Mg. Protein (Kjeldahl)		376.0	580.0	625.6
Mg. Protein Recovered		337.4	473.8	509.5
Total Protein Mg./Endo.		29.1	13.2	13.9
No. Endosperms/4g		12.9	44.0	44.9
% Protein (Kjeldahl)		9.4	14.5	15.7

*Crude protein including free amino acids

**Sodium lauryl sulfate

When tested for activity of various enzymes concerned with starch synthesis, the mutant extracts have generally had normal levels of activity when stated on the basis of protein content. The exceptions to this observation have been the starch granule-bound nucleoside diphosphate glucose-starch glucosyl transferase of de*-92 which is very low relative

to normal or any mutant except waxy and the amylo-1,6-glucosidase of de*-92. At the same time, the amylose content of the starch produced by de*-92 is only 12% as compared to 25% in normal and 0% in waxy. It is not established, however, that the low glucosyl transferase activity and the resultant low amylose content of the de*-92 stock investigated is conditioned by the de*-92 mutation. It could have its basis in an intermediate waxy allele present in the de*-92 line. See the accompanying report by Warren Bryce for more detailed information on amylo-1,6-glucosidase activity in endosperms of de*-92.

It is not concluded that the low protein content of these mutants indicates inefficiency at a step in transcription or protein synthesis. The shrunken-4 mutant, which also has a low protein content at all stages of development of the seed, appears to be partially blocked in pyridoxal phosphate synthesis as the primary mutational lesion. The lower protein content is a secondary consequence of the lessened availability of pyridoxal phosphate. There are obviously other possible defects in reactions not directly concerned with protein synthesis that could result in lowered rates of protein synthesis.

Don Foard*
Yu Ma
Oliver Nelson

*Biology Division, Oak Ridge National Laboratory, operated by Union Carbide Corporation for the Atomic Energy Commission.

2. The endosperm proteins of opaque-6.

We reported no change in the amino acid profile of the collective endosperm proteins of opaque-6 (MNL 46:203). A detailed re-examination of the endosperm proteins of the homozygous opaque-6 and normal seeds from the same ear has been done by the modified Osborn-Mendel procedure. Since the results are not in accord with our previous report, we take this opportunity to offer a correction. The results of protein fractionation and amino acid analysis are given in Tables 1 and 2, respectively.

As shown in Table 1, the major differences between the mutant and its normal counterpart occurred in water-soluble and 70 percent ethanol-soluble fractions. The amount of zein (70 percent ethanol-soluble proteins) in normal endosperms was 9.6 mg per endosperm, which constituted 62.6

Table 1

Results of protein fractionation of defatted corn endosperms of o6(R) and its normal counterpart by a modified Osborn-Mendel method

Fraction		Genotype			
		+/-[1]		o6(R)	
		a	b	a	b
H_2O	mg	37.9	0.49	210.7	2.26
	c	3.2		23.4	
	d	3.2		20.7	
H_2O-soluble Protein Nitrogen	mg	33.3	0.43	132.3	1.42
	e	87.9		62.8	
	d	2.8		13.0	
H_2O-soluble Non-protein Nitrogen	mg	4.6	0.06	78.4	0.84
	e	12.1		37.2	
	d	0.4		7.7	
5 percent NaCl	mg	33.8	0.44	60.6	0.65
	c	2.9		6.7	
	d	2.8		6.0	
70 percent Ethanol	mg	750.2	9.60	270.8	2.90
	c	63.8		30.1	
	d	62.6		26.6	
0.2 percent NaOH	mg	356.9	4.57	356.9	3.83
	c	30.3		39.7	
	d	29.8		35.0	
0.2 percent NaOH Precipitate	mg	274.7	3.52	285.1	3.06
	e	77.0		79.9	
	d	22.9		28.0	
0.2 percent NaOH Supernatant	mg	82.2	1.05	71.8	0.77
	e	23.0		20.1	
	d	6.9		7.0	
Total soluble protein	mg	1176.7	15.10	899.0	9.64
	d	98.2		88.3	

Table 1 (Continued)

Fraction		Genotype			
		+/-[1]		o6(R)	
		a	b	a	b
Residue	mg	22.2	0.28	119.7	1.28
	d	1.8		11.7	
Total protein recovered	mg	1198.9	15.38	1018.7	10.92
	d	98.0		95.3	
Total endosperm protein	mg	1223.0	15.65	1069.0	11.47
No. kernels/10 gr.		78.1		93.2	

a - per 10 gr. of endosperm, b - per endosperm, c - percentage of the soluble protein, d - percentage of the total protein

percent of the total endosperm protein, whereas the mutant had a drastic decrease to 2.9 mg per endosperm, which was only 26.6 percent of the total endosperm proteins. In opaque-6, the total water-soluble fraction was increased over its normal counterpart by factors of 4.6 and 5.5 on weight and endosperm basis, respectively. This fraction was distributed among water-soluble proteins, albumins, and the non-protein nitrogen (free amino acids and small peptides).

Differences were also found in 5 percent NaCl-soluble fraction, globulins, and the insoluble proteins. The mutant seeds contained nearly two times more globulin on a weight basis than their normal counterpart. However, this fraction was only a minor portion of the total protein. Therefore, doubling the amount of this fraction in the mutants did not account for a large percent of the total proteins. The protein which could not be extracted by the four solvents utilized was referred to as residue and was found to be higher in mutant than normal by 5.4 times on a weight basis and 4.6 times on a per endosperm basis.

The protein fraction which showed no significant difference between opaque-6 and normal was the 0.2 percent NaOH-soluble fraction. When this fraction was separated into two subfractions, the differences between the mutant and the normal seeds, on a dry weight basis, were still not significant. However, on an endosperm basis, both subfractions in the mutant had less protein than normal because of the reduction of kernel size in the mutant.

The pronounced decrease in the alcohol-soluble fraction and the increase of the water- and salt-soluble fractions have previously been observed in opaque-2, opaque-7, and floury-2.

The amino acid composition analysis of each protein fraction was carried out by ion exchange chromatography following acid hydrolysis. The amino acid content of mutant and normal endosperm is given in Table 2.

Table 2

The amino acid composition of the defatted-endosperms of opaque-6(R) and its normal counterpart

Amino acids	+/-[1]	o6(R)
Lysine	1.55	3.32
Histidine	2.93	3.00
Arginine	2.98	3.93
Aspartic Acid	5.74	9.80
Threonine	3.48	3.65
Serine	5.12	4.78
Glutamic Acid	20.54	22.95
Proline	10.59	7.09
Glycine	2.54	3.38
Alanine	8.24	7.38
Valine	3.23	3.23
Methionine	2.55	3.18
Isoleucine	3.92	3.62
Leucine	15.96	11.76
Tyrosine	4.12	3.80
Phenylalanine	6.51	5.13
Data corrected to 100 percent recovery		
Actual recovery	101.9	91.5

The amino acid composition of the collective endosperm proteins of opaque-6 mutant is rather similar to that of the opaque-2, opaque-7, and floury-2 mutants. The same amino acids (lysine, arginine, aspartate, glycine) are increased as in those mutants. The decreases observed for proline, alanine, and leucine are also characteristic of these other mutants. It is interesting that in this mutant a familiar pattern of amino acid shifts is associated with a lethal seedling condition.

Yu Ma
Oliver Nelson

3. Amylo-1,6-glucosidase activity in maize endosperm.

Amylo-1,6-glucosidase (de-branching enzyme) activity was examined in maize endosperms collected 22 days after pollination, frozen on dry ice, and stored at -20°C. The enzymatic activities in the endosperm of several carbohydrate mutants and in the hybrid W64A x W182E (+) endosperms and pollen were determined using a modification of the procedure described by Nelson and Larner (Analyt. Biochem. 33:87-101, 1970). Their method relies on the incorporation of ^{14}C-glucose into glycogen as a means of quantitatively measuring the de-branching activity. ^{14}C-glucose residues that are incorporated into polysaccharide are precipitated by aqueous ethanol on filter paper, while free glucose molecules are soluble in aqueous ethanol.

The pericarp and embryos were removed from endosperms, which were homogenized (in a ratio of 1 g. fresh weight endosperm/1 ml extraction buffer and 1 g. fresh weight pollen/5 ml extraction buffer) in a Virtis blender in chilled 0.01 M citrate buffer (containing 10^{-3}M dithiothreitol) at pH 6.75. This homogenate was filtered through cheesecloth and centrifuged at 31,000 x g for 20 minutes. A 0% to 70% saturation ammonium sulfate cut was taken and, after centrifugation, the precipitated protein was redissolved in extraction buffer. This preparation, after dialysis for about 14 hours against extraction buffer, was used to assay amylo-1,6-glucosidase activity.

The 400 ul incubation mixture contained 100 ul enzyme prep.; 100 ul of D-glucose-^{14}C-UL solution, (15 uCi and 7.5 umoles/ml); and 200 ul

0.10M citrate buffer pH 6.0 containing 4 mg dissolved phytoglycogen. This reaction mixture was incubated at 37°C.

Sixty microliter aliquots were taken from the reaction mixture at time intervals of 0, 10, 35, and 45 minutes after the start of the reaction and added to testtubes containing 0.5 ml 60% ethanol. After centrifugation at 31,000 x g for 20 minutes, the supernatant was decanted, and the precipitated phytoglycogen was rinsed with 95% ethanol and recentrifuged. The ethanol supernatant was decanted, and the phytoglycogen precipitate was dissolved in 0.5 ml 0.2M phosphate buffer, pH7.0. Immediately before filtration on a Millipore filter apparatus, 1.0 ml of 95% ethanol was added and the phytoglycogen was trapped on glass fiber filter paper. The glass fiber filters were dried, added to vials containing PPO-POPOP scintillation fluid, and counted.

There was a lag period of about 30 minutes before appreciable ^{14}C-glucose incorporation was observed. Three 60 ul aliquots were taken 45 minutes after the start of incubation. The incorporation of ^{14}C-glucose was linear with respect to time between 30 and 60 minutes.

In Table 1, amylo-1,6-glucosidase activity is expressed as mumoles glucose incorporated/ 45 minute incubation period/mg. protein and also as mumoles glucose incorporated/45 minute incubation period/endosperm on a 400 ul total incubation volume basis.

Some mutants examined were in heterogeneous genetic backgrounds; this may explain some of the variation in enzymatic activity observed between different mutants.

Amylo-1,6-glucosidase activity was detected in both the pollen and endosperm of the hybrid W64A x W182E. Considerable enzymatic activity was observed in the two waxy alleles examined, wx-B1 and wx-C. The absence of amylose in waxy starch does not appear to be due to the lack of a functional de-branching enzyme.

Two mutants, miniature (mn) and de*-92, had greatly reduced amylo-1,6-glucosidase activities compared to the hybrid W64A x W182E and many of the other mutants examined. The activities of mn and de*-92, expressed on a per endosperm basis, were 5.1% and 7.6%, respectively, of the activity of the hybrid W64A x W182E. When enzymatic activities are

Table 1

Amylo-1,6-glucosidase activity in maize endosperm harvested 22 days after pollination and in mature W64A x W182E(+) pollen

Genotype	Year harvested	Soluble protein[1] (mg/ml)	Soluble protein[1] (mg/endosperm)	mumoles glucose incorporated/45 min. incubation period/mg protein (mean + std. error)	mumoles glucose incorporated/45 min. incubation period/ endosperm (mean + std. error)
+ pollen	1973	3.12	----	1.97 ± 0.36	----
+ endosperm	1973	3.92	0.87	1.82 ± 0.28	1.57 ± 0.27
wx-C	1973	3.36	0.53	2.11 ± 0.54	1.12 ± 0.30
wx-B1	1973	4.16	0.61	1.43 ± 0.35	0.87 ± 0.21
bt-c	1973	4.64	0.92	1.13 ± 0.14	1.04 ± 0.13
bt2-r	1973	3.52	0.63	3.12 ± 0.37	1.97 ± 0.23
cp	1973	5.28	0.64	1.41 ± 0.25	0.91 ± 0.16
cp2	1973	3.60	0.62	0.59 ± 0.05	0.36 ± 0.03
sh	1971	3.36	0.44	2.70 ± 0.10	1.19 ± 0.05
sh2-c2	1973	3.44	0.80	1.61 ± 0.55	1.28 ± 0.43
sh4-c	1973	1.62	0.46	1.33 ± 0.58	0.61 ± 0.27

Table 1 (Continued)

Genotype	Year harvested	Soluble protein[1] (mg/ml)	Soluble protein[1] (mg/endosperm)	mumoles glucose incorporated/45 min. incubation period/mg protein (mean + std. error)	mumoles glucose incorporated/45 min. incubation period/ endosperm (mean + std. error)
ae-st	1972	3.44	0.47	2.34 ± 0.40	1.10 ± 0.18
du-st	1972	4.24	0.55	2.65 ± 0.64	1.46 ± 0.35
su	1970	6.48	1.09	0.63 ± 0.24	0.68 ± 0.26
W64A su2	1971	4.72	0.53	0.63 ± 0.25	0.33 ± 0.13
mn	1973	6.88	0.31	0.24 ± 0.05	0.08 ± 0.02
de*-Kg	1973	3.12	0.49	0.60 ± 0.25	0.30 ± 0.12
de*-Ke	1972	4.24	0.33	1.93 ± 0.45	0.64 ± 0.15
de*-Kn	1971	4.64	0.73	0.43 ± 0.11	0.31 ± 0.07
de*91	1973	3.68	0.59	1.24 ± 0.42	0.55 ± 0.11
de*92	1971	3.76	0.23	0.51 ± 0.02	0.12 ± 0.01
de*95	1972	4.08	0.56	1.02 ± 0.13	0.57 ± 0.07
de*7005	1972	3.04	0.45	0.78 ± 0.06	0.35 ± 0.03

[1]A 0%-70% saturation ammonium sulfate cut was taken.

expressed on a protein basis, mn and de*-92 activities were 13.2% and 28%, respectively, of the hybrid W64A x W182E activity.

De-branching enzyme activity was qualitatively detected in 3.75% acrylamide-agarose gels which had 0.7% phytoglycogen incorporated into them. A band which stains a deep blue color with I_2-KI was visible in the hybrid W64A x W182E endosperm and pollen and it was detectable in most of the mutants (including wx-C) examined but was absent in mn and de*-92.

Warren Bryce

4. Identical twins of dizygotic origin.

The proportion of twins formed by cleavage polyembryony in plants has been estimated from the excess over one-half of twins concordant in phenotype for a gene heterozygous in one parent. In this form the procedure is a direct extension of the estimation in man of one-egg twins from the excess of like-sexed pairs. Caution concerning applicability of these procedures to twins induced through action of the indeterminate gametophyte (ig) mutation was indicated in an earlier report (Amer. Jour. Botany 58:1-7). Twins were invariably concordant for a gene heterozygous in the ear parent; about one-fourth were discordant when the gene was heterozygous in the pollen parent. The corresponding estimates of one-egg twins are 100% and 50%. The discrepancy was explained by a class of ig female gametophytes that differentiate more than one cell capable of functioning as egg. Two such eggs, of identical genotype, evidently can be fertilized by separate sperm.

Might the excess of twins of concordant phenotype observed when the marker gene was introduced via the pollen parent also originate by some means other than cleavage polyembryony? If the two sperm from one pollen grain were to fertilize two eggs of one embryo sac, the resulting embryos would be fully identical even though of dizygotic origin. One class of twins, comprising about one-fourth of all cases in the previous study, suggested this mode of origin. Although the seedling phenotypes were concordant for the marker gene (R:r), they derived from a kernel of noncorresponding aleurone phenotype. Clearly, sperm from two male gemetophytes had participated in double (triple?) fertilization. To test

whether the paternal contribution to twins was identical in such cases, a group of twin sets was established following crosses to ig ig females of a multiple heterozygous male (W22 colored aleurone/Mangelsdorf's tester). Eight genes on as many chromosomes were followed and evaluated following self-pollination. The seedling population screened numbered 816.

Classifying 37 twins according to number of concordant gene pairs revealed two classes, as seen in the following distribution.

Concordant gene pairs	1	2	3	4	5	6	7	8
Twin sets (No.)	1	4	5	7	1	2	0	17

Excluded from the summary are ten cases: two instances of triplets, two triploid-diploid and one haploid-diploid twin sets, a case of two shoots emerging from a single coleoptile, and four instances of incomplete marker classification.

Seventeen of the 37 twin sets were concordant for all eight markers and therefore are presumed to be identical. When the R allele carried by a set was compared with the aleurone phenotype of the kernel from which they were derived, nine cases corresponded whereas eight indicated presence of the contrasting R allele. Correspondence in only one-half the cases for a single endosperm marker would be expected if two male gametophytes were involved in origin of this class. Heterofertilization, that is, approached 100%. The requirement for heterofertilization is understandable if the two sperm of a single pollen grain both fertilize eggs. On the other hand, if cleavage polyembryony were the basis for identical twinning, there would be no obvious requirement for involvement of two male gametophytes.

The current evidence favors the view that ig induced twins are regularly dizygotic. They are identical maternally because the eggs derive from a single female gametophyte. The eggs may be fertilized by sperm from different pollen grains (20 pairs in the current study) or from the same pollen grain (17 pairs). If three of the four sperm from two pollen grains were utilized at random in such cases, the proportion of fully identical twins would be one-third. The present and previous studies both show somewhat more than one-third identicals, indicating a preference for fertilization of the eggs by sperm from one male gametophyte.

Jerry Kermicle

5. TB-10 breakpoints and marker genes on the long arm of chromosome 10.

Entered in the accompanying Table are results based on intercrossing as male 38 TB-10 translocation stocks with strains carrying the chromosome 10 marker genes, golden-1 (g_1), lineate (li), blue fluorescent-2 (bf_2), dull-1 (du_1), and zebra necrotic (zn). The translocation stocks all have breakpoints between R and the centromere of 10 since R was used as a selective marker in their derivation (MGCNL 46:193). The hypoploid offspring, generated through use of the translocation lines as male, are deficient for segments of 10L extending from its tip through R to the translocation breakpoint. Presence of the mutant phenotype among plants of the intercross progenies (recessive mutant x TB-10 stock) is indicated by a "+" and absence of mutant offspring by a "-".

The pattern of mutant gene "uncovering" in hypoploid plants defines five translocation categories which form the following nierarchical ranking: one translocation, TB-10(32), failed to uncover any of the five mutants; 33 expressed only golden-1; one expressed bf_2 and li as well as g_1; one gave dull kernels as well as g_1, bf_2 and li plants; and two gave zn plants in addition to expressing the four other markers. Beckett's evidence (MGCNL 47:145) concerning two previously established B-10L translocations places TB-10a in the second category and TB-10b in the third. The 200 combinations involving the five mutants and 40 translocations are without exception in indicating a gene order of centromere-zn - du_1 - (bf_2 - li) - g_1 - R. None of the 40 breakpoints separates bf_2 from li.

A sixth marker, white-tip, was tested against the translocations since it had shown linkage with T2-10 (6061) but not with two other chromosome 2 translocations (Burnham, MGCNL 44:145). No plants of white-tip phenotype were observed, however, in the F_1 progenies obtained following crosses with the 38 TB-10 translocation stocks.

Three-point linkage data involving the breakpoint (T), golden, and R are available for 36 of the translocations. Testcrosses involved g r^g/g r^g as female parents with N g R^r/T G R^{scm} as male. Kernels lacking aleurone color and therefore inheriting the translocation were classified for golden and seedling root color after sprouting.

The level of recombination varied widely. Not only was this true for the T-g interval, which is of variable length from case to case, but

Table 1

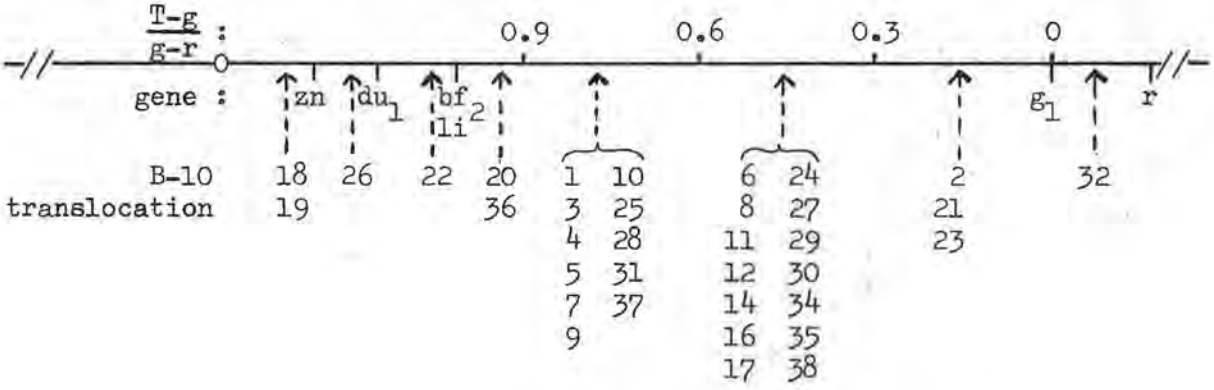
Tests for marker uncovering and three-point linkage analysis involving 38 B-10 translocations

B-10 translocation	Marker genes					T-g-R recombination		
	zn	du_1	bf_2	li	g_1	Population	g-R c.o (%)	$\frac{T\text{-}g}{g\text{-}R}$
1	-	-	-	-	+	239	16.7	0.80
2	-	-	-	-	+	261	6.5	0.17
3	-	-	-	-	+	241	16.2	0.61
4	-	-	-	-	+	362	16.0	0.89
5	-	-	-	-	+	261	20.7	0.61
6	-	-	-	-	+	278	13.6	0.32
7	-	-	-	-	+	342	15.2	0.77
8	-	-	-	-	+	86	23.2	0.50
9	-	-	-	-	+	115	15.6	0.61
10	-	-	-	-	+	298	18.1	0.65
11	-	-	-	-	+	274	19.7	0.33
12	-	-	-	-	+	189	11.6	0.41
13	-	-	-	-	+	-	-	-
14	-	-	-	-	+	305	12.6	0.45
15	-	-	-	-	+	188	0	-
16	-	-	-	-	+	218	13.7	0.40
17	-	-	-	-	+	251	23.1	0.42
18	+	+	+	+	+	335	25.3	1.35
19	+	+	+	+	+	417	21.6	1.30
20	-	-	-	-	+	315	15.5	1.04
21	-	-	-	-	+	385	12.7	0.30
22	-	-	+	+	+	305	21.3	0.98
23	-	-	-	-	+	270	15.5	0.29
24	-	-	-	-	+	189	15.3	0.34
25	-	-	-	-	+	215	18.1	0.67
26	-	+	+	+	+	510	22.9	1.21
27	-	-	-	-	+	204	5.4	0.45
28	-	-	-	-	+	226	17.7	0.82
29	-	-	-	-	+	577	19.9	0.39
30	-	-	-	-	+	76	22.3	0.35
31	-	-	-	-	+	186	15.6	0.86
32	-	-	-	-	-	240	9.6	-
33	-	-	-	-	+	-	-	-
34	-	-	-	-	+	206	19.4	0.47
35	-	-	-	-	+	441	18.6	0.41
36	-	-	-	-	+	498	20.5	1.14
37	-	-	-	-	+	216	10.2	0.63
38	-	-	-	-	+	230	10.4	0.54
TB-10a	-	-	-	-	+	-	-	-

also for g to R. In part, the g-R variation can be attributed to the limited sample size for a given translocation. Agreement between subsamples was sufficiently good, however, to suggest differential effects associated with the translocation stocks. Accordingly, the T-g values have been normalized by dividing the percent T-g recombination in any given case by the percent g-R recombination recorded for that translocation.

The resulting T-g recombination index (right-hand column of the Table) bears a definite relationship to the translocation categories defined by marker genes. The three highest values, to cite an outstanding example, belong to the three translocations having breakpoints proximal to dull. A potential use of the index is to distinguish among translocations belonging to the same marker category. This is best illustrated in the present case by the 33 translocations categorized as having a breakpoint between g_1 and (bf_2 - li). For this group, the index ranged between 0.17 and 1.14.

Results of both studies are summarized in the following diagram.



Bor-yaw Lin

6. A seed-size effect associated with certain B-10 translocations.

In the course of establishing the set of 38 translocations whose breakpoints in the centromere-R segment of chromosome 10 are reported in the preceding note, a detrimental effect of six of them on seed development was observed. The effect is specific for kernels of the hypoploid endosperm class, produced following crosses of the translocation as pollen parent. The reduction is parallel, therefore, to effects

described by Roman and Ullstrup (1951) and Bianchi (1961) involving translocations B-1a, B-1b and B-4a. For the six TB-10 cases, consideration of the extent of reduction and the position of the breakpoint permits a tentative assignment of the minimum number and regional placement of the genetic factor involved.

Measurements of seed size were based on the cross, $10(\underline{r}^g)/10(\underline{r}^g) \times 10(\underline{r}^g)/10^B/B^{10}(\underline{R}^{scm})/B^{10}(\underline{R}^{scm})$, where the female parent is inbred W23 and the translocation stock is a subline of inbred W22. With TB-10a, established by Roman, the weight of the hypoploid endosperm kernels (colorless aleurone, colored scutellum) was, on the average, 95% that of the euploid kernels (colorless aleurone and scutellum) formed by meiotic nondisjunction of the two B^{10} chromosomes. The hyperploid endosperm class, distinguished by colored aleurone but colorless scutellum, did not differ detectably from the control group. Thirty-two of the 38 additional B-10L translocations conform to the pattern set by TB-10a. Of the remaining six, reductions in weight of approximately 15% and 25% were observed in translocations TB-10(20) and TB-10(36), respectively, whereas a 50% reduction was characteristic of TB-10(18), TB-10(19), TB-10(22) and TB-10(26). Kernels of the hyperploid endosperm class did not differ significantly from the euploid control in any of the six cases. Likewise, kernels having both endosperm and embryo hyperploid, following functioning of 10 B^{10} microspores, were not subnormal. Thus, kernel size reduction may be attributed to segmental deficiency in the endosperm of that portion of chromosome 10 carried by B^{10}.

The slight reduction associated with 32 of the translocations suggests the presence of a factor of minor influence located beyond the point of break in the most distal case, i.e., TB-10(32). By similar reasoning, the four translocations associated with the most pronounced effect should have the most proximal breakpoints. Only these four of the 38, in fact, "uncovered" $\underline{bf}_2$ and $\underline{li}$. Translocations 20 and 36, the two with intermediate effects, are among the larger group that uncover golden but not $\underline{bf}_2$ or $\underline{li}$. They were, nevertheless, the two translocations of this group that mapped most distant from golden. The observations suggest then the presence of three factors of relatively major cumulative effects carried in a region distal to $\underline{du}_1$ or distal to

the breakpoint of TB-10(22) and a fourth factor of minor effect located distal to golden.

Bor-yaw Lin

ADDENDUM:

UNIVERSITY OF CONNECTICUT
Storrs, Connecticut

1. Modulator as viewed through the pericarp.

Previous published works have stressed the importance of the conclusion that all transpositions of Modulator from the P locus result in only potential twin mutations. Potential in that a pair of differently mutant cells always arise from a transposition, but only sometimes do they condition pericarp phenotypes visibly definable as twin spots of red and light variegated tissue. While the pre-transposition cell has a $P^{rr}Mp$ complex (medium variegated), the post-transposition pair of cells are altered, and one carries only P^{rr} with or without Mp somewhere within its nucleus but not at the P locus (potentially red-forming tissue), while the other member of the pair carries a $P^{rr}Mp$ complex plus an Mp within its genome (potentially light variegated tissue).

The conclusion that there is equality in mutant frequency comes from a model of the mechanism of transposition rather than the published counts of equal red vs. light variegated frequencies. In fact, due to the effect of intervening events such as meiosis, only indirect evidence has been offered to support the model (Greenblatt, Genetics, 1968) which demands that mutant classes arise as equals since only in twin spots are they found in that ratio.

New data on untwinned mutant sectors and a reevaluation of previously published data derived from homozygous variegated pedigrees offer direct support for the contention of a 1 red: 1 lt. variegated ratio at the time of transposition. The two tables which follow are abstracted from a forthcoming publication in Genetics. Table 1 shows that mutant spots one kernel and larger in size in the pericarp tissue are most often twinned. Among those that are not twinned, the two mutant classes occur

in equal frequency. Thus when only the uncertain consequences of ear morphogenesis intercede between transposition and visualization of its consequences, the mutant types are in the expected 1:1 ratio.

Table 1

Frequency of sectors larger than one kernel in size on heterozygous medium variegated pericarp ears in the 4Co63 genetic background

Sector phenotype	Number of sectors			
	Small (1 kernel)	Medium (2-8 kernels)*	Large (>9 kernels)*	Totals
Untwinned light variegated	155	19	2	176
Untwinned red	136	17	6	159
Twinned red/light variegated	453	278	21	752

*When both sectors occurred together as twins, the larger sector was used to define size of sector.

Table 2

Segregation of major colored pericarp phenotypes among the offspring from the mating W23 $\underline{P^{rr}}Mp/\underline{P^{rr}}Mp$ X $\underline{P}^{wr}/\underline{P}^{wr}$ (Data extracted from Brawn, 1956)*

Pericarp phenotype	Number of ears	Percent colored
Medium variegated	6114	90.53
Red	302	4.47
Light and very light variegated	337	4.99
Total	6753	100.00

*The relatively rare orange variegated and colorless pericarp classes are omitted for reason of clarity.

Table 2 lists the phenotypic classes arising from the backcross of a homozygous medium variegated parent. It can be seen that red and light variegated types are in equal frequency. In comparison, in a similar mating, with a heterozygous variegated parent, the red class is repeatedly found in a significantly higher frequency than the light variegated. This difference in relative frequency of mutant class is due to the recombination of the Modulator elements which have transposed from the P locus. In the case of heterozygous medium variegated, potential light variegated individuals among the progeny are converted to medium variegated due to recombination of tr-Mp with the P^{rr}Mp, (tr-Mp segregates with the colorless allele, P^{ww}), and thus are not recorded as mutants. Whereas, in the progeny of a homozygous medium variegated, the recombining tr-Mp will segregate with another P^{rr}Mp complex and thus the tr-Mp will not be lost to the total count of mutants. Thus, recombination in homozygous medium parents does not alter the expected mutant ratio while in heterozygous medium parents the ratio is expected to be altered by the loss of light variegated types.

Irwin M. Greenblatt

2. Proximal-distal polarity of Modulator transpositions upon leaving the P locus.

Modulator has been previously shown to transpose from the P locus on chromosome 1 to receptor sites throughout the genome in a nonrandom pattern. The major prior findings were that the nonrandom movement resulted in a disproportionately large number of transpositions to chromosome 1 locations and that the number of receptor sites increased as the distance from the P locus decreased. Findings reported here for the first time show that this high frequency of transposition to chromosome 1 locations occurs more often to distal locations than proximal ones relative to P. As striking as this left/right total difference is the additional finding that a region proximal to P and extending approximately three recombinational units from P is totally refractory to Modulator, while the distal equivalent length of chromosome contains the highest frequency of receptor sites.

Studies of Modulator locations after transposition from the P locus were conducted employing the P locus, transposed Modulator, and the

breakpoint of the reciprocal translocation Tl-2b as a three marker system in a backcross mating. The location of the receptor site was estimated in 105 independent cases in which (1) a twin spot of multiple kernels was found in the pericarp, (2) the red sector was analyzed for the presence or absence of Modulator, and (3) semisterile light variegated offspring resulted from the backcross seed within the light variegated sector of the twin mutation.

Of these 105 cases, 41 showed transposed Modulator recombining at random with P, 39 were linked and distal to P, while only 18 were linked and proximal to P. The remaining 7 cases all showed Modulator linked to P but a proximal/distal relationship was not obtained. Thus, there are twice as many recovered transpositions to distal sites as to proximal sites on chromosome 1. Any sites on chromosome 1 which result in high recombination frequencies (42% or more) are tallied with the random group; thus the sites listed here as distal or proximal represent only those within a detectable linkage arc with the P locus. In addition, the proximal portion of the chromosome, three map units from P in length, was void of any receptor sites. This contrasts with the equivalent distal portion of the chromosome, which produced seventeen events. This difference in receptor site positioning adjacent to the P locus is the most striking aspect of this three-point linkage study. When transpositions occur, the condition of the immediate proximal length of chromosome is obviously in a very different state than the equivalent length of chromosome distal to the P locus.

Irwin M. Greenblatt

3. Modulator: A modifier of crossing over.

During the analysis of recombination data using P, transposed Mp and the breakpoint of Tl-2b, an unexpected find was uncovered; Modulator increases the frequency of crossing over in chromosome segments adjacent to its position! Specifically, the interval T to P was found to produce recombination rates which increase when Mp is located adjacent to, but not within, the interval (see Table 3).

Table 3

The effect of Modulator's position on the frequency of recombination within a test interval on chromosome 1 marked by P and the breakpoint of T1-2b. (Mp is not within the interval.)

Group	Modulator location	Percent recombination
1	Totally absent from genome	2.30
2	Absent from P and T but present elsewhere in the genome	2.74
3	Present at P but not T and present elsewhere in the genome	3.27
4	Present at P and immediately adjacent to T only	4.18

The plant materials used in the above assessment of recombination rates were all grown in the same field, the same year, and have any genetic heterogeneity spread evenly throughout the four groupings which are compared. Group one in Table 3 represents the measured rate of recombination for a series of different red pericarp types, mutants from medium variegated, and judged to be totally void of any Modulator by use of C-Ds testers.

Group two, also a series of mutant red types from medium variegated, had been found to carry Modulator by the C-Ds tester. That means Modulator is absent from the P locus (= red phenotype) and located in an array of sites believed not too different from those mentioned in article 2 above. Their exact locations are as yet unknown, but since there are numerous different ones, there should be a good number adjacent to P but a significant number recombine at random with P.

Group three represents a collection of independently occurring light variegateds from the same medium variegated parentage as the above reds. In this group one Mp is, of course, at the P locus and the other is at sites located throughout the genome with the exception that the sites found near the T breakpoint are not included. Those cases omitted from group three are compiled as a special group four with one Mp

at P and the second, on the same chromosome strand, adjacent and proximal to the T breakpoint. When all four groups are viewed, it is obvious that the percent recombination increases when Modulator is adjacent to the test interval.

Irwin M. Greenblatt

4. Discussion of the above reports.

It is clear that transposition of Modulator from the P locus results in the mutant phenotype, red pericarp. It now appears clear that these same transpositions also cause the mutant phenotype, light variegated, to occur in equal frequency with the red mutant class. This conclusion was first advanced by Greenblatt (1968) and here tested by direct count of spots within the pericarp and again by progeny counts among the backcross offspring of a homozygous medium variegated parent. In both cases, a 1:1 ratio of red to light variegated was found. The conclusion that potential red and light variegated occur in equal frequency applies to all transpositions; there are no transpositions which would produce a red type without a concurrent light variegated type. As pointed out by Greenblatt (1968), this means that transpositions occur during that restricted period in the cell cycle when the P locus is being replicated--not before this time and not after this time.

The discovery that the proximal-distal regions adjacent to the P locus on chromosome 1 receive Modulator in a most strikingly dissimilar manner is exactly the result expected if transpositions were occurring during replication of the chromosome. As outlined in Genetics (1968), the Modulator that moves from the P locus is the one that is newly replicated, i.e., it is the one which is not from the strand that will serve as the receptor site and, in terms of semi-conservative replication, it moves from the newly forming strand to the original strand. It can only move to the original strand in those regions which are themselves in the process of replicating. As reported elsewhere, at the receptor site replication may occur a second time during the single replication of the chromosome. The interpretation of the polarity differences in site locations rests on the pattern of the

chromosome's replication. Thus, the proximal location to P, found to be void of receptor sites, is that region which has already replicated at the time the P locus is replicated. The region immediately distal to P is, at the time Mp is replicated, about to replicate and serves as the most probable receptor site due to the state of replication and proximity to the source of Mp.

The formal interpretation of the pattern of receptor sites is that a short region, extending minimally 3-4 crossover units (it may be larger due to the crossover suppression expected within the immediate vicinity of the breakpoint of a heterozygous reciprocal translocation, the third marker used in these linkage tests), exists which replicates prior to the P locus, and that there is a polar progression of the replication sequence past P into the distal region for an undisclosed length. The recovery of Mp in sites proximal to P beyond the length of chromosome void of Mp is interpreted to mean that the replication sequence is also progressing proximally. Such a pattern of replication has already been described by means of cytological techniques (see Lark, et al., 1971, J. Mol. Biol. 58:873). Such a pattern has given rise to the notation of a unit of replication known as a Replicon.

These studies of Modulator transpositions have served to build a model of chromosome replication; the pulse-radioactive labeling of chromosomes has also yielded a view of the pattern of chromosome replication; these two different approaches yield a congruent image of the pattern. Once again, genetic and cytological analysis point to the same conclusion concerning the chromosome. This time the conclusion is that the replicon can now be defined the same way cytologically and genetically.

The data in article 3 above were, to say the least, unexpected, but nonetheless gratifying. Clearly, Modulator causes an increase in crossing-over. It is very difficult to envision such a result if Modulator is within the linear length of the chromosome; if it were, the disparity in homology adjacent to the test interval would be increased and the expectation would be a decrease in crossing-over. As postulated in 1968, Modulator is thought of as being capable of synapsing with but not joined within the linear length of the chromosome and thus it serves to functionally increase synapsis in a region known to be under physical stress at the time of synapsis due to the heterozygous reciprocal translocation.

Irwin M. Greenblatt

UNIVERSITY OF RHODE ISLAND
Kingston, Rhode Island
Department of Botany

1. Transposition of the control element affecting bz-x3.

Data were presented in the 1970 Newsletter on the induction by x-rays of three unstable mutants at the bronze locus. They were designated bz-x3, bz-x4 and bz-x5. Tests with bz-x3 have indicated that control is autonomous, i.e., there is a single regulating element which is either tightly or completely linked to the bronze gene. (For a complete discussion, see Theoret. and Applied Genet. 43: 190-195, 1973.)

Three hypotheses which could account for the data are: 1) the x-rays activated a dormant element similar to Ac or Spm which was present in the treated stock; 2) the regulatory gene which normally controls the bronze cistron (assuming that Bz is a structural gene) was altered resulting in instability; and 3) the x-rays created an element with the property of causing instability. The third possibility is highly unlikely since it would require a constructive alteration and the action of x-rays is primarily destructive.

To distinguish between the first two hypotheses, studies were conducted to determine if the element would undergo transposition. A regulatory gene normally at the bronze locus would not be expected to transpose but if the element were similar to Ac or Spm, transposition might occur.

Stocks containing one or two doses of bz-x3 and homozygous for A, A2, C, and C2 were crossed with four tester stocks, a, a2, c and c2. The bz-x3 plants were used as pollen parents. If the element at the bronze locus transposed to the sites of any of the four endosperm characters, colorless kernels or kernels mosaic for colored and colorless tissue would appear in the testcross progeny. Results of these crosses are listed in Tables 1-4.

The phenotypic classes in the testcross progeny were divided into five groups as follows: 1) total loss represents colorless kernels in which the dominant phenotype is completely absent; 2) partial loss indicates fractional kernels in which a portion of the endosperm has lost the

Tables 1-4

Colorless and mosaic progeny from crosses of bz-x3 plants used as pollen parents and a, a2, c and c2 tester stocks. For an explanation of the column headings, see text.

Table 1.	♂ parent			F_1 progeny exhibiting no color or A/a mosaicism							
Plant #	Genotype	Kernel phenotype	Plant phenotype	Total loss	Partial loss	Loss & reversion	BBF	Misc	Total	Pop. size	% mosaic or colorless kernels
2977-1	bz-x3 / bz	early reversion	non-str.	2	4	0	1	1	8	1,832	0.4
2978-2	"	late reversion	"	0	2	0	0	0	2	410	0.5
2982-1	"	"	"	1	1	1	0	0	3	641	0.5
2986-K	"	"	"	0	4	0	0	0	4	376	1.1
2986-M	"	"	striped	0	4	0	0	2	6	1,297	0.5
2986-N	"	"	"	2	8	1	0	2	13	2,243	0.6
2986-P	"	"	"	5	19	2	0	3	29	4,721	0.6
2986-W	"	"	"	0	1	0	0	0	1	186	0.5
2987-1	bz-x3 / ?	full reversion	"	0	0	0	0	1	1	67	1.5
2989-1	"	"	non-str.	5	4	0	0	0	9	1,258	0.7
2990-1	bz-x3 / bz	early reversion	striped	3	7	1	0	3	14	1,566	0.9
2993-1	bz-x3 / ?	medium reversion	"	16	23	1	5	3	48	4,172	1.2
3006-1	"	"	non-str.	0	8	0	1	0	9	670	1.3

Table 1. (cont.)	♂ parent			F_1 progeny exhibiting no color or A/a mosaicism							
Plant #	Genotype	Kernel phenotype	Plant phenotype	Total loss	Partial loss	Loss & reversion	BBF	Misc	Total	Pop. size	% mosaic or colorless kernels
3011-N	$\frac{bz\text{-}x3}{bz\text{-}x3}$	late reversion	striped	0	11	0	0	0	11	1,343	0.8
3011-P	$\frac{bz\text{-}x3}{bz}$	"	"	5	48	4	0	0	57	4,059	1.4
3013-2	"	"	non-str.	0	3	0	0	0	3	293	1.0
total	-	-	-	39	147	10	7	15	218	25,134	0.8

Table 2.	♂ parent			F_1 progeny exhibiting no color or C/c mosaicism							
Plant #	Genotype	Kernel phenotype	Plant phenotype	Total loss	Partial loss	Loss & reversion	BBF	Misc	Total	Pop. size	% mosaic or colorless kernels
2982-1	bz-x3 / bz	late reversions	non-str.	0	15	0	4	15	34	7,665	0.4
2985-1	"	medium reversions	?	13	47	7	2	7	76	6,037	1.3
2986-H	"	late reversions	non-str.	1	46	6	22	6	81	4,048	2.0
2986-K	"	"	"	2	14	2	6	2	26	1,927	1.3
2986-W	"	"	"	2	74	2	4	8	90	4,082	2.2
2989-2	bz-x3 / ?	full reversion	?	0	3	0	0	0	3	423	0.7
2990-1	bz-x3 / bz	early reversion	striped	0	9	0	0	1	10	730	1.4
3012-1	"	late reversion	non-str.	4	26	1	4	1	36	1,952	1.8
3013-1	"	"	striped	0	38	6	1	0	45	2,219	2.0
3013-2	"	"	non-str.	0	41	3	5	6	55	2,899	1.9
3014-1	"	"	"	1	34	3	7	7	52	2,915	1.8
total	-	-	-	23	347	30	55	53	508	34,899	1.6

Table 3.	♂ parent			F_1 progeny exhibiting no color or A2/a2 mosaicism							
Plant #	Genotype	Kernel phenotype	Plant phenotype	Total loss	Partial loss	Loss & reversion	BBF	Misc	Total	Pop. size	% mosaic or colorless kernels
2986-W	bz-x3 / bz	late reversions	striped	0	0	0	0	1	1	191	0.5
2993-1	bz-x3 / ?	medium reversions	"	5	37	2	6	4	55	2,177	2.5
3011-P	bz-x3 / bz	late reversions	"	0	11	0	2	12	25	1,747	1.4
total	-	-	-	5	48	2	8	17	81	4,115	1.5

Table 4.	♂ parent			F_1 progeny exhibiting no color or C2/c2 mosaicism							
Plant #	Genotype	Kernel phenotype	Plant phenotype	Total loss	Partial loss	Loss & reversion	BBF	Misc	Total	Pop. size	% mosaic or colorless kernels
2990-1	bz-x3 / bz	early reversion	striped	1	4	0	0	0	5	2,171	0.2
2993-1	bz-x3 / ?	medium reversions	"	1	10	1	5	6	23	2,168	1.1
3011-P	bz-x3 / bz	late reversions	"	1	2	0	3	1	7	1,599	0.4
total	-	-	-	3	16	1	8	7	35	5,938	0.6

dominant phenotype; 3) loss and reversion means total or partial loss of the dominant phenotype with subsequent reversion resulting in a colorless background with small sectors of colored tissue; 4) BBF stands for the bridge-breakage-fusion pattern; and 5) Misc. includes phenotypes similar to blotched, r mottling and other patterns which do not fit the above categories. The columns in the tables entitled "Kernel phenotype" refer to the bronze/purple patterns of the kernels giving rise to the various male parents. Late, medium and early reversions refer to bronze kernels with small, medium and large sectors of purple, respectively, while full reversions represent completely purple kernels.

It must be stressed that these data constitute incomplete results since transmission of the unstable phenotypes must be demonstrated to be certain that transposition has occurred. Certain observations, however, can be made. Although no controls were performed to determine the frequency of endosperms in normal stocks with loss of the dominant characters, in the F_1 population of a cross between bz ♀♀ and Bz ♂♂ where pollen of the dominant stock was treated with x-rays, the frequency of kernels mosaic for Bz and bz was 1.4%. Since the frequencies in the radiation and transposition experiments are similar, it is probable that a substantial number of mosaic kernels in the current studies are not due to spontaneous events but rather to transposition of the element affecting bz-x3.

There appears to be no relationship between the instability pattern of the bz-x3 kernel or plant and the frequency of mosaic kernels in the testcross progeny. In progeny of bz-x3 individuals exhibiting full, early, medium and late reversions, the frequencies of mosaic offspring are similar. The same is true for progeny of striped and non-striped plants.

Conclusions on the frequency of transposition must await the results of studies on transmission of the mosaic phenotypes in the testcross offspring. Currently, plants in a greenhouse crop from some of the A/a kernels are mosaic for purple and green striping. Thus, the mosaicism is being transmitted in some of the cases.

John P. Mottinger

UNIVERSITY OF WISCONSIN
Madison, Wisconsin
Department of Botany

1. Freezing the genetic landscape - the preservation of diversity in cultivated plants as an urgent social responsibility of the plant geneticist and plant taxonomist.

Natural selection, and thus the continuation of evolution, is dependent on the amount of variability present in a population. In cultivated plants, variability allows selection for valuable goals such as disease resistance or high yield and, considering man's absolute dependence on his food plants, needs absolute protection. In a cultigen, variability is usually greatest in its evolutionary "cradle" region, where wild, weedy, and primitive cultivated forms tend to mingle in highly heterozygous and hybridizing populations of truly irreplaceable scientific value. In primitive societies, these nodes of variability persisted unimpaired for millenia, even after a crop became highly evolved elsewhere. Today, "progress"-oriented agriculture and massive technology, often blindly conspiring with greed, hunger, population pressures and ignorance, deliberately replace this low-yielding primitive diversity with high-yielding advanced uniformity. The corn blight of 1970 is but an ill omen of the disasters such uniformity will bring to man in the future.

Taxonomists and geneticists must counteract these ill-advised trends in several ways: first, by doing sound taxonomic-geographic-evolutionary work; second, by explaining the biological-genetic issues to the scientific public; and last, by urging drastically new and ingenious approaches to the preservation of genetic variability, especially in cultivated phylads. The widely supported preservation of genetic diversity in cold-storage gene banks, as in the national seed bank at Ft. Collins, Colorado, has short-term utility for research but is easily susceptible to accidents, such as power failures, and to loss of seed viability. It provides no long-term solution. The only way we can hope to save a crop's dynamic evolutionary potential is to literally protect the diverse "ancestral" genotypes in their cradle region from modern agricultural interference, in effect, by "freezing" the genetic

landscape, even to the extent of subsidizing primitive agricultural systems. In the case of truly wild "ancestral" species we need to preserve them outright and manipulate their habitats, as in a wildlife preserve, in situ.

Only by the deliberate and permanent preservation of selected specific local genetic landscapes, scientifically justified, politically negotiated, and perhaps internationally subsidized, and by the deliberate exclusion of agricultural "improvements" as represented by the "Green Revolution" and modern agricultural technology, is there any hope for long-range success in continuing the evolution of our crops. By placing specific regions "off limits" to agricultural aid, one to several areas for each crop, the slow processes of primitive cultigen evolution would be allowed to continue without marked loss of variability. In the case of potatoes, one could set aside the Lago Titicaca basin as an International Potato Diversity Zone. Here, not only the Indians' potato fields with their 500 odd named cultivars could survive, but also their adjoining weedy and wild potato populations would be protected from well-meaning agricultural experts and their genetically uniform strains. In the case of corn, it is imperative to protect several local regions of high diversity in Mexico, Guatemala, Colombia, Peru and Brazil from genetic "improvement." In addition, the 5 major races of Zea (Euchlaena) mays mexicana sensu lato, the wild grass 'teosinte', are in urgent need of preservation. Even though one or several of these races gave rise to cultivated Zea mays, erroneous taxonomy produced a veritable maize mystique which so confounded evolutionary understanding that these potentially highly valuable taxa, ancestral to corn as they are, were considered nothing but inconsequential weedy hybrids. Sound taxonomic and genetic work enables recognition of a crop's ancestral taxa, and thus compels their preservation. Only by the rigid protection of specific, primitive regional genetic landscapes will man be able to preserve the vast array of potentially valuable genotypes and phenotypes and give crop breeding a solid future. The taxonomist and geneticist here have a crucial and indispensable role.

Hugh H. Iltis

IV. REVISED GENETIC NOMENCLATURE FOR MAIZE

During the 1974 Allerton Park meetings, there was consideration of the proposed nomenclature changes (1973 MNL 47:229-230). Following discussion of possible difficulties, the group voted to accept the recommended changes which are outlined below. It is hoped that these changes will be implemented in all journal papers written after this date.

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 newly assigned symbols have 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 in the designation of the first locus identified, i.e., the first locus identified would be *sh* and the second *sh2*. 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 dominant allele at a locus should be designated by the gene symbol with a capital letter, *Sh2*. Where it is desirable to designate a particular dominant, this can be done as *Sh2-W22*.

The mutation by which a locus was first detected should be designated by a capital R or Ref. as *sh2-R* to indicate the reference allele.

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 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 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.

Since these recommendations provide only a framework for changes, uncertainties in application are certain to arise. It is suggested that all queries be referred to Dr. R. J. Lambert, Maize Genetics Cooperative, University of Illinois, Urbana, Illinois 61801. Dr. Lambert has agreed to act as a clearinghouse for all questions relating to gene symbols.

C. R. Burnham
E. H. Coe
O. E. Nelson
E. B. Patterson
M. M. Rhoades

V. REPORT ON MAIZE COOPERATIVE

During 1973 the Maize Genetic Cooperative received 144 requests for maize genetic stocks. There were 109 (76%) domestic and 35 (24%) foreign requests. Requests from Geneticists were 60%, Physiologists 17%, Plant Breeders 10% and Educational 13% of the total number received. A total of 1577 seed packets were sent to fill these requests. On a percentage basis 66% of the seed packets sent were for domestic and 34% for foreign use. In the past twenty years 2,120 seed requests have been received by the Maize Cooperative. A total of 25,785 seed packets have been sent in the past twenty years to fill these requests.

Seed stocks of chromosome 10 and some additional stocks were grown for increase in 1973. In addition, certain selfed cultures were grown to find new or lost traits in the collection. In addition, certain cultures increased in 1972 were grown to confirm mature plant and seedling traits. This procedure insures that confirmed stocks are available to fill seed requests. Also, certain reciprocal translocation stocks with low seed viability were increased.

A list of reciprocal translocation stocks available from the Co-op is published in the Co-op report in News Letter volume 43, 1969, or can be obtained upon request.

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^{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

ts_2 P^{WW} bm_2

P^{CR}

P^{RR}

P^{RW}

P^{CW}

P^{MO}

P^{VV}

P^{RR} as br_1 f_1 an_1 gs_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 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 Kn

br_1 f_1 Kn Ts_6

br_1 f_1 Kn bm_2

Chromosome 1 (Continued)

br_1 bm_2

Vg

Vg an_1 bm_2

Vg br_2 bm_2

bz_2^m m ; A_1 A_2 C_1 R Pr

bz_2^m M ; A_1 A_2 C_1 R Pr

an_1 bm_2

an_1 bz_2 6923 (apparent deficiency including an_1 and bz_2)

br_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}

ms_{14}

$mi_{8043} = mi_1$

D_8

Chromosome 1 (Continued)

TB-1a (1L.20)

TB-1b (1S.05)

Chromosome 2

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 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 fl_1 v_4

ws_3 lg_1 gl_2 b ts_1

ws_3 lg_1 gl_2 b v_4

al

al lg_1

al lg_1 gl_2 B sk v_4

al lg_1 gl_2 b sk v_4

lg_1

lg_1 gl_2 B

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 sk v_4

lg_1 gl_2 B v_4

lg_1 gl_2 b

lg_1 gl_2 b gs_2

Chromosome 2 (Continued)

lg_1 gl_2 b gs_2 sk Ch

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 w_3

lg_1 gl_2 w_3 Ch

lg_1 gl_2 Ch

lg_1 b gs_2 v_4

lg_1 Ch

$d_5 = d_{037-9}$

B gl_{11}

B ts_1

$gl_{11} = gl_{8712}$

wt_1

mn_1

fl_1

ts_1

v_4

Chromosome 2 (Continued)

w_3

w_3 Ht_1

w_3 Ch

Ht_1 A source

Ht_1 B source

ba_2

R_2; r_1 A_1 A_2 C_1

Ch

TB-2_{6270} (2S)

TB-2_{4463} (2L)

Primary Trisomic 2

Chromosome 3

cr_1

cr_1 d_1

cr_1 d_1 Lg_3

cr_1 ts_4 na_1

d_1 Tall = d_{6016} = tn

d_1 rt_1 Lg_3

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

Chromosome 3 (Continued)

ra_2

$ra_2\ ys_3\ Lg_3\ Rg$

$ra_2\ ys_3\ Rg$

$ra_2\ Rg\ lg_2$

$ra_2\ pm\ lg_2$

$ra_2\ lg_2$

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

$Lg_3\ Rg$

$gl_6\ lg_2\ A_1$; $A_2\ C_1\ R$

$gl_6\ lg_2\ A^b$ 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

$ts_4\ ba_1\ na_1$

$ts_4\ lg_2\ a_1^m$; $A_2\ C_1\ R\ Dt_1$

$ts_4\ lg_2\ gl_7$

Chromosome 3 (Continued)

$ts_4\ na_1\ a_1^m$ et; $A_2\ C_1\ R\ Dt_1$

$ts_4\ a_1^m$; $A_2\ C_1\ R\ Dt_1$

ba_1

$lg_2\ A^b$ 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^d-3l; $A_2\ C_1\ R$

A_1^d-3l; $A_2\ C_1\ R\ pr\ dt_1$

A_1^d-3l; $A_2\ C_1\ R\ B\ Pl\ dt_1$

A_1^d-3l; $A_2\ C_1\ R\ Dt_1$

A_1^d-3l; $A_2\ C_1\ R\ pr\ Dt_1$

A_1^d-3l sh_2; $A_2\ C_1\ R\ B\ Pl\ dt_1$

A_1^d-3l sh_2; $A_2\ C_1\ R\ Dt_1$

A_1^d-3l sh_2; $A_2\ C_1\ R\ B\ Pl\ Dt_1$

A_1^d-3l et; $A_2\ C_1\ R\ Dt_1$

a_1^m; $A_2\ C_1\ R\ dt_1$

a_1^m; $A_2\ C_1\ R\ B\ Pl\ dt_1$

a_1^m; $A_2\ C_1\ R\ Dt_1$

a_1^m; $A_2\ C_1\ R\ B\ Pl\ Dt_1$

$a_1^m\ sh_2$; $A_2\ C_1\ R\ B\ Pl\ dt_1$

$a_1^m\ sh_2$; $A_2\ C_1\ R\ B\ Pl\ Dt_1$

Chromosome 3 (Continued)

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 et; A_2 C_1 R Dt_1

a_1^{st} et; A_2 C_1 R 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_{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

Chromosome 4 (Continued)

Ts_5

Ts_5 fl_2

Ts_5 su_1

Ts_5 su_1 zb_6

Ts_5 su_1 zb_6 o_1

Ts_5 Tu

la su_1 Tu gl_3

la su_1 gl_3

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

su_1

su_1^{am}

su_1 bm_3

su_1 zb_6

su_1 zb_6 Tu

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 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

Chromosome 4 (Continued)

$bt_2 = bt_4 = bt_{60\text{-}158} = bt_{Williams}$

$bt_2\ gl_4$

$bt_2\ gl_4\ j_2$

$gl_4 = gl_{16} = gl_{Stadler}$

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; $A_1\ A_2\ C_1\ R$

$C_2^{Idf\ (Active\text{-}1)}$; $A_1\ A_2\ C_1\ R$

v_{17}

gl_7

o_1

ra_3

TB-4a (4S.20)

$TB\text{-}4_{4692}$ (4L)

Primary Trisomic 4

Chromosome 5

lu_1

$lu_1\ sh_4$

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; $A_1\ C_1\ R$

$A_2\ bt_1$ 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; $A_1\ C_1\ R\ B\ P1$

$a_2\ bm_1\ bt_1\ bv_1$ 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; $A_1\ C_1\ R$

$a_2\ bm_1$ pr ys_1; $A_1\ C_1\ R$

$a_2\ bm_1$ pr v_2; $A_1\ C_1\ R$

$a_2\ bt_1\ v_3$ 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; $A_1\ C_1\ R$

Chromosome 5 (Continued)

a_2 pr; 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

yg_1

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}

Y_1 pg_{11}; wx pg_{12}

y_1 pl

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

Chromosome 6 (Continued)

Y_1 wi pl

Y_1 wi Pl

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 py; P^{RR}

Pt

w_1

$w_{8657} = w_{025-12} = w_{035-2} =$
$w_{5946} = w_{8050} = w_{6853} =$
$w_{1-7_{4302}}$

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

In^D gl_1

o_2

o_2 v_5

o_2 v_5 ra_1 gl_1

Chromosome 7 (Continued)

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 gl_1

o_2 gl_1 sl_1

o_2 bd

in

in gl_1

v_5

vp_9

vp_9 gl_1

ra_1 gl_1 ij bd

$gl_1 = gl_9$

gl_1^m

gl_1 Tp_1

gl_1 o_5

gl_1 g_2

Tp_1

ij

Bn

bd

Pn

o_5

g_2

va_1

Chromosome 7 (Continued)

Dt_3; a_1 A_2 C_1 R

v_{8647}

yel_{7748}

TB-7b (7L.30)

Primary Trisomic 7

Chromosome 8

gl_g

$v_{16} = v_{8661}$

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^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 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

Chromosome 9 (Continued)

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

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

Chromosome 9 (Continued)

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 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 wx; A_1 A_2 R y_1

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 v_1

bp wx; P^{RR}

bp wx; P^{RW}

bp wx; P^{WW}

lo_2

$wx = wx^a$

w_{11}

wx d_3

Chromosome 9 (Continued)

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

$d_3 = d_{015\text{-}12} = d_{072\text{-}7} = d_{fg} = d_{8054} = d_{x\text{-ray}}$

$v_1 = v_{8587}$

gl_{15}

gl_{15} bm_4

bk_2 Wc

Wc

bm_4

l_6

l_6; l_1

l_7

l_7; l_1

$yel_{034\text{-}16}$

yg zb_{5588}

w_{4889}

w_{8889}

w_{8951}

Chromosome 9 (Continued)

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 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 du R; A_1 A_2 C_1

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 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

Chromosome 10 (Continued)

du

du g_1 r; A_1 A_2 C_1

zn_1

zn_1 g_1 r; A_1 A_2 C_1

Tp_2 g_1 r; A_1 A_2 C_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

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 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 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

Chromosome 10 (Continued)

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

Lc

w_2

w_2 l_1

l_1

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

Unplaced Genes (Continued)

l_4

Rs_1

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

Multiple Gene Stocks (Continued)

A_1 A_2 C_1 R Pr wx y_1

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 gl_2 wt_1 ; a_1 Dt_1 A_2 C R

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

Popcorns (Continued)

Supergold

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)	
WF 9	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.

Chromosome rearrangements

The following rearrangements are being maintained primarily for use in determining the chromosome locations of new traits. All are marked with closely-linked endosperm or seedling traits.

The cytological positions of Inv 2a were determined by Dr. Morgan; those of Inv 9a were determined by Dr. Li. The indicated interchange points of the reciprocal translocations are taken from published work of Dr. Longley.

Inversions

*gl_2 Inv 2a (also available with Ch)	2S.7; 2L.8
*wx^2 Inv 9a	9S.7; 9L.9

Reciprocal translocations

*wx 1-9c	1S.48; 9L.22
*wx 1-9 4995	1L.19; 9S.20
*wx 1-9 8389	1L.74; 9L.13
*wx 2-9b	2S.18; 9L.22
*wx 3-9c	3L.09; 9L.12
wx 3-9 5775	3L.09; 9S.24
*wx 4-9b	4L.90; 9L.29
*wx 4-9 5657	4L.33; 9S.25
*wx 4-9g	4S.27; 9L.27
*wx 5-9a	5L.69; 9S.17
*wx 5-9c	5S.07; 9L.10
*wx 5-9d	5L.14; 9L.10
wx 5-9 4817	5L.06; 9S.07
*wx 6-9a	6S.79; 9L.40
*wx, y 6-9b	6L.10; 9S.37
wx 6-9 4505	6L.13; 9 cent
wx 6-9 4778	6S.80; 9L.30
*wx 7-9a	7L.63; 9S.07
*wx or gl_1 7-9 4363	7 cent; 9 cent
*wx 8-9d	8L.09; 9S.16
*wx 8-9 6673	8L.35; 9S.31
*wx 9-10b	9S.13; 10S.40

*These constitute a basic series of twenty rearrangements for use in locating unplaced genes.

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