

MAIZE GENETICS COOPERATION

NEWS LETTER

43



April 15, 1969

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

Department of Botany
Indiana University
Bloomington, Indiana

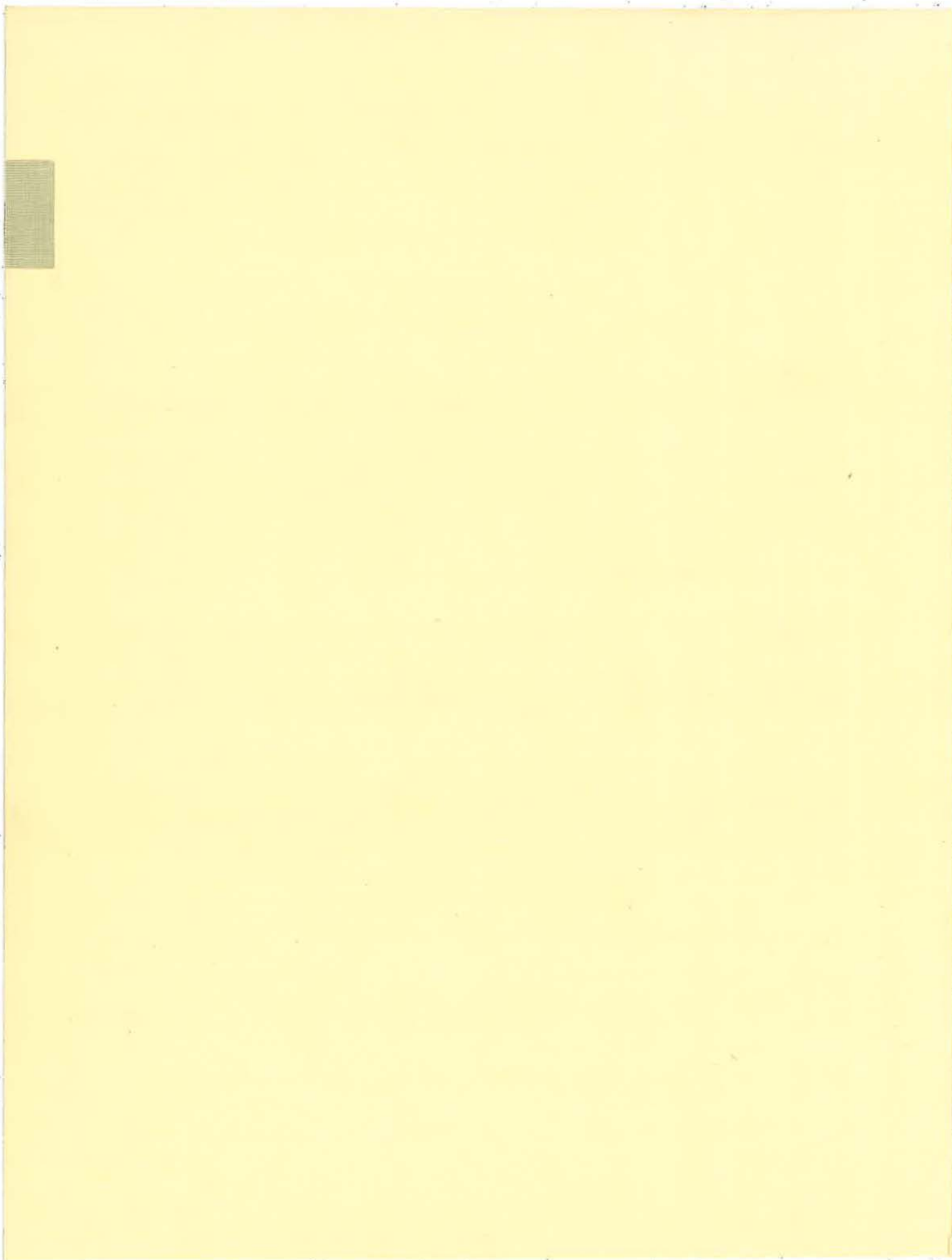


TABLE OF CONTENTS

	Page
I. Foreword	1
II. Special Notices	2
III. Reports from Cooperators	3
Andhra University, Waltair, India	3
Bhabha Atomic Research Centre, Trombay, Bombay, India	10
Birla Institute of Scientific Research, Rupa, Punjab, India	17
Boston College	20
Brookhaven National Laboratory	23
Centro Internacional de Mejoramiento de Maiz y Trigo, Londres, Mexico	34
Cornell University	40
Defiance College	46
Illinois, University of	48
Indiana University	50
Iowa State University	70
Iowa, University of	78
Istituto di Genetica Vegetale, Università Cattolica, Piacenza, Italy	88
Istituto Sperimentale per l'Orticoltura, Sezione di Ascoli Piceno, Italy	89
Massachusetts, University of, and Harvard University	92
Macdonald College of McGill University, Quebec, Canada . . .	101
Milano, Università di, Milan, Italy	101
Minnesota, University of	110
Missouri Botanical Garden	117
Missouri, University of	117
Missouri, University of, and United States Department of Agriculture	121
National Colonial Farm	134
North Carolina State University and Instituto de Genética, Piracicaba, Brazil	135
Osmania University, Hyderabad, India	136

	Page
Pennsylvania State University	138
Purdue University	145
Rhode Island, University of	148
São Paulo, Universidade de, Piracicaba, Brazil	155
Saskatchewan, University of, Saskatoon, Canada	166
Station d'Amelioration des Plantes, Dijon, France	168
Stellenbosch, University of, Stellenbosch, South Africa	171
Sydney, University of, Sydney, Australia	176
Texas, University of	178
Tufts University	179
Tulane University	182
Victoria, University of, Victoria, Canada	183
Western Ontario, University of, London, Canada	186
Wisconsin, University of, and Indian Agricultural Research Institute, New Delhi, India	201
Addendum:	
Osaka Prefecture, University of, Osaka, Japan	208
IV. Report on Maize Cooperative	215
V. Recent Maize Publications	231

I. FOREWORD

For the past 15 years, Miss Ellen Dempsey has been increasingly responsible for all of the editorial and supervisory work entailed in publishing the annual Maize Genetics News Letter. My contributions in the past have been negligible and during the preparation of the 1969 volume, they reached zero because of illness. All credit is therefore due her for this year's News Letter and I am confident that I speak for all maize workers in expressing our thanks and appreciation for her efficient, dedicated and competent services. Publication of the News Letter is no small task; we are truly in her debt. Recognition of the voluntary assistance of Gregory Anderson, Edward Ward, Reid Palmer, and William Laughner in proof reading is gratefully acknowledged.

Volumes 1-29 and Volume 33 have been placed on microfilm. Copies can be obtained from this laboratory for \$8.50. Checks should be made out to M. M. Rhoades.

The cost of publishing this year's News Letter has been met from a grant by the National Science Foundation to the Maize Genetics Stock Center. We are truly appreciative of this financial help.

M. M. Rhoades

II. SPECIAL NOTICES

1. Maize geneticists throughout the world will be interested in the recent publication "The Mutants of Maize" by M. G. Neuffer, L. Jones, and M. S. Zuber. The more useful and accurately placed mutants are depicted in more than 150 full color illustrations; the majority of them are excellent. The revised linkage map includes a great deal of unpublished data unselfishly provided by many maize workers and it undoubtedly will be of great service. The book may be obtained from the Crop Science Society of America, 677 S. Segoe Road, Madison, Wisconsin 53711. The softcover edition is priced at \$5.00 and the hardcover at \$6.00. There is an additional charge of \$.50 for foreign orders.

2. Selected stocks of reconstructed wild maize developed by Dr. P. C. Mangelsdorf may be obtained from Dr. W. C. Galinat, University of Massachusetts, 240 Beaver St., Waltham, Massachusetts, 02154. These pod popcorn lines contain the tunicate inhibitor and are homozygous for tu^{md}. This stock, as well as the various tunicate alleles, will be deposited with the Maize Genetics Collection at the University of Illinois.

III. REPORTS FROM COOPERATORS

ANDHRA UNIVERSITY
Waltair, India
Department of Botany

1. Experimental evidence on apomixis in *Coix lacryma-jobi*.

Occurrence of multiple embryo sacs in the ovules was taken as an evidence of apomixis in *Coix lacryma-jobi* (MNL 41:8, 1967). For obtaining genetic evidence on the occurrence of apomixis, an experiment involving the crossing of two types of *C. lacryma-jobi* with contrasting characters was done in 1967-68. The two types, Anantagiri *Coix* and Campus wild *Coix*, differ in the following easily recognizable characters.

No.	Character	Anantagiri <i>Coix</i>	Campus wild <i>Coix</i>
1.	Style colour	White	Purple
2.	Plant colour (at the base of the plant in seedling stage)	Green	Purple
3.	Hairs on upper surface of leaves	Long hairs present	Hairs absent

The two types were found to breed true indicating homozygosity for the above characters.

In June 1967, a potted plant of Anantagiri *Coix* was surrounded with potted plants of Campus wild *Coix*. The former plant was emasculated before the anthers exerted to make it serve as a female parent and it was allowed to cross pollinate with the surrounding plants. 219 seeds were harvested from the emasculated Anantagiri *Coix* in November-December, 1967. In June 1968 plantings, 113 of these germinated. 4 seedlings showed green plant colour and 109 showed purple plant colour. The seedlings with green plant colour at maturity showed white style and long hairs on the upper surface of the leaves and those with purple plant colour showed purple style and leaves with only short hairs. Apparently the 109 plants exhibiting the characters of Campus wild *Coix* with respect

to purple style and purple plant colour are F_1 hybrids and the 4 plants having characters of Anantagiri Coix are produced through apomixis, since the possibility of these being selfed progeny of the female parent was eliminated beyond doubt by careful emasculations. The occurrence of a large number of crossed plants in this experiment shows that the species (or at least the type, Anantagiri Coix, under investigation) is largely sexual in reproduction. However, apomixis also occurs although in low proportions (3.54%) and the species may therefore be termed as a facultative apomict.

The purple colour of style and purple plant colour appear to be dominant over white style and green plant colour, respectively. As the F_1 plants exhibited only short hairs on the leaves, the condition of leaves having long hairs on the upper surface seems to be incompletely dominant over glabrous leaves. The F_1 plants were selfed and also test crossed for further studies.

J. Venkateswarlu
Panuganti N. Rao

2. Twins (?) in Coix aquatica.

Twins are of considerable interest because they offer a potential source for haploids. In Coix aquatica, three seeds have been found to give rise to twin plants out of a total of 3,681 germinating seeds in June, 1968 (0.0008%). These were transferred to pots and both the plants in each of these seeds were marked separately. One of the plants from one seed died early owing to mechanical injury. The remaining 5 plants grew to maturity and their meiotic study showed the following chromosome numbers and behaviour (see table at top of next page). The female parent of 68k-30A on cytological examination last year showed a chromosome number of $2n=11$ with the same meiotic behaviour as that of 68k-30A-1. Six other plants of the open pollinated progeny of this same parent have shown chromosome numbers $2n=10$ in three plants and $2n=11$ in the other three.

In Coix, the use of the word "seed" is rather deceptive and usually refers to the structure enclosing the caryopsis, which is really the metamorphosed or indurated or hardened leaf sheath called variously

Culture No.	Twin No.	Chromosome No.	Meiotic behaviour
68k-78A	1	$2n = 10$	5 ii or an occasional chain of three
	2	$2n = 10$	5 ii
68k-39A	1	$2n = 10$	5 ii or an occasional ring of four
	2 (died early)	--	--
68k-30A	1	$2n = 11$	5 ii + 1 i
	2	$2n = 10$	5 ii

as capsular spathe, false fruit, shell, involucre etc. Since these are the actual materials sown and during germination, the plumule emerges through the mouth of the spathe, just as it pierces through the protective husk in rice, the word "seed" may be used loosely to designate this structure as long as the problems of twins and multiple seedlings are not involved. In discussions of such problems, however, the distinction between the seed proper and the protective sheath ought to be made. In Coix, each capsular spathe contains a fertile pistillate spikelet consisting of two florets, only one of which normally functions to produce a single seed or caryopsis while the other aborts. However, occasionally the second floret also seems to become functional and develops into a seed; thereby two seeds, instead of one, may be formed in one spathe in which case it is something like a fruit with more than one seed. When twin plants arise in such a low frequency it is not practicable to make a developmental study of the embryogeny and there is only a good deal of speculation about their origin. In the present study, since all the twins showed only a diploid chromosome number, they could have come from twin embryos of a single seed or from the two rarely formed seeds in a spathe. To find out how frequently two seeds occur in one spathe, a random sample of 2,200 normal, hard capsular spathes were crushed and examined. Of these, 10 had two seeds (0.0045%), 10 had no seed at all (0.0045%) and the rest had one seed in each (99.991%). The observed frequency of twin plants

(0.0008%) in the June planting is, therefore, well within the frequency of the occurrence of double seeds in each spathe. Further, the distribution of chromosome numbers in twin plants 68k-30A-1 and 2 ($2n=11$ and $2n=10$, respectively) is comparable with that in the other progeny from the same parent. This evidence suggests that the occurrence of twin plants in C. aquatica is due to double seeds in one spathe rather than to twin embryos developing from the same seed, although the reported existence of the phenomenon of apomixis in C. aquatica (MNL 39:184, 1965; MNL 40:164, 1966) points to the possibility of the latter event also. It is likely, therefore, that the two plants arising from the same spathe have no closer relationship than each may have with any other plant coming from the same parent.

Two approaches to the problem of twins in Coix seem to be straightforward and likely to yield more direct evidence on the nature of twins.

(i) When two seedlings are found emerging from one spathe, break open the shell and determine if it contains two seeds. Some injury to the seed or seeds inside is inevitable in most cases while breaking open the spathe and it might not be possible to grow the seedlings further. In view of the risk involved and since the twins were spotted for the first time, no attempt was made to break open the shells in the present investigation.

(ii) If some of the spathes, at the time of flowering, are found to show two bifid styles, instead of the normal one, it indicates that the second floret, in the pistillate spikelet, is likely to function. Then such spathes may be harvested at maturity separately and some of them may be examined to see if they contain two seeds while the remaining ones are germinated to see if they produce twin plants with greater frequency than spathes with only one bifid style.

Panuganti N. Rao

3. Cytological study in a progeny of an open pollinated plant of Coix aquatica with a chromosome number of $2n = 12 + 1$ fragment.

In the 1967 plantings of Coix aquatica, among the progeny of a plant 66k-8-2, one plant 67k-I 8-2-2 showed a chromosome number of $2n = 12 + 1$ fragment. At meiosis the 12 chromosomes formed 6 bivalents and

the fragment associated itself with one of the bivalents. Frequently univalents varying from 2 to 4 were also observed. This plant was allowed to open pollinate in the field and its progeny was raised during June, 1968. Pollen mother cells of 58 plants of this progeny were examined for chromosome numbers. The observations are summarized in the table below. In each of the 58 plants, cytological material was collected from 5 different tillers in view of the chromosomal instability in plant 66k-8-2 recorded last year (MNL 42:2-3, 1968).

Chromosome No.	No. of plants	Percentage	Remarks
2n=10	9	15.53	Including one plant showing chromosomal instability.
2n=11	17	29.31	Including one plant showing suspected trisomic condition.
2n=11+1f*	1	1.72	Showing desynapsis.
2n=12	14	24.14	Including two plants showing suspected tetrasomic condition.
2n=13	15	25.86	Including two plants showing chromosomal instability with 2n=12 in some cells. In one of the plants when the number is 2n=13 regularly an association of 4 chromosomes of apparently equal size was observed. But in cells with 2n=12 only an association of 3 chromosomes of similar nature was found. This is suggestive of tetrasomic and trisomic condition in the respective cells.
2n=13+1f*	1	1.72	The fragment associated itself with the nucleolar bivalent. Tetrasomic condition also seems to be present. In some spikes 2n=13 was observed and in this case the fragment was missing.
Polyploid	<u>1</u>	<u>1.72</u>	3n(?)
	58	100.00	Total.

*fragment

Preliminary observations on meiosis showed the following general features. The fragment observed in the parent plant was found in a very

few plants in the progeny. Bridges, laggards, and irregular distribution at anaphase were common. A heteromorphic bivalent was found in many of the plants. Many plants frequently showed the occurrence of 2 to 4 univalents per cell and one plant was found to be desynaptic. In most of the progeny there were higher associations involving 3, 4 or more chromosomes. In plants with a regular diploid complement ($2n=10$) these higher associations may be taken as due to chromosomal interchanges. In cytological behaviour, the plants with the chromosome numbers of $2n=11$, 12 and 13 may be tentatively grouped into the following four categories.

(i) Those showing higher associations involving chromosomes of apparently equal size suggesting an aneuploid condition.

(ii) Those having higher associations involving chromosomes with discernible size differences indicating the presence of chromosomal interchanges.

(iii) Those having higher associations involving equal as well as unequal chromosomes indicating the presence of both aneuploidy and segmental interchanges.

(iv) Those in which the chromosome associations were strictly as bivalents with the odd chromosome, when present, remaining as a univalent.

Detailed meiotic studies are underway for a precise understanding of the nature of chromosomal associations in these plants.

The variability in chromosome number and behaviour occurring both within and between plants of the progeny arising from a single open pollinated individual shows that the genome in *C. aquatica* is highly plastic. Further, since all the chromosomal variants in the progeny are capable of survival and reproduction, the individual plants of a population have an adaptive ability that is significant in preserving and enlarging the cytogenetic diversity. Although morphological differences between plants carrying different chromosome numbers are not apparent, differences of a quantitative nature may exist, since Venkateswarlu and Chaganti (J. Cytol. Genet. 1:14-21, 1966) stated that at least a part of the high amount of variability in populations of *C. aquatica*, analyzed by them, was due to chromosomal variants.

Panuganti N. Rao

4. Cytological studies in a progeny of autotetraploid *Chionachne koenigii*.

Meiosis in autotetraploid *Chionachne koenigii* ($4n=40$) was reported earlier (MNL 41:6-7, 1967). The seed setting was very poor in the tetraploid and from the few good seeds obtained a progeny of 27 plants was raised in June, 1968. Cytological studies showed that all 27 plants had a tetraploid chromosome number ($4n=40$) and behaviour. The tetraploids and diploids grew almost to the same height, but in tetraploids the male and female spikelets and the inflated spathe enclosing half the inflorescence are larger in size than in diploids. The tetraploids generally have leaves which are pale green in colour.

Panuganti N. Rao

5. Annual and perennial habit in *Chionachne koenigii*.

Weatherwax (1926) described *Chionachne koenigii* under the name *Polytoca barbata* as a tall, slender annual grass with no tendency to perennate. Nirodi (1955) described *C. koenigii* as an erect grass with a perennial stem. In the collections maintained at the Experimental Farm, there are two populations of *C. koenigii*, one raised from the seed obtained from Maharashtra (Type 1) and the other raised by transplanting young plants got from Andhra Pradesh (Type 2). Externally the two types differ in a few noticeable features. Type 1 under cultivation is a vigorous, often aggressive, annual grass with thick culms and broad light-green leaves, on both surfaces of which and on the leaf sheath are somewhat stiff hairs. These plants produce abundant fertile seed. However, when plants of this type occur as weeds on the field embankments they are small and produce only a few seeds. The plants of Type 1 die soon after seed production. Type 2 is a perennial grass with a small woody root stock, thin culms and narrow dark-green leaves devoid of conspicuous hairs. The plants of this type also produce fertile seeds and perpetuate through the root stock putting forth several new shoots, which will be in various stages of development and some of which will not flower till the following year. Both types have a chromosome number of $2n=20$. The tetraploid ($4n=40$) plants of Type 1 obtained through colchicine treatment also

produced some seeds but died soon after. Type 2, in the place where it was collected, seems to propagate mainly through the perennial root stock as the same clumps are usually seen year after year although small plants developed from seeds also could be found, in the rainy season, scattered nearby and competing with other plants in the area for survival and growth. The ability of these young plants to become established and to perennate perhaps depends on the chance availability of conditions ideal for the purpose. Otherwise, they remain restricted in growth, produce a few seeds, and perish. Once they get established, they perennate. It may be concluded that in C. koenigii different types with variation in habit exist, reproducing either entirely by seed (annual) or by both vegetative and sexual means (perennial).

Panuganti N. Rao

BHABHA ATOMIC RESEARCH CENTRE
Trombay, Bombay-74, India
Biology Division

1. Complementation between some colorless recessive mutations obtained from I and C.

We have isolated 3 i mutants (from I) and 9 c mutants (from C) all of which breed recessive to C and in homozygous condition yield only colorless kernels. All these mutants were reciprocally crossed to test for complementation. Extensive complementation was noted in i-3 x c-6, i.e., the majority of the kernels were partially pigmented. Slight complementation was also noted in i-3 x c-2 and i-3 x c-8. All other combinations were completely colorless. The data are summarized in Table 1.

The response of these mutants to Bh (Blotched) is variable. The observations are noted in Table 2. Only i-3, c-2, c-6 and to a slight extent c-9 gave a blotched expression. i-1 and c-1 gave completely colored kernels. All other mutants gave only colorless kernels. The significance of these observations is not clear.

Table 1
Reciprocal crosses between different i and c mutants
and the expression of F₁ aleurone colour.

Mutants	<u>i</u> -1		<u>i</u> -2		<u>i</u> -3	
	Male	Female	Male	Female	Male	Female
<u>c</u> -1	-	-	-	-	Colorless	-
<u>c</u> -2	Colorless	Colorless	Colorless	Colorless	Colorless	Two kernels partially pigmented
<u>c</u> -3	Colorless	-	-	Colorless	-	-
<u>c</u> -4	Colorless	-	-	-	Colorless	Colorless
<u>c</u> -5	Colorless	-	Colorless	-	Colorless	Colorless
<u>c</u> -6	-	-	Colorless	Colorless	All kernels partially pigmented	Few kernels partially pigmented
<u>c</u> -7	-	-	Colorless	-	-	-
<u>c</u> -8	-	-	Colorless	-	Six kernels partially pigmented	Colorless
<u>c</u> -9	-	-	Colorless	-	-	-

Table 2
Response to Bh (Blotched) of i and c mutations

Mutagen	Mutant	<u>Bh</u> (Blotched)	
		Male	Female
Diethyl sulfate (DES)	<u>i</u> -1	-	Completely colored
Ethyl methane sulfonate (EMS)	<u>i</u> -2	Colorless	Colorless
Radiation (gamma rays)	<u>i</u> -3	'blotched'	'blotched'
EMS	<u>c</u> -1	Colored	Colored
"	<u>c</u> -2	-	'blotched'
"	<u>c</u> -3	-	-
"	<u>c</u> -4	-	Colorless
"	<u>c</u> -5	Colorless	Colorless
"	<u>c</u> -6	Slightly 'blotched'	Colorless
"	<u>c</u> -7	-	Colorless
"	<u>c</u> -8	Colorless	Colorless
"	<u>c</u> -9	Slightly 'blotched'	-

We are repeating these experiments to confirm our results.

Chandra Mouli
N. K. Notani

2. Breeding behaviour of mutations from I and C.

Colored kernels isolated from the progeny of the cross $\frac{I \ Sh \ Bz \ Wx}{I \ Sh \ Bz \ Wx}$ (seed treated) x $\frac{C \ sh \ bz \ wx}{C \ sh \ bz \ wx}$ generally do not breed true for i mutation. Out of 16 kernels isolated this way, only 3 bred true for the change I to i. In a new series from 1442 progenies, 22 kernels were isolated, none of these showed the change from I to i on further testing. In contrast, $\frac{C \ Sh \ Bz \ Wx}{C \ Sh \ Bz \ Wx}$ (seeds treated) x $\frac{c \ sh \ Bz \ wx}{c \ sh \ Bz \ wx}$ yielded 6 colorless kernels in 794 progenies. Out of these 6, 3 failed to propagate but the other three had changed from C to c and bred true. Essentially then the findings are the same as those reported last year, i.e.:

(i) There is a very high proportion of non-concordant changes of I. C, in general, yields concordant changes.

(ii) The mutation rate of I and i must be much lower than C to c.

All the observations are suggestive that I and C may occupy different loci and may in fact have a functional relationship that is yet to be clarified.

Chandra Mouli
N. K. Notani

3. The basis of somatic instability in maize.

Various mutagenically-effective treatments for the Sh₂ locus failed to produce any back mutation of the closely-linked A₁Ds marker complex. Nor were any Ac-like elements generated (Maize News Letter 42:6-7). An excision-repair model is proposed to explain the stability of Ds (mutations) in the absence of Ac and its high mutability in the presence of Ac. According to this model, Ds is considered to be a mutation that produces a specific kink in the chromosome. The Ds mutation can occur once or more than once in a structural gene and also anywhere in the genome. The presence of this kink is specifically recognized by the product of Ac. The product of Ac may be visualized as an excision enzyme concerned with monitoring the fidelity of the chromosomes and excising the Ds-type damage. Ac can occur in either active or inactive phase and probably in more than one location. Two major states of Ds are assumed to present themselves somewhat differently to the Ac excision enzyme. The Dissociation-type state is excised as a chromosome break and the back-mutation type is excised so that it is repairable. Intracistronic recombination and therefore the site-referability of different Ds 'insertions' as observed by Nelson (cited in Dawson, G.W.P. 1966. The Physiology of Gene and Mutation Expression. Proc. Symp. Prague 67-70) is also readily explained. Under the present scheme, the phenomenon of transposition cannot be explained as a unique transfer of material substance from one location to the other but rather as the occurrence of a new Ds kink at another location (presumably in the wake of chromosomal breakage).

Chandra Mouli
N. K. Notani

4. Irradiation of growing I Sh Bz Wx/C sh bz wx/C sh bz wx endosperms.

Irradiation of heterozygous growing endosperm allows detection of numerous losses of the dominant markers. When C sh bz wx plants were pollinated by I Sh Bz Wx pollen and then transferred to a gamma field for their entire post-fertilization life, the F_1 kernels showed phenotypes that mimicked those obtained from mutable alleles. Subsequently, we irradiated the fertilized plants for fixed periods such as 6-24 hrs, 24-48 hrs, 48-72 hrs and so on. The frequency of sectors or dots was counted on the mature kernels. The following general observations were made:

- (i) The sector size is correlated with the time of irradiation. The earlier the irradiation, the larger the sector of loss or change.
- (ii) There is practically no effect of irradiation about the time the aleurone-pigment genes express themselves. This suggests that the expression of I and C is unaffected and that all the changes that occur are due to a loss or a change in the genetic material.
- (iii) Although the number of I losses can be estimated by counting the C sectors, the data are complicated by the varying number of nuclei or cells present at the time of irradiation, lethality etc.

Chandra Mouli
W. K. Notani

5. Cryptic deletion on the short arm of chromosome 9.

A locus has been detected on the short arm of chromosome 9 which completely prevents the transmission of the male gametes carrying it. The female gametes bearing it also have a slightly, but significantly, lower rate of transmission (Table 3). The position of the locus was mapped 1.13 map units distal to I. The four markers linked to this locus show a polarity of transmission, the transmission of I, Sh, Bz and Wx being 1.13%, 2.26%, 4.49% and 27.18% respectively. For I, it was verified that the transmitted gametes were due to crossing-over between I and the distorting locus. The basis of the aberrant transmission was considered to be either a mutation of a gametophytic factor or a cryptic deletion. Cytologically, no aberration was detectable and stainability of pollen was normal. The data, however, appear to be more compatible

Table 3

Transmission of linked factors through pollen and egg cells of heterozygote

$$\frac{I \text{ Sh Bz Wx}}{C \text{ sh bz wx}}$$

Kernel type	Cross				
	$\frac{O}{+} \frac{C \text{ sh bz wx}}{I \text{ Sh Bz Wx}} \times \frac{I \text{ Sh Bz Wx}}{C \text{ sh bz wx}} \sigma$			$\frac{O}{+} \frac{I \text{ Sh Bz Wx}}{C \text{ sh bz wx}} \times$	$\frac{I \text{ Sh Bz Wx}}{C \text{ sh bz wx}} \otimes$
	Upper half	Lower half	Total	$\frac{C \text{ sh bz wx}}{C \text{ sh bz wx}} \sigma$	
* $\frac{I \text{ Sh Bz Wx}}{C \text{ sh bz wx}}$	26	25	51	1540	763
	1603	1655	3258	1681	465
* $\frac{I \text{ sh bz wx}}{C \text{ Sh Bz Wx}}$	0	1	1	15	10
	42	38	80	17	30
* $\frac{I \text{ Sh bz wx}}{C \text{ sh Bz Wx}}$	0	0	0	9	-
	26	38	64	9	17
* $\frac{I \text{ Sh Bz wx}}{C \text{ sh bz Wx}}$	0	0	0	283	117
	530	496	1026	308	308
* $\frac{I \text{ sh Bz Wx}}{C \text{ Sh bz wx}}$	0	0	0	2	2
	5	6	11	0	-
* $\frac{C \text{ sh Bz wx}}{C \text{ Sh Bz wx}}$	1	2	3	1	-
	2	3	5	2	-
$\frac{C \text{ Sh bz Wx}}{C \text{ Sh bz Wx}}$	4	2	6	1	-
			4515	3868	1712

*In the presence of I, Bz or bz cannot be classified. The classification of Bz or bz given along with I is the more probable one.

with the deletion model since a slightly lower transmission through the female would be unexpected from a gametophytic mutation and the expected type of cross-sterility remains undiscovered.

Chandra Mouli
N. K. Notani

6. Electrophoresis of analogous enzymes in teosinte and maize-teosinte hybrids.

Six different races of teosinte (Chalco, Balsas, Guatemala, Huehuetenango, Nobogame and Central Plateau), a maize (Wilbur's flint) x teosinte hybrid and the parental maize line (seed of all these stocks was kindly supplied by Dr. H. G. Wilkes) were examined for analogous enzymes. Endosperms from dry seeds were extracted in 0.01 M sodium pyrophosphate and separated by disc electrophoresis. Esterases, peroxidases, alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH) were investigated.

Esterase zymograms of the teosinte lines were not very different from those of maize (Wilbur's flint). Migration of the major esterase band relative to the front (Rf) was the same for teosinte and maize. The teosinte line Chalco showed a different esterase pattern from other races. Peroxidase zymograms of teosinte were different from maize. In teosinte race Guatemala, the major peroxidase band showed a different Rf value. MDH of teosinte and maize migrated to the same position. ADH activity could not be detected in the endosperm extracts from teosinte. The zymograms for the maize x teosinte hybrid were similar to the maize lines used as the female parent for all the enzymes considered. The limited electrophoretic data gathered so far, suggest close structural homologies between teosinte and maize enzymes.

C. R. Bhatia
S. E. Pawar
N. K. Notani

7. Buoyant density in cesium chloride of DNAs of maize and teosinte.

DNAs from maize and teosinte (races Chalco, Balsas, and Guatemala) when banded in cesium chloride density-gradients yielded only a single peak in each case. Both ^{32}P -labelled maize DNA and ^3H -labelled teosinte

DNA in each case banded in the same position indicating that overall base-composition of maize and the tested teosinte DNAs is the same.

S. E. Pawar
N. K. Notani

8. Basis of cytoplasmic male-sterility (Texas type).

We have made the following observations so far: (i) Two sectorially pollen-fertile plants arose in the male-sterile mitomycin-treated series; (ii) One of the two plants in further breeding tests indicated that the change had occurred at the cytoplasmic level; (iii) Mitomycin inhibits overall DNA synthesis (MNL 41:9-10); (iv) Combined treatment with colchicine and gamma rays yielded a progeny of plants half (5 out of 11) of which were pollen fertile; (v) These on further testing showed that the change had occurred at the cytoplasmic level, although there were two exceptions; (vi) Total DNAs extracted from the germs of male-sterile, maintainer, and restorer lines when banded in cesium chloride gradients yielded only a single peak. No satellite band was observed.

These observations have so far not provided any unique supporting evidence for the involvement of plasmids or episomes in male-sterility. In fact, these observations are compatible with another hypothesis, namely the mutation of a cytoplasmic DNA (plastid or mitochondrial).

D. Sen
N. K. Notani

THE BIRLA INSTITUTE OF SCIENTIFIC RESEARCH
Rupar, Punjab, India

1. Inheritance of male-sterility in Llera III variety of maize.

Last year (MNL 42:12) a few male-sterile plants were reported from Llera III but it could not be determined then as to how this variety inherited male-sterility. In order to resolve this point, five randomly selected male-sterile plants (even numbered plants in Table 1) were sib-pollinated by five different randomly selected male-fertile plants (odd numbered plants in Table 1). The latter five plants were self pollinated also. The progenies of all the ten plants were grown during summer 1968

Table 1

Proposed genetic constitution, observed frequency, and expected ratio
of male-sterile and fertile plants

Plant no.	Pedigree	Proposed genetic constitution	Observed frequency		Expected ratio		χ^2
			Fertile	Sterile	Fertile	Sterile	
1	Llera III ♂	$m_1m_1 m_2m_2 ms ms$	44	0	1	0	
2	Llera III ♂ St- # plant 1	$m_1m_1 m_2m_2 Ms ms$	57	43	1	1	1.96
3	Llera III ♂	$m_1m_1 M_2m_2 ms ms$	83	0	1	0	
4	Llera III ♂ St- # plant 3	$m_1m_1 m_2m_2 Ms ms$	63	27	3	1	1.20
5	Llera III ♂	$M_1m_1 M_2m_2 ms ms$	70	0	1	0	
6	Llera III ♂ St- # plant 5	$m_1m_1 m_2m_2 Ms ms$	75	5	7	1	2.86
7	Llera III ♂	$M_1m_1 m_2m_2 Ms Ms$	61	17	3	1	0.42
8	Llera III ♂ St- # plant 7	$m_1m_1 m_2m_2 Ms ms$	36	36	1	1	
9	Llera III ♂	$M_1m_1 M_2m_2 ms ms$	44	0	1	0	
10	Llera III ♂ St- # plant 9	$m_1m_1 m_2m_2 Ms ms$	39	1	7	1	3.65

and male-sterile and fertile plants counted. Table 1 presents the observed frequency and expected ratio of sterile and fertile plants.

It will be noted from Table 1 that the observed frequency of fertile and sterile plants showed a good fit to the expected ratio. From the various segregation ratios we presume that male-sterility in Llera III is caused by a dominant gene, \underline{Ms} , whose action is modified by two modifiers, \underline{M}_1 and \underline{M}_2 . Sterility will be produced if the gene, \underline{Ms} , is present in homozygous dominant or heterozygous condition and the two modifiers, \underline{M}_1 and \underline{M}_2 , in homozygous recessive condition. Therefore the genetic constitution of male-sterile plants will be $\underline{m}_1\underline{m}_1 \underline{m}_2\underline{m}_2 \underline{Ms} \underline{Ms}$ or $\underline{m}_1\underline{m}_1 \underline{m}_2\underline{m}_2 \underline{Ms} \underline{ms}$. Any other alternative form of any one of these three genes will result in a fertile plant. On the basis of this hypothesis the genetic constitution of the ten plants is also presented in Table 1.

Further studies are in progress to determine the linkage relationship of the three genes reported above.

B. K. Bhat*
M. C. Pande

*Present address: Regional Research Laboratory (C.S.I.R.),
Sanat Nagar, Srinagar, Kashmir, India.

2. "Notched leaf" a new trait in maize.

In a population of about 200 plants of Caribbean Flint Composite, grown during summer 1967, two plants were observed which very slightly resembled "knotted leaf". The two plants were selfed and also crossed with another variety not having the trait. The S_1 generation of both the plants segregated into the "so called knotted" and normal leaf types whereas the F_1 generation did not (S_1 and F_1 generations were grown during summer 1968). On closer comparison of those plants showing the trait with the description of the knotted leaf in the literature (Bryan, A.A. and J.E. Sass (1941), J. Hered 32:343-346) it was presumed that the trait was different from the knotted leaf and therefore it was designated as "notched leaf". The characteristics of this trait were:

- (i) only the top leaves (9th leaf and above) developed notch like structures about 1.0 cm on either side of the mid-rib and at about 11.5 cm from the leaf base (in three plants, out of a

population of 139 plants, notch had been noticed right at the leaf base and in one plant at a distance of 26.0 cm from the leaf base);

- (ii) notched leaf appeared only on those plants having a total of thirteen or more than thirteen leaves;
- (iii) after the 9th leaf any one leaf or two to five consecutive leaves developed the notch;
- (iv) invariably two notches per leaf appeared on either side of the mid-rib at about an equal distance from the leaf base;
- (v) unlike the knotted leaf each notch was a distinct structure against a smooth and clear background of the leaf surface;
- (vi) the number of notches per plant varied from one to ten depending upon the number of leaves bearing the notch;
- (vii) the expressivity of the concerned gene or genes varied from mild streaking to a big and prominent notch extending to about 2.5 cm;
- (viii) the segregation of S_1 plants into notched and normal leaf types might have been due to incomplete penetrance of the gene or genes.

The exact mode of inheritance of notched leaf is not yet known but our preliminary results indicate that it is due to a recessive gene with varying expressivity and incomplete penetrance. Studies are in progress to find out its exact mode of inheritance and the position on the linkage map. A more detailed paper including photographs will be reported very soon.

B. K. Bhat
M. C. Pande

BOSTON COLLEGE
Chestnut Hill, Massachusetts
Department of Biology

1. Chromosomes of Guanajuato teosinte from Mexico.

Nine F_1 hybrid plants of maize X Guanajuato teosinte were cytologically examined. At pachytene, nine chromosome knobs were observed. The

knobs on the long arms of chromosomes 1 and 3 were small. Large knobs each on the long arms of chromosomes 4 and 7 and on the short arm of chromosome 5 were present. The knob on the first knob position of the long arm of chromosome 6 was also large. However, the two knobs on the long arm of chromosome 8 and the one on the second knob position of the long arm of chromosome 6 were medium-sized. All of these knobs were intercalary.

There was a second type of chromosome 4 in which two large terminally located knobs were observed. This was found in only one plant.

A paracentric inversion on the short arm of chromosome 8 was identified. It was designated as In 8 of Guanajuato teosinte. The average length of four separate measurements of the inverted segment was 8.0 u. It occupied about 60 per cent of the short arm of this chromosome, and it is the same In 8 as that found in maize and the other teosintes. Bridges and fragments were observed at anaphases I and II. No duplication on the short arm of chromosome 8 was found.

Y. C. Ting
Mary E. Dougall

2. The effect of EMS on haploid and diploid maize.

Since last summer the well-known mutagen EMS (ethyl methane sulfonate), an alkylating agent, has been employed to treat haploid and diploid maize seeds. In order to break dormancy the seeds were presoaked in tap water one day before treatment and kept on a moistened filter paper in a petri dish. Two strengths of the aqueous mutagen solution were prepared; one was 0.5 per cent, the other 0.25 per cent. For each treatment, 100 ml of the solution was applied. The seeds were soaked in the solutions for four hours. Then they were rinsed in tap water seven times and placed on a filter paper overnight. These seeds were again rinsed seven times and planted.

Last summer, 50 diploid and 50 haploid maize seeds were treated with 0.5 per cent EMS, and a duplicate sample was treated with 0.25 per cent of this mutagen. At the same time, 50 diploid and 50 haploid maize seeds were selected as control. The control seeds were subjected to the same treatment procedures except that the EMS solutions were substituted

by tap water. All of the seeds were planted in the field on the same day. Subsequently, all of the surviving haploid plants showed a chlorophyll-deficiency characteristic, and it varied in degree and extent from plant to plant. As compared with untreated sibs, these haploid plants were consistently shorter and less tillered. Among the diploid plants, 25 per cent of those receiving 0.25 per cent EMS treatment showed chlorophyll deficiency, while 75 per cent of those receiving 0.5 per cent EMS treatment manifested the same symptom. No chlorophyll-deficiency mutants were observed among the controls.

This experiment was repeated last November in the greenhouse. The same effects were found. In addition among the surviving haploid plants receiving 0.5 per cent EMS treatment, 60 per cent showed a slashed-leaf (sl) appearance. No plants of the controls demonstrated this characteristic.

Pachytene chromosomes from 5 diploid and 6 haploid M_1 plants, as well as from over 20 F_1 plants involving the cross of both diploid and haploid M_1 plants with the inbred Wilbur's Flint, were cytologically investigated. No gross chromosome aberrations of any kind were consistently found.

Y. C. Ting
Mary E. Dougall

3. Lethal homozygotes of T6-9t.

During the last three years, over 120 maize plants from the selfed progeny of plants heterozygous for T6-9t were cytologically studied. No plants homozygous for this interchange were identified. Last summer, 36 seeds (bz/bz) from the same pedigree, were planted in the field. As reported previously, the gene bz was located within the translocated segment of the short arm of chromosome 9. It was found that none of those seeds was viable. Thus, it suggests that the T6-9t homozygotes are lethal.

Y. C. Ting

BROOKHAVEN NATIONAL LABORATORY*
Upton, New York
Biology Department

1. Induction of endosperm mutations in maize with ethyl methanesulfonate.

Methods for evaluating the relative genetic effect of mutagenic treatments and for obtaining endosperm mutations throughout the entire genome of an agronomically desirable line of maize are needed to facilitate fundamental and applied mutation research in maize. One method commonly used by maize workers is to treat a dominant stock, cross it to a genetic tester stock that is recessive for several known loci, and score for recessive mutants in the M_1 generation. However, this method is not well suited for identifying and accumulating endosperm mutations occurring elsewhere in unmarked regions throughout the chromosomes.

Most of the mutations used by geneticists and plant breeders have arisen spontaneously, hence at a low frequency. Therefore, the chemical mutagen ethyl methanesulfonate (EMS) was used because its ability to induce a high mutation frequency has been well established. The use of this mutagen with an efficient screening procedure can show the relative genetic response to mutagenic treatments; in addition, mutations potentially useful to geneticists and plant breeders may be obtained.

Inducing endosperm mutations directly into an agronomically desirable line of maize may have three possible uses to plant breeders: (1) Mutants may be produced that are not known to have arisen spontaneously and their usefulness can be determined. (2) The amount of a specific desirable product may be increased by increasing the number of genes that govern its production. (3) Mutation may be more expedient than backcrossing to incorporate genes into desired lines. Endosperm mutations can also be useful to geneticists as genetic markers and in studies on biochemical pathways.

Mutants in many self-pollinated plants, e.g., barley, are readily recovered by growing seeds of individual heads of the M_1 plants, since the male and female organs are in the same flower. Even if the mutant

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

involves only a small sector, most segregate in the M_2 generation. In maize, however, small mutant sectors may not involve both the ear (female inflorescence) and tassel (male inflorescence) and will not segregate in the M_2 generation. A mutation in either the ear or tassel would result in a heterozygous plant in the second generation which segregates in the third generation. Singleton and co-workers, who have done research on this problem, state that this difficulty could be overcome by using the one-celled proembryo as the experimental material, which affords an opportunity of obtaining a nonchimeric plant (Chatterjee et al., Genetics 52:1101-1111, 1965). Singleton (Genetics 52:475, 1965) has further pointed out that mutagenic treatments on seeds could be used more effectively if the treated seeds were planted in an isolated field and allowed to interpollinate for one generation before an attempt was made to recover the mutants. In this manner mutants could be readily recovered if a generation of random mating (M_1) were followed by self-pollination of the M_2 generation. Every mutant would be in the heterozygous condition and involve a whole plant, not a sector. The purpose of carrying mutagen treated material into subsequent generations in this research was to provide plant geneticists and breeders an opportunity to develop plant and endosperm mutations in the entire genome of maize for use in their research as well as to obtain an estimate of the effects of mutagenic treatments.

Seeds of the inbred line M14 were treated with 0.005 or 0.01 M EMS (Eastman Organic Co.) for 10 h at $25^\circ \pm 0.02^\circ C$ in a 0.02 M aqueous phosphate buffer (pH 7.5). The seeds had been stored at 60% relative humidity and had a moisture content of approximately 11.5%. After treatment they were rinsed in distilled water and sown immediately in the field. The material was planted in isolation, and the M_1 plants were self-pollinated. Forty-five M_1 ears were obtained from the 0.005 M treatment and 55 M_1 ears from the 0.01 M treatment. In the next generation, 30 seeds were planted from each M_1 ear, and the plants were later thinned to 15 per row. These plants were self-pollinated, and M_2 ears with M_3 seed on them were harvested and visually scanned for mutants segregating on the ears.

The procedure used in handling the treated material through subsequent generations was as follows:

M_1 seed
↓
 M_1 plant (ears)
↓ self-pollinate
 M_2 seed on ear

FIRST POLLINATION

M_2 seed
↓
 M_2 plant (ears)
↓ self-pollinate
 M_3 seed on ear

SECOND POLLINATION

An M_1 seed (normal or wild-type seed treated with a mutagen) is planted; this seed produces an M_1 plant, which is self-pollinated and has M_1 ears with M_2 seed on them. These M_2 seeds are planted in a subsequent growing season and in turn produce M_2 plants, which are self-pollinated and have M_2 ears with M_3 seed on them. Mutants produced by treating the M_1 seed with a mutagen will segregate in the M_3 seed generation. Since the M_1 seed has tissue made up of mutated and nonmutated cells, this procedure of self-pollinating the plants twice essentially isolates the progeny of a single mutated cell.

The mutants were detected in the M_3 seed generation, but the material was pedigreed from an M_1 ear. Therefore, the number of independently occurring mutations was divided by the number of M_1 ears to give the M_1 mutation frequency. To obtain the M_3 mutation frequency the number of independently occurring mutations was divided by the number of ears with M_3 seed on them.

Sterility occurring on ears bearing M_3 seed could be separated into two classes, i.e., $\frac{1}{4}$ or $\frac{1}{2}$ sterility. When sterility occurred with the mutants (Tables 1 and 2), its percentage was calculated by a method similar to that used for obtaining the mutation frequency. That is, to calculate sterility on an M_1 ear basis for a particular phenotypic class the number of M_1 ears was divided into the number of mutant ears that had sterility in that phenotypic class. The M_3 sterility frequency was calculated in a like manner, with the number of M_3 ears being the divisor. If an ear is $\frac{1}{4}$ sterile the reason for it is assumed to be genetic; if the ear is $\frac{1}{2}$ sterile the reason is assumed to be chromosomal, i.e., a chromosomal rearrangement. If several ears in a progeny row (ears with M_3 seed) were

Table 1

Phenotypes and mutation and sterility percentages from an ethyl methanesulfonate treatment (0.005 M , seeds planted wet)

Phenotype	All mutants			Mutants grown			Mutants that produced plants			Mutants that produced seed		
	M_1	M_3	Nr.	M_1	M_3	Nr.	M_1	M_3	Nr.	M_1	M_3	Nr.
Slightly opaque	11.11	0.99	5	6.67	0.59	3	6.67	0.59	3	6.67	0.59	3
Sterile*	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1
Translucent, tarnished												
Translucent, shrunken	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1
Wrinkled, glassy												
Opaque												
Floury	13.33	1.18	6	6.67	0.59	3	4.44	0.39	2	4.44	0.39	2
Lemon												
Orange												
White												
Miscellaneous												
Total	26.66	2.37	12	15.56	1.38	7	13.33	1.18	6	13.33	1.18	6
Sterile*	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1	2.22	0.20	1
45 M_1 ears												
507 M_3 ears												

*¼ sterility

segregating for sterility, it was assumed to be due to a single gene or to one chromosomal rearrangement and was used in this way to calculate the M_1 and M_3 sterility percentages. However, the sterility could be from separate gene mutations or chromosomal rearrangements.

Barley researchers use a system to classify the different chlorophyll deficient mutants. To make this maize mutation research more meaningful a classification system for the mutants was devised. The phenotypes used in this classification system, as well as mutation and sterility percentages from two EMS treatments, are given in Tables 1 and 2. The phenotypes of the mutants are based on the classification system as reported by Kramer et al. (Agron. J. 50:207-210, 1958) and by Creech (Genetics 52:1175-1186, 1965). However, additional phenotypic classifications were needed for this research. The phenotypic classification (Tables 1 and 2) does not imply genotype or allelism. Therefore, one must be cognizant of the definite distinction between the phenotypic classification in these tables and the genotypic classification of existing genes that have the same names, e.g., as used by Kramer and in this paper the opaque phenotype is the waxy genotype, and there are genotypes that are opaque (as opaque-1 and opaque-2). The classification system has enough different phenotypes to include the mutants that have occurred in the various experiments to date, even though in these two experiments mutants were not observed for each of the phenotypic classes; also additional phenotypes could easily be added to the classification system. The miscellaneous class was used for rarely occurring mutants or ones that were combinations of the other phenotypes.

The number of mutations and mutation frequencies by phenotypic classification is given for both the M_1 and M_3 generations (Tables 1 and 2). The mutants are classed as "all mutants," i.e., all of those detected. The classification "mutants grown" included those actually selected for planting in the field. The classifications "mutants that produced plants" and "mutants that produced seeds" are self explanatory. The total mutation frequency for an experiment and the total number of M_1 ears and the number of ears that had M_3 seed on them are given at the bottom of the tables.

Table 2

Phenotypes and mutation and sterility percentages from an ethyl methanesulfonate treatment (0.01 M, seeds planted wet)

Phenotype	All mutants			Mutants grown			Mutants that produced plants			Mutants that produced seed		
	M ₁	M ₃	Nr.	M ₁	M ₃	Nr.	M ₁	M ₃	Nr.	M ₁	M ₃	Nr.
Slightly opaque	32.73	3.42	18	9.09	0.95	5	3.64	0.38	2	3.64	0.38	2
Sterile*	3.64	0.38	2	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Sterile**	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Translucent, tarnished	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Translucent, shrunken	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Wrinkled, glassy	1.82	0.19	1	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Sterile*	1.82	0.19	1	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Opaque	3.64	0.38	2	3.64	0.38	2	3.64	0.38	2	1.82	0.19	1
Floury	10.91	1.14	6	7.27	0.76	4	3.64	0.38	2	0.00	0.00	0
Sterile**	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Lemon	7.27	0.76	4	7.27	0.76	4	5.45	0.57	3	3.64	0.38	2
Sterile*	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
Orange												
White	1.82	0.19	1	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Miscellaneous	5.45	0.57	3	3.64	0.38	2	0.00	0.00	0	0.00	0.00	0
Sterile*	3.64	0.38	2	1.82	0.19	1	0.00	0.00	0	0.00	0.00	0
Total	67.28	7.03	37	38.19	3.99	21	20.01	2.09	11	12.74	1.33	7
Sterile*	10.92	1.14	6	7.28	0.76	4	1.82	0.19	1	1.82	0.19	1
Sterile**	3.64	0.38	2	1.82	0.19	1	1.82	0.19	1	1.82	0.19	1
55 M ₁ ears; 527 M ₃ ears												

*¼ sterility

**½ sterility

The M_1 mutation frequency for the 0.005 M EMS treatment is 26.66%, and at this dose level not all of the phenotypic classes have mutants in them. However, a dose of 0.01 M EMS produced an M_1 mutation frequency of 67.28% and gave a wider spectrum of mutations, i.e., mutants occurred in all but one phenotypic category. Also, the sterility percentage was increased with a 0.01 M EMS treatment compared with 0.005 M .

After the mutants were observed on the ears, the usual mutation frequencies were calculated ("all mutants"). Some mutants ("mutants grown") were selected to be grown to the M_4 generation, since one objective of this experiment was to determine whether viable and useful mutants could be obtained. The main reason not all mutants were grown to the M_4 generation was that some, in addition to being endosperm mutants as listed in the tables, had seeds that were miniature.

If sterility occurred in a particular phenotypic classification, the type ($\frac{1}{4}$ or $\frac{1}{2}$ sterility) is designated and the total sterility of the mutants in the experiment is given at the bottom of the table. For example, in Table 1 five mutants were classified as slightly opaque, and one of the five mutant ears was $\frac{1}{4}$ sterile.

A disadvantage of EMS has been that sterility is produced in the progeny of treated material. However, this research indicates that it is possible to obtain mutants that do not have sterility (Tables 1 and 2).

The usual nursery procedure is to overplant and then thin to 15 plants per nursery row; however, to conserve seed only 15 seeds of each mutant were planted, and in many cases the survival in a row was less than this number. Also, some of the mutants (entire rows of M_3 seed), did not survive in the field. With the 0.005 M EMS treatment 86% of the mutants planted grew and produced seed. With the 0.01 M treatment 52% of the mutants grew and 33% produced seed.

Mutations can be obtained after one self-pollination in maize if the same mutated cell goes to make up both the tassel and the ear. In only one instance in these two experiments were all the kernels on the ear (M_3 seed) of the mutant class, i.e., the mutation was present after the first pollination but was not detected. As proposed by Singleton and shown by this research, the material should be carried to the M_3 generation to obtain any appreciable number of mutations.

The type of induced mutations (point mutations or deletions) at the waxy locus has been studied at this laboratory. The method reported here should produce homoallelic waxy mutants and in the same genetic background as that to which the standard waxy alleles have been backcrossed. Singleton has proposed allowing the M_1 plants to open pollinate (rather than selfing them as we did) and then selfing them in the next generation, at which time the heterozygous plants will segregate. If the material were allowed to open pollinate, a single mutation in the tassel could be disseminated in the field and be detected several times, but in fact it would be only one mutation. Also, we were interested in calculating the M_1 and M_3 mutation frequencies, and to do this we pedigreed the material by keeping the progeny from a particular M_1 plant. The second generation has to be self-pollinated. We self-pollinated both generations for the above reasons and because in our nursery procedure the number of M_1 plants is less by approximately a factor of 15 than the number of plants in the second generation.

When the endosperm mutants (M_3 seed) were planted, some of them segregated for chlorophyll deficient or dwarf plants; this indicates that probably a gene for chlorophyll deficiency or dwarfism mutated and was segregating independently from the endosperm mutation. Some of the endosperm mutants produced all chlorophyll deficient or dwarf plants--this may be an example of pleiotropism. Among the chlorophyll deficient seedlings, a case was observed which may represent the lemon-white (lw) series of genes, i.e., lemon endosperm-white seedling.

It should therefore be possible to obtain plant mutations at frequencies dependent on the dose of EMS if the M_3 seeds were planted, even if endosperm mutants had not been detected on the ears. For example, chlorophyll deficient and morphological mutants should be observed. Some of these mutants may be useful to investigators also.

For mutagen treatments to be used to introduce a particular mutant into a line and hence eliminate backcrossing, it is vital to have no genetic or chromosomal sterility concomitant with a mutant; also, one mutant per plant is desired, which may be attainable with a proper dose of EMS. After an induced mutant is obtained in a line, it may be necessary to backcross it to the original line to eliminate sterility or a

second mutant gene. However, fewer backcrosses would be required than if an unrelated non-recurrent parent were used with conventional backcross procedures.

Apparently EMS can be used to produce cytoplasmic mutants in plants (Dulieu, Mutation Res. 4:177-189, 1967) and may be useful to produce cytoplasmic sterility in maize by the above outlined procedures; probably more important, cytoplasmic sterility may be produced in other species with some modification of these procedures.

Robert W. Briggs

2. Modification of the efficiency and effectiveness of ethyl methanesulfonate treatments in maize.

The action of ethyl methanesulfonate (EMS) on seeds was investigated by altering post-treatment conditions so as to modify the genetic effects and physiological damage produced. Genetic effects were measured by the frequency of yellow green (yg_2) sectors in the leaves of Yg_2/Yg_2 seedlings; physiological damage by reduction in plant height. If seeds are dried immediately after treating with 0.01 M EMS (10 h, 25° C), the height of seedlings will be reduced significantly. If, however, the seeds are soaked for 4 days (at 3° C) after EMS treatment, then dried, the physiological damage is minimized and equals the control value. This post-treatment condition also reduces mutation rate, but it maximizes the treatment efficiency (ratio of yg_2 frequency to plant height reduction). Post-soaking apparently removes EMS and its hydrolysis products which are particularly harmful when the seeds are dried. However, post-soaking EMS-treated seeds before drying reduced the effectiveness of the treatment, as measured by the ratio of yg_2 frequency to dose of mutagen. This is probably because unhydrolyzed (active) EMS was removed from the seeds.

Robert W. Briggs

3. Esterase isozymes: new loci.

The E_1 through E_4 esterases were described by Drew Schwartz and his students at Indiana. The present note briefly describes several new anodal esterase loci and a single transaminase locus found during an ongoing classification of enzyme polymorphisms in flowering plants carried out at the University of Hawaii.

Re₄. This locus interacts with 'prime' alleles at the E₄ esterase locus to cause cessation of enzyme production. Two alleles were found: Re₄, which is functional with respect to interacting with the prime E₄ locus, and Re₄^o, which is nonfunctional and does not interact with the E₄ locus.

Oe₄. The Oe₄ site represents the interacting, or regulatory portion of the E₄ locus. Linkage between the Oe₄ and the E₄ sites has not been broken and, at present, they must be considered part of the same locus. Two 'alleles' of the Oe₄ 'locus' were found. Oe₄ represents the regulatory portion of a prime allele (designated as Oe₄E₄ⁿ, where n = one of the electrophoretic variants of the E₄ esterase isozymes). Plants containing the prime allele and the functional Re₄ 'regulator' allele do not produce E₄ esterase isozymes. The other 'allele', Oe₄^c, represents the regulatory portion of a standard allele (designated as Oe₄^cE₄ⁿ, where n = one of the electrophoretic variants of the E₄ esterase isozymes). The standard allele is not affected by the Re₄ locus and is of the 'constitutive' type.

To summarize then, two independently segregating loci control the E₄ esterase isozymes. Prime E₄ alleles interact with the functional 'regulator' allele of the Re₄ locus resulting in cessation of enzyme production. Prime alleles, in the presence of the nonfunctional 'regulator' allele, and standard alleles produce functional E₄ esterase isozymes.

It should be pointed out that in addition to the null phenotype produced through the interaction of prime alleles and functional 'regulator' alleles, null phenotypes were also produced due to nonfunctional 'structural' portions of the E₄ locus (E₄^{null}).

The genetic designations proposed for the E₄ esterases are not meant to imply that the prime and standard alleles represent operons. It has not been shown that the Oe₄ and the E₄ portions of this locus represent discrete cistrons due to the lack of crossover data. If one were to draw analogies, however, the Oe₄ portion of the locus would represent the operator gene of an operon and the E₄ portion of the locus would represent the structural gene of an operon.

E₅ Esterases. Two loci are involved in the control of the E₅ esterases. Two phenotypes were observed and each consisted of four

isozymes arranged in two pairs. Complex-I was assigned to inbreds containing the faster migrating pairs of isozymes while Complex-II was assigned to inbreds containing the slower migrating pairs of isozymes. Genetic analysis led to the postulating of two loci (\underline{E}_5 -I and \underline{E}_5 -II) which segregate independently. Each locus contained two alleles (\underline{E}_5 -I^A, \underline{E}_5 -I^a and \underline{E}_5 -II^A, \underline{E}_5 -II^a). Complex-II was found only in double recessive individuals (\underline{E}_5 -I^a/ \underline{E}_5 -I^a; \underline{E}_5 -II^a/ \underline{E}_5 -II^a). All other combinations resulted in Complex-I.

\underline{E}_6 Esterases. Two phenotypes were noted for the \underline{E}_6 esterases. A single isozyme was either present or absent in inbreds. A simple monogenic control mechanism was indicated from the genetic analysis. Two alleles were postulated (\underline{E}_6^A and $\underline{E}_6^{\text{null}}$). Individuals homozygous for the null allele showed no \underline{E}_6 esterase isozyme. The heterozygote and homozygous \underline{E}_6^A individuals contained the isozyme.

\underline{E}_7 Esterases. Two phenotypes were noted for the \underline{E}_7 esterases. A pair of closely associated isozymes was either present or absent in inbreds. Genetic analysis indicated that a single locus controlled the \underline{E}_7 esterases and two alleles were proposed (\underline{E}_7^A and $\underline{E}_7^{\text{null}}$). Individuals homozygous for the null allele lacked the \underline{E}_7 esterase isozymes. The heterozygote and homozygous \underline{E}_7^A individuals contained the isozymes.

\underline{E}_8 Esterases. Two phenotypes were noted for the \underline{E}_8 esterases. A single isozyme was either present or absent in inbreds. Genetic analysis indicated that the \underline{E}_8 esterases were controlled by a single locus and two alleles were postulated (\underline{E}_8^A and $\underline{E}_8^{\text{null}}$). Individuals homozygous for the null allele lacked the \underline{E}_8 isozyme. The heterozygote and homozygous \underline{E}_8^A individuals contained the isozyme. This isozyme is inhibited by fluoride.

\underline{E}_9 Esterases. Three phenotypes were observed to be controlled by a single locus. Three alleles were postulated. \underline{E}_9^A , when homozygous, demonstrated a single isozyme which migrated faster than the isozyme demonstrated in \underline{E}_9^B homozygotes. The third allele, $\underline{E}_9^{\text{null}}$, lacked both of the isozymes when homozygous. Heterozygotes between \underline{E}_9^A and \underline{E}_9^B contained both isozymes. Heterozygotes between \underline{E}_9^A and $\underline{E}_9^{\text{null}}$ contained the faster isozyme. Heterozygotes between \underline{E}_9^B and $\underline{E}_9^{\text{null}}$ contained the slower isozyme. The two isozymes of the \underline{E}_9 locus were activated by atropine.

E₁₀ Esterases. The E₁₀ esterases were confined to immature endosperm tissue. Two phenotypes were noted for the E₁₀ esterases. Individuals homozygous for E₁₀^A demonstrated a single isozyme which migrated faster than the single isozyme found in E₁₀^B homozygotes. The heterozygote E₁₀^A/E₁₀^A/E₁₀^B demonstrated both isozymes; however, due to the dosage effect the faster isozyme was stronger than the slower isozyme. The heterozygote E₁₀^A/E₁₀^B/E₁₀^B also demonstrated both isozymes, but in this case the slower isozyme was stronger than the faster isozyme. A single locus was postulated.

Ta₁ Transaminases. Two transaminase variants were found to be controlled by a single locus. The symbol Ta₁ was assigned to this locus and two alleles were noted (Ta₁^A and Ta₁^B). Ta₁^A homozygotes showed the faster variant. Heterozygotes demonstrated three isozymes: the faster variant associated with Ta₁^A homozygotes, the slower variant associated with Ta₁^B homozygotes, and a third isozyme intermediate in migration between the two parental types. It was postulated that the functional transaminase isozyme is dimeric in structure and that the intermediate isozyme represents an allodimer or hybrid enzyme containing the two parental type subunits. The faster isozyme would then be composed of two A type subunits, the slower would be composed of two B type subunits, and the hybrid isozyme would contain one of each. The transaminase isozyme migrated towards the anode at pH 8.2.

Timothy Macdonald

CENTRO INTERNACIONAL DE MEJORAMIENTO DE MAIZ Y TRIGO
Londres 40, Mexico 6, D.F., Mexico

1. Studies on the transmission of the B type chromosomes in maize.

Chromosome morphology studies made by Longley and Kato (1965) and Kato (1964) on different Mexican maize varieties have shown that some tend to accumulate a high number of B type chromosomes. Other varieties present very low frequencies of this type of chromosome and still other populations have no B chromosomes.

It is known that B chromosomes cause chromatid non-disjunction in the second mitosis of the microspore, and that on the female side the non-disjunction does not occur at all or very rarely. Also, it is known that the so called "directed fertilization" or "selective fertilization" occurs in plants possessing B chromosomes. Both of these phenomena could be genetically controlled. But the genetics of this control and whether these mechanisms behave differently in different populations is not known.

Therefore, one possible approach in solving the problem, as to why different maize populations differ in relation to B chromosome content, is to find out if either or both of the aforementioned phenomena behave differently in different maize populations. Also, information could be obtained on the nature of the genetic control of these mechanisms.

The present informal report has the intention to present some preliminary data obtained regarding this problem and some ideas developed from them.

During the summer of 1966 at Chapingo several reciprocal crosses between plants having OB and 1B chromosome were made. Single B chromosome plants were chosen for this purpose because of the convenience in starting the study from the most simple condition. The varieties used were Mexico 210 and Mexico 211 of the race Palomero Toluqueño and Puebla 262 of the race Arrocillo.

These varieties have a low frequency of B chromosomes. Studies with populations having a very high frequency of these type chromosomes are underway.

The seeds obtained from the above crosses were planted at Tepalcingo, Morelos during the winter of 1966-67. Sporocytes were collected from a sample of approximately 50 plants of each cross progeny. The cytological analysis of this material is presented in Table 1.

According to Roman (1947, 1948) B chromosomes undergo nondisjunction of the chromatids during the second mitotic division of the microspore. As a result, 3 types of pollen grains are formed: without B chromosomes; with one B chromosome in both gametes; and with 2B chromosomes in one gamete and the other gamete without any B.

The data obtained from the progenies of the crosses OB x 1B (Table 1) show that only 2 types of pollen grains were formed when the male

Table 1

B type chromosome constitution of the progeny plants of several crosses between plants of OB and 1B Palomero Toluqueño, Arroccillo and Chalqueño

Progeny Tep. 66-67	Type of cross	Plants crossed CH - 66*	No. plants of progeny with				Total plants examined	% plants with B
			OB	1B	2B	3B		
131Y	OB x 1B	5K-14 x 1K-30	35	0	12	0	47	25.53
134Y	"	5K-26 x 1K-23	33	0	15	0	48	31.25
136Y	"	3K x 1K-5	18	0	13	0	31	41.93
144Y	"	7K-1 x 7K-11	36	0	13	0	49	26.53
145Y	"	7K-8 x 7K-9	27	0	22	0	49	44.89
147Y	"	7K-15 x 7K-9	34	0	16	0	50	32.00
148Y	"	5993-18 x 6K-15	34	0	14	0	48	29.16
149Y	"	5993-18 x 7K-11	31	0	13	0	44	29.54
133Y	1B x OB	1K-23 x 5K-26	20	20	0	0	40	50.00
135Y	"	1K-5 x 3K	22	20	0	0	42	47.62
143Y	"	7K-11 x 7K-1	26	24	0	0	50	48.00
146Y	"	7K-9 x 7K-8	21	29	0	0	50	58.00
							548	

* 1K, 3K and 5K plants are Palomero Toluqueño type.

7K plants are Arroccillo type

5993 is a plant of Hidalgo 8 of the Chalqueño type.

parent plant had a single B, i.e., those without B chromosomes and those with 2B in one gamete and without any B in the second gamete. This means that all the microspores having a single B underwent nondisjunction of the B chromosome. This deduction is made because all the progeny plants contained either OB or 2B chromosomes, and none was found with a single B (see Figure 1). These results are in contradiction with those of Roman (1947, 1948). The discrepancy probably resides in the fact that Roman worked with a B chromosome translocated reciprocally with an A chromosome and in the present work only normal B chromosomes were used.

In the case of the crosses 1B x OB (Table 1) again only 2 types of progeny plants were found: (1) without any B and (2) with a single B. This result indicates clearly that in the female parent, B chromosomes behave normally; that is, in all the meiotic divisions of the megasporocytes and further mitotic divisions of the megaspores during the formation of the embryo sacs, B chromosomes invariably carry on normal disjunction of their chromatids.

In relation to the crosses of the type, 1B x OB, it can also be said that there is no preferential segregation of the B chromosomes in the megaspore that forms the embryo sac. This should be so since the frequencies of plants with and without B chromosomes appeared to be equal.

The most interesting feature of the present study resides in the frequencies with which the 2B plants appeared in the progenies of the crosses, OB x 1B. The percentage of plants with 2B in these crosses (Table 1) can be grouped into 3 general groups: one group comprises those crosses giving approximately 25% plants with 2B, the second group has approximately 40-45% plants with 2B, and a third group has approximately 30% of the plants with 2B.

The progenies 145Y, 147Y and 148Y (Table 1) suggest the female side may carry a factor which controls the fertilization of the ovules by pollen grains possessing B chromosomes. In other words, progenies 145Y and 147Y were the product of crossing two different female plants by the same male plant, but they gave different frequencies of 2B plants in their progenies, 44.89% and 32.00%, respectively. On the other hand, progenies 148Y and 149Y, which gave the same frequency of 2B plants in their progenies, were the product of crossing the same female plant by two different males.

Under the assumption that there exists a single pair of alleles with an incomplete dominance, say \underline{A} and \underline{A}^1 , and considering the type of microsporogenesis which has been deduced from the data in Table 1 and shown schematically in Figure 1, two possible mechanisms can be used to explain the results obtained in the crosses OB x 1B:

(1) The pollen tubes formed by all pollen grains (with and without B chromosomes) grow at the same speed in the female stigmatic tissues, penetrating the ovules at random. The female genotype \underline{AA} tends to guide preferentially the 2B gametes of the pollen grains with B chromosomes to fertilize the egg cells and the OB gametes to fertilize the polar nuclei which will produce the endosperm tissues. This would occur in ovules penetrated and fertilized by pollen tubes having B chromosomes. In ovules penetrated by pollen tubes without B chromosomes the fertilization process would be normal. Thus 50% of the progeny would be 2B plants and 50% OB plants. In the case of a female plant with a genotype $\underline{A}^1\underline{A}^1$ the pollen tubes again would grow at the same speed and the fertilization process would be more or less random between 2B and OB gametes. The resulting progenies would be approximately 25% 2B plants and 75% OB plants. In the case of the female plant with \underline{AA}^1 genotype the development of the pollen tubes would be normal and the fertilization process would take place in an intermediate fashion in comparison with the females \underline{AA} and $\underline{A}^1\underline{A}^1$.

(2) The second possibility is that the female genotype controls the development of the pollen tubes and has no influence on the fertilization process; in other words, the fertilization is at random.

The pollen tubes with B chromosomes would grow at a higher rate of speed than those without B chromosomes in stigmatic tissues of \underline{AA} genotype plants. Therefore, the pollen tubes with B chromosomes would reach the ovules first. Fertilization by OB and 2B gametic nuclei would be at random, resulting in 50% of the progenies being 2B plants.

In the stigmas of $\underline{A}^1\underline{A}^1$ plants the growth rate of the pollen tubes with and without B chromosomes would be the same. Since 25% of the gametic nuclei have 2B chromosomes and fertilization is at random, 25% of the progeny would be 2B plants.

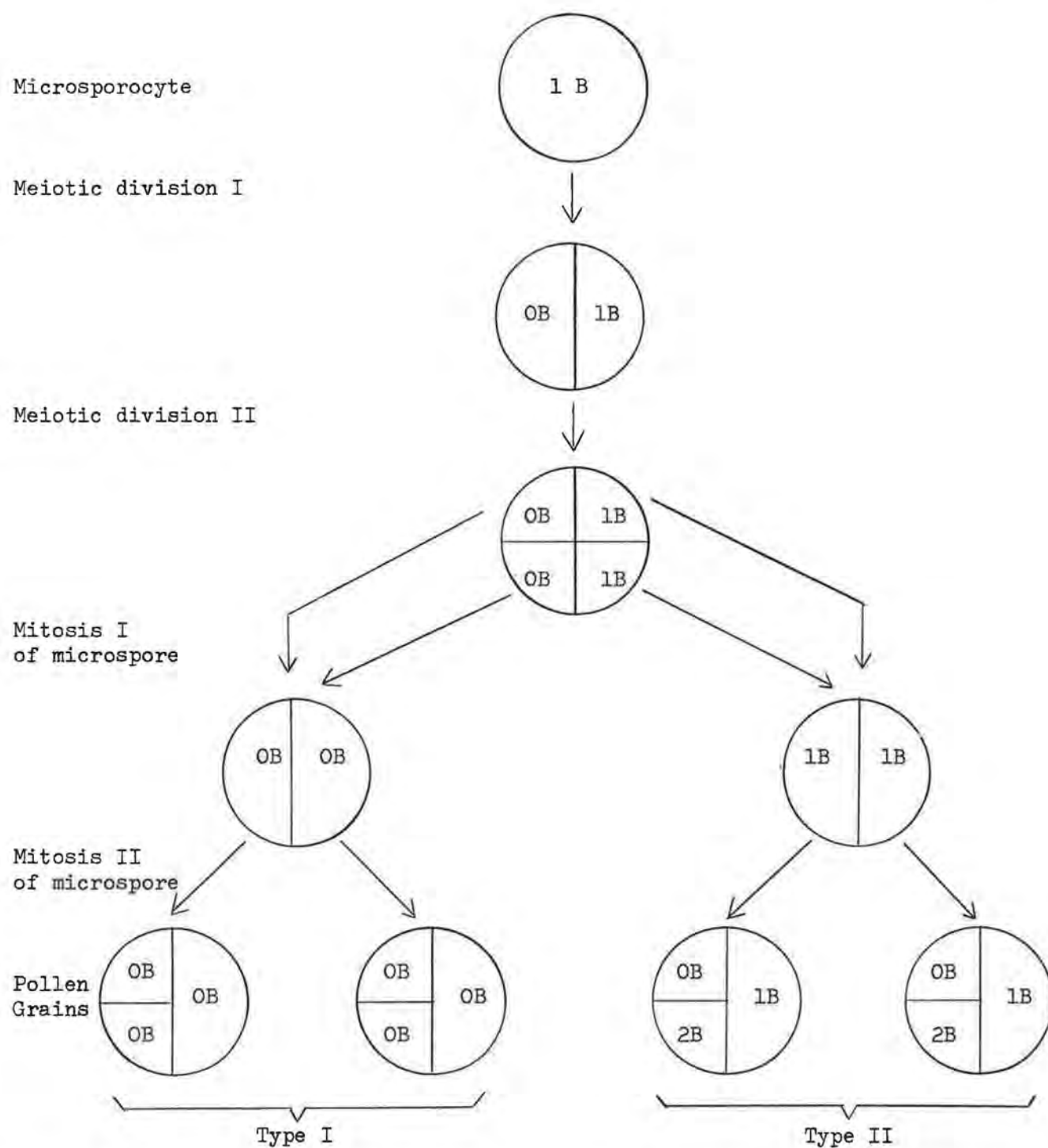


Figure 1. Diagram showing the type of microsporogenesis carried on by plants with a single B chromosome. This scheme was deduced from the data presented in Table 1.

In female plants with the \underline{AA}^1 genotype the growth rate of the pollen tubes with and without B chromosomes would be intermediate between that in female plants with \underline{AA} or $\underline{A}^1\underline{A}^1$ genotypes. Assuming fertilization is at random, the frequency of 2B plants in the progeny would be intermediate, i.e., between 25% and 50% expected in \underline{AA} and $\underline{A}^1\underline{A}^1$ genotype plants.

Other explanations may be developed but these presented are worthy of consideration as working hypotheses since they can be tested experimentally.

The failure of the OB x 1B crosses (Table 1) to give exactly 50% 2B progeny is probably due to the presence of environmental or genetic modifier factors.

Some experimental studies regarding these two working hypotheses have been started during 1968.

T. Angel Kato Y.

CORNELL UNIVERSITY
Ithaca, New York

1. Further evidence for sister-strand crossing over in maize.

Schwartz (Genetics 38:251, 1953) presented evidence that sister-strand crossing over is a general phenomenon in meiotic cells of maize. Essentially that work has been repeated here using plants heterozygous for a ring and its homologous rod chromosome 10.

The ring chromosome 10 was derived from a long derivative of abnormal chromosome 10. Figure 1 gives a diagrammatic representation of the formation of the ring following a crossover between 10L and a second 10L fragment attached to the short arm of the chromosome. The knob is a large portion of the abnormal chromosome 10 knob.

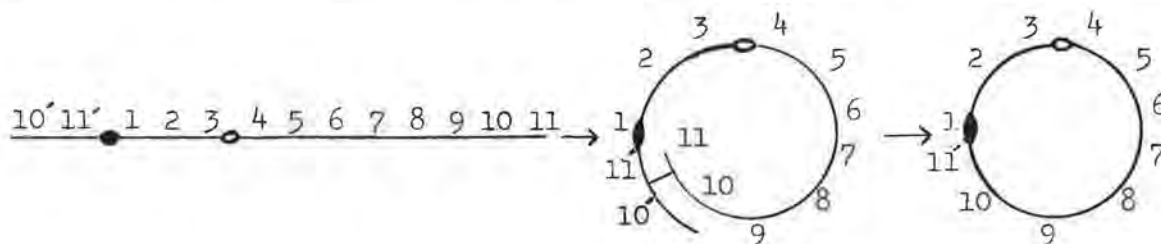


Figure 1. Diagrammatic representation of the formation of ring 10.

Certain crossovers between a ring and its homologous rod chromosome result in bridges being formed at anaphase I and/or at anaphase II. For example, a tetrad with a single crossover results in a single bridge at AI but no bridge at AII. Double exchanges result in specific AI and AII configurations depending on whether the exchanges are 2-strand doubles, type I 3-strand doubles, type II 3-strand doubles or 4-strand doubles, and on whether the two crossovers are in the same arm (Class A doubles) or are separated by the centromere (Class B doubles). The anaphase configurations resulting from single and double crossovers are shown in Table 1.

Table 1

Crossover type	Anaphase I (bridges)	Anaphase II (bridges)
None	None	None
Single	Single	None
Class A double crossovers (in one arm)		
2-strand double	None	None
3-strand double I	Single	None
3-strand double II	Single	Single
4-strand double	Double	None
Class B double crossovers (separated by centromere)		
2-strand double	None	None
3-strand double I	Double	None
3-strand double II	None	Single
4-strand double	Double	None

Schwartz used a ring chromosome 6 and could assume no crossing over in the very short arm. However when a ring chromosome 10 is used eight types of double crossovers must be considered rather than four. This consideration of crossing over in both arms makes the argument more complex but eventually leads to the same conclusion.

Three plants heterozygous for the ring chromosome 10 and its homologous rod were examined for anaphase I and anaphase II configurations. The frequencies of the various anaphase configurations are listed in Table 2. Anaphase I data are given for single cells counted; anaphase

Table 2

Anaphase configurations observed in plants heterozygous for a ring
and its homologous rod chromosome 10

	Anaphase I				Anaphase II (daughter cell pairs)			
	Single bridge	Double bridge	No bridge	Total	Single bridge	Double bridge	No bridge	Total
Number	332	70	126	528	242	36	386	664
Percent	62.8	13.3	23.9	100	36.4	5.4	58.2	100

Table 3

Theoretical expectations from crossovers between a ring and its homologous rod chromosome 10

Crossover type	Anaphase I			Anaphase II		
	Single bridge	Double bridge	No bridge	Single bridge	Double bridge	No bridge
Part a. - non-sister chromatid crossovers only.						
None	-	-	100%	-	-	100%
Single	100%	-	-	-	-	100%
Double : Class A	50%	25%	25%	25%	-	75%
Double : Class B	-	50%	50%	25%	-	75%
Part b. - non-sister chromatid crossovers plus 50% effective sister-strand crossing over.						
None	-	-	100%	-	50%	50%
Single	100%	-	-	50%	-	50%
Double : Class A	50%	25%	25%	25%	12.5%	62.5%
Double : Class B	-	50%	50%	50%	-	50%

II data have been converted to daughter cell pairs which show a single or double bridge in one of the two cells.

The observed results (Table 2) can be compared with the theoretical expectations from each of the exchange possibilities (Table 3). The theoretical expectations are expressed as the percent of the total for each type of exchange. Table 3 is divided into Part a and Part b. Part a gives the expected results if crossing over is allowed between non-sister chromatids only. Part b will be discussed below.

Two discrepancies between the observed data and that expected if no sister-strand crossing over is allowed are obvious. The first is the high frequency of single bridges in anaphase II. When no sister-strand crossing over is allowed, these result from 3-strand type II double exchanges exclusively. If all 36.4% AII single bridges were due to one kind of double crossover, the total of the four types of double crossovers would exceed 100%. Since this is impossible, it is necessary to look for another source of AII single bridges.

The high frequency of AII single bridges can be accounted for by sister-strand crossing over. If the number of sister-strand crossovers per bivalent is high, an odd number occurring in any one region will appear as a crossover while an even number will appear as a noncrossover. Therefore, when a high number of sister-strand crossovers occurs per bivalent an effective sister-strand crossover occurs 50% of the time. Table 3 Part b indicates the frequency of anaphase bridges resulting from single and double crossovers plus 50% effective sister-strand crossing over. Note particularly that with 50% effective sister-strand crossing over AII single bridges result from half of the single exchanges and one-half of the Class B double exchanges.

To calculate the total percent of AII single bridges from all sources it is necessary to determine first the percent of AII single bridges expected irregardless of sister-strand crossing over, i.e. the percent of both Class A and Class B type II 3-strand double exchanges. First, assume that because of no chromatid interference 2-strand double exchanges equal 3-strand I double exchanges equal 3-strand II double exchanges equal 4-strand double exchanges. Second, assume the chromosome 10 arm ratio for crossing over is 2.8:1. It follows from the second

assumption that 61.2% of the double crossovers are in the same arm (Class A doubles) and 38.8% have the two crossovers separated by the centromere (Class B doubles). Third, observe that the 13.3% AI double bridges are the result of the two classes of 4-strand double exchanges and Class B 3-strand I double exchanges (Table 1), and that the proportions of these three classes are not changed by the occurrence of sister-strand crossing over. (Table 3). Combining these three statements it can be calculated that Class A double crossovers occur 23.6% of the time and Class B double crossovers occur 14.8% of the time, with 5.9% of each type of Class A exchange and 3.7% of each type of Class B exchange. Therefore only 9.6% ($5.9\% + 3.7\%$) AII single bridges are expected on the basis of non-sister chromatid crossing over.

Single bridges in anaphase I were observed 62.8% of the time. These result from single crossovers and half of the Class A double crossovers. The frequency of one-half of the Class A double crossovers is expected to be 11.8% ($\frac{1}{2} \cdot 23.6\%$). This leaves 51.0% AI single bridges due to single non-sister chromatid crossovers. As a result of sister-strand crossing over, half of these or 25.5% will form single bridges at AII. The third source of AII single bridges is Class B double exchanges. When 50% effective sister-strand crossing over occurs, 3.7% single bridges are expected at AII (Table 3). Thus, 38.8% ($9.6\% + 25.5\% + 3.7\%$) single bridges are expected on the basis of abundant sister-strand crossing over; the observed frequency was 36.4%.

The second observation which can not be explained on the basis of no sister-strand crossing over is the appearance of double bridges (dicentric rings) at anaphase II. Double bridges at AII are not expected from either single or double non-sister chromatid crossovers. However, AII double bridges arise from sister-strand crossing over in tetrads which had no other crossovers and those which had 2-strand double exchanges in the same arm. The frequency of noncrossover tetrads is equal to the frequency of all AI cells showing no bridges less the percent of no bridge AI cells coming from double exchanges. The percent of no bridge AI cells coming from double exchanges is 13.3% since the number of these expected is the same as for double bridge AI cells (Table 1). Thus, 10.6% ($23.9\% - 13.3\%$) of all tetrads had no non-sister chromatid

crossovers. As a result of sister-strand crossing over, half of these or 5.3% will form double bridges at anaphase II. Class A 2-strand double exchanges occur 5.9% of the time; with 50% effective sister-strand crossing over half of these or 2.9% will form AII double bridges. Thus, 8.2% (5.3% + 2.9%) double bridges at AII are expected; the observed frequency was 5.4%.

The disparity between the observed anaphase configurations and those expected on the basis of non-sister chromatid crossing over between the ring chromosome 10 and its homologous rod is interpreted as due to sister-strand crossing over since the hypothesis of at least one sister-strand crossover per bivalent accounts for the experimental results. Two assumptions basic to the argument are no chromatid interference and a 2.8:1 arm ratio for crossing over in chromosome 10. Although the assumption of no chromatid interference probably holds in maize, the experimental results also discount the possibility of negative chromatid interference accounting for the high percent of AII single bridges. If there were negative chromatid interference we would expect more 2-strand doubles than type II 3-strand doubles. Since only part of the 23.9% AI cells with no bridges could be due to 2-strand doubles the 36.4% AII single bridges must not be a true indication of the number of type II 3-strand doubles. The 2.8:1 arm ratio was assumed since it is the cytological arm ratio. However, the arm ratio chosen is not critical to the argument. When the arms are assumed to be the same length, 40.3% AII single bridges and 7.4% AII double bridges are expected; both figures are reasonably close to the observed values. A third point is the dismissal of three or more crossovers to account for the data. One through seven exchanges were considered with an arm ratio varying from 20:1 to 1:1;* at no time did the percent of single bridges in AII exceed the percent of double bridges in AI. The observed percent of AII double bridges was reached only in situations where every tetrad had at least three crossovers and when the arm ratio was at least 19:1. Obviously, higher exchange levels could not account for the data.

*Computer program written by Roland Littlewood.

DEFIANCE COLLEGE
Defiance, Ohio

1. Relationship of the number of photoperiod cycles to paramutant R-gene expression.

In MGCNL 1968 we reported that light-dark cycles at the time of tassel initiation controlled the level of paramutant R-gene expression. The present report shows that the number of light-dark cycles at the time of tassel differentiation can determine the level of paramutant R expression in testcross kernels.

Test plants were grown under constant light conditions supplied by ten 200W fluorescent lamps supplemented by twelve 50W incandescent lamps; temperatures were maintained at 26°C. During the first 25 days of plant development, plants were kept in constant light except for the dark periods administered at specific plant ages. Since preliminary experiments showed plants were ready for tassel induction at 13 days, dark cycles were administered to seedlings beginning at ten days.

Seed genotypes in inbred W22 background were RRst heterozygotes; the Rst allele used was known to give paramutant-R (called R' hereafter) scores with intermediate levels of pigmentation. Groups of 12 plants each were tested for the effect of one, two, three or six light-dark (L:D) cycles consisting of 12 hours of light followed by 12 hours of darkness. Following L:D treatment of each group, plants were held in constant light until the 25th day; all experimental groups were then transplanted to field conditions until maturity. At anthesis, all L:D-treated plants were testcrossed to females grown under field conditions. Because variation in R' expression has been noted in single tassels, several pollinations were made from single plants on different days during the week of pollen shed. All pollinations from a single plant were recorded as single plant means and reported in the experimental group means. Testcross kernels were scored at harvest time by methods outlined in our previous MGCNL reports. Differences found in scores of R' in test-cross kernels, as a result of L:D treatments, had to be pollen transmitted.

Table 1 shows that the pigment scores for R' expression increased with the number of light-dark cycles given the seedlings; treatment means show this relationship is linear for the period tested. Seedling

sensitivity to L:D cycles begins toward the end of the second week of seedling development--our preliminary experiments had shown this to be the period seedlings are ready for floral induction at the temperature and light intensities available.

Table 1

R¹ testcross scores following L:D treatments administered during tassel initiation

No. of L:D Cycles	Age of Plant	No. of Plants Tested	No. of Pollinations Scored	Group Means	Treatment Means
1	12-13	7	11	9.29	9.17
	14-15	10	20	8.80	
	16-17	5	7	8.31	
	18-19	11	29	10.29	
2	11-13	10	28	9.76	10.70
	13-15	11	32	9.94	
	15-17	10	33	12.40	
3	11-14	11	36	11.03	11.49
	15-18	9	28	11.95	
6	10-16	12	47	12.02	13.20
	12-18	11	44	14.04	
	16-22	8	22	13.53	

To provide for optimal pigment formation in testcross seeds under late summer conditions, August 18 was selected as a pollination cut-off date. Plants which received fewer L:D cycles reach anthesis later; therefore fewer plants with one L:D cycle could be included in the above data. However, objections that score differences reported reflect differences in pollination time are not supported by our existing data since the latest pollinations from plants given six L:D cycles were still consistently darker than those receiving one L:D cycle where both pollinations were made the same day.

The data above raise many more questions than answers. What internal mechanism in somatic tissue is capable of response to environment at

this tassel initiation stage of development? What memory mechanism receives and stores this information in the pollen and then reflects the environmental response in pigment cell differentiation in the endosperm of the testcross parent? Such a mechanism must possess an additive capability for the one to six L:D cycles during flower initiation. While this short-term memory offers no special conceptual problems beyond those of differentiation of cells in the individual treated, to account for the above data as a carry-over effect from the pre-tassel somatic tissue, through the pollen, to the endosperm formed in the testcross on the female, does strain existing genetic models.

We have previously reported the paramutational additive effect on R-expression of Rst through ten generations; it is possible that the environmental effects recorded above may now help to determine the ontogenetic times that R is susceptible to additive "suggestion" from both paramutation and the environment.

Appreciation is expressed to the Center for the Biology of Natural Systems, Washington University, St. Louis, under Public Health Service Grant No. ES-00139-01, for making the early stages of this work possible.

Bernard C. Mikula

UNIVERSITY OF ILLINOIS
Urbana, Illinois
Department of Plant Pathology

1. The effect of linkage and breakage between the elements of locus Rp₁.

It has been shown (Saxena and Hooker, 1968) that several dominant "alleles" at Rp₁ (Rp₁^a, Rp₁^c, Rp₁^k) consist of closely linked genes for resistance and susceptibility to different biotypes of Puccinia sorghi. The elements conditioning resistance to a portion of the rust biotypes in two complex "alleles" can be recombined with resultant broadening in spectrum of resistance. Results reported here are from a study in progress undertaken to observe the effect of breakage on these recombined elements for resistance.

The recombinant Rp₁^c-Rp₁^k (linkage $0.16 \pm 0.03\%$) was used for this study and the following testcross: Rp₁^c-Rp₁^k a₁ a₂ c r pr / rp₁ a₁ a₂

c r pr x rp₁ A₁ A₂ C R Pr / same was made using the susceptible plant (rp₁ A₁ A₂ C R Pr) as pollen parent. All pollinations were handmade. Since no suitable markers are available close to Rp₁, and an outcross could be impossible to differentiate from a crossover product, the pollen parent with dominant genes for purple aleurone was used as an added precaution against pollen contamination.

"Allele" Rp₁^c conditions resistance to P. sorghi culture 936c but not to 941bR; "allele" Rp₁^k conditions the reciprocal reaction. Both alleles give resistance to 901aba. The recombinant Rp₁^c-Rp₁^k is resistant to all three cultures. Thus, parentals can be easily distinguished from nonparentals in the progeny test.

To eliminate all parentals, the progeny was first tested with 901aba, and subsequently with a mixture of cultures 936c and 941bR. From a total of 12,038 seedlings thus tested, 18 suspected nonparentals were saved and allowed to grow to maturity. These were testcrossed by susceptible plants and the seed saved for further progeny testing.

On progeny testing, 11 out of 18 plants saved proved to be parentals and two nonparentals. Progeny of three are still undergoing testing. For further characterization, the progeny of the two nonparentals was tested with 15 biotypes of P. sorghi. One of these proved to be indistinguishable from Rp₁^k. "Allele" Rp₁^k is susceptible to cultures 904d and 936c whereas the modified Rp₁^k is susceptible to only 936c. Further testing is in progress.

K. M. S. Saxena

INDIANA UNIVERSITY
Bloomington, Indiana

1. Inherited variation of catechol oxidase.

Toru Endo (unpublished) first observed inherited variation of catechol oxidase behavior between inbred maize lines. When extracts of shoots from 3-4 day old seedlings were subjected to starch gel electrophoresis at pH 8.3, and the gels incubated in 0.01 M catechol at pH 4.2 to reveal the enzyme bands, three true breeding classes were observed.

Class I: zymograms showed a cathodally migrating band.

Class II: showed no band of enzyme activity. However, if the seeds were presoaked in 0.01 M maleic hydrazide, the same cathodally migrating band was revealed.

Class III (Null class): no enzyme band could be detected either with or without maleic hydrazide treatment.

The inheritance of enzyme variants.

In the present study a slower moving electrophoretic variant of a Class II line was found in a survey of inbred lines. Genetic studies indicate that the common fast migrating isozyme (F), the variant slow isozyme (S), and the null form (Class III) are specified by different alleles of a gene, designated here as catechol oxidase or Cx.

Using the wx linked translocations, Cx has been located on Chromosome 10. In crosses to the endosperm marker dull (du) a recombination frequency of 0.16% (2/1197) was observed indicating very close linkage of du and Cx.

The difference between Class I and Class II.

Zymograms of extracts from shoots of Class II maize lines only show a catechol oxidase band if the seeds have been presoaked in 0.01 M maleic hydrazide. Concomitant treatment with protein synthesis inhibitors (cycloheximide and chloramphenicol) does not prevent the maleic hydrazide mediated appearance of a band of enzyme activity. This suggests that the appearance of a band after maleic hydrazide treatment does not involve a de novo protein synthesis.

There is evidence suggesting that Class II seedlings contain a "modifier" which rapidly reacts with catechol oxidase during extraction, so that the enzyme no longer migrates in electrophoresis. The existence of a non-migrating enzyme in extracts of untreated Class II seedlings can be demonstrated by electrophoresis on cellulose acetate strips. Zymograms from these strips show a band of catechol oxidase activity remaining at the origin.

The amount of "modifier" present in untreated Class II seedlings is limited, but sufficient to prevent the appearance of the cathodally migrating band. Thus, when shoots from Class II seedlings carrying the Cx^F allele are homogenized in the presence of increasing concentrations of S enzyme extract (partially purified on a sephadex column), the F enzyme band is eventually revealed in electrophoresis.

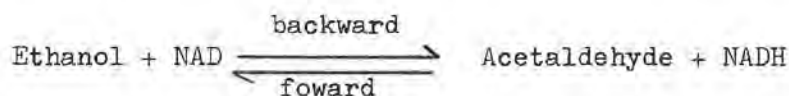
We suggest that the difference between Class I and Class II lines is due to the amount of "modifier" present. The amount of "modifier" is sufficient in Class II lines to inhibit the migration of all of the catechol oxidase, but insufficient in Class I, thus allowing the appearance of an enzyme band in zymograms from untreated seedlings. On this basis, the treatment of seeds with maleic hydrazide in some way affects the amount or availability of "modifier" in shoots, so that the migration of enzyme in Class II seedlings is not affected and extracts give rise to a cathodal band in electrophoresis.

Tony Pryor

2. Substrate inhibition of allelic isozymes of alcohol dehydrogenase in maize.

Differences in the pH optima of the enzyme forms specified by the $Adh_1^{C(m)}$ and Adh_1^S alleles were reported previously (Schwartz & Laughner, Maize News Letter 42:83, 1968). Further studies on the kinetic properties of these alcohol dehydrogenase isozymes have revealed striking differences in their inhibition by specific substrates.

Alcohol dehydrogenase (E.C. 1.1.1.1.) catalyzes the following reaction:

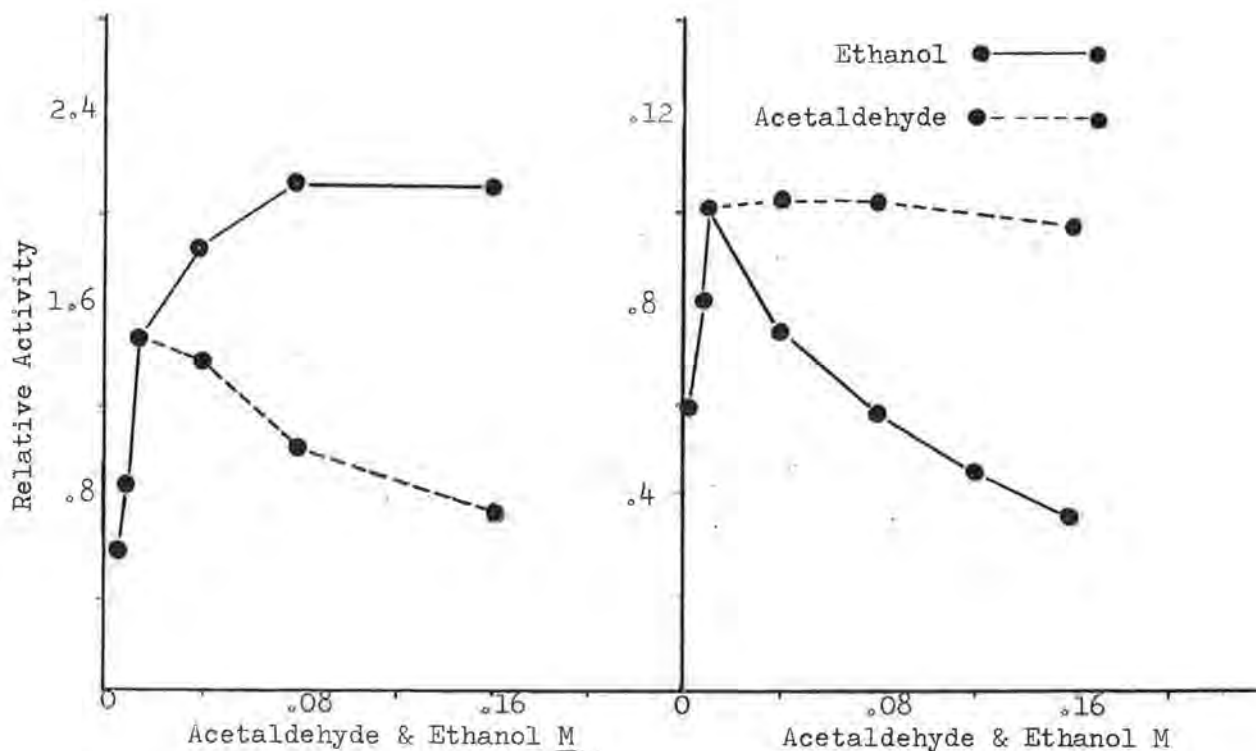


Oxidation of ethanol to acetaldehyde is accompanied by reduction of NAD to NADH and the reverse. When the activity of specific isozymes at different substrate concentrations was determined in the back and forward reactions (by following the absorbance of NADH at 340m μ), it was found (fig. 1) that the enzyme specified by the $\text{Adh}_1^{\text{C(m)}}$ allele was inhibited by ethanol concentrations which were not inhibitory to the enzymes specified by the Adh_1^{F} , Adh_1^{S} , and $\text{Adh}_1^{\text{C(t)}}$ alleles.

Similarly, the enzymes specified by the Adh_1^{F} , Adh_1^{S} , and $\text{Adh}_1^{\text{C(t)}}$ alleles were inhibited by concentrations of acetaldehyde which were not inhibitory to the C^{m} enzyme. Although $\text{ADH}^{\text{C(t)}}$ and $\text{ADH}^{\text{C(m)}}$ show the same migration rate in starch gel electrophoresis, the two enzymes differ much more from each other than do the three electrophoretically distinguishable enzymes specified by the Adh_1^{F} , Adh_1^{S} , and $\text{Adh}_1^{\text{C(t)}}$ alleles.

William Laughner

Figure 1. Relative activity (calculated by setting the activity at .01M substrate concentration as 1) vs substrate concentration.



3. EMS induced mutations of the Sh_1 locus.

Schwartz (Genetics 45:1419-1427, 1960) presented evidence to show that the Sh_1 gene controls the synthesis of a major protein component (designated as the Sh_1 protein) in the endosperm. This protein is completely lacking in the $sh_1/sh_1/sh_1$ material. We have obtained 16 new sh_1 mutants by ethyl methanesulfonate (EMS) treatment of Sh_1Wx seeds. The mutants were detected by crossing the treated material to a recessive sh_1wx tester. On the basis of electrophoretic and immunochemical analysis of 20 day old endosperm these mutants were grouped into the following four classes:

Class	Characterization of the sh_1 mutants		Number of mutants	Remarks
	Electrophoretic behavior on the starch gels	Immunochemical behavior in Ouchterlony plates		
I	No Sh_1 protein band.	CRM^-	9	similar to previously analyzed sh_1 mutants.
II	No Sh_1 band detectable by protein staining.	CRM^+ (very faint precipitation band)	1	probably indicates low concentration of Sh_1 protein.
III	Sh_1 protein band with altered migration rate.	CRM^+	2	one is faster and another is slower migrating in relation to Sh_1 protein band.
IV	Sh_1 protein band present (unaltered migration rate)	CRM^+	4	indistinguishable from wild type protein by these two criteria.

These results show that the Sh_1 protein has high specificity in activity since its function in kernel development can be eliminated by point mutations which cause little or no change in net charge or size (Classes III & IV). Although the Sh_1 protein in the Class IV mutants shows the wild type migration rate, it must differ in primary structure. A majority of amino acid replacements in a protein resulting from base changes would not alter the charge of the molecule and would remain

undetected by electrophoretic analysis. The presence of Class II and III type mutations offers strong support, along with the previously presented evidence, that this protein is specified by the Sh₁ gene, since qualitative and quantitative changes in the protein are associated with the appearance of the sh₁ phenotype.

Prem S. Chourey

4. An effect of B chromosomes on crossing over in chromosome 5.

It was reported in last year's News Letter (p. 63) that B chromosomes might cause an increase in crossing over in the A₂-Bt₁-Pr region of chromosome 5. This work was followed up during the summer of 1968, when plants of two related families (536 and 537) which were heterozygous for A₂Bt₁Pr were root-tipped and scored for B chromosomes, then transplanted to the field and backcrossed as females and as males to a₂bt₁pr testers.

The results are shown below:-

Family no.	Used as:	No. of plants	No. of B's	No. of kernels	% Recombination			
					<u>A-Bt</u>	<u>Bt-Pr</u>	Total	% increase
537	♀	8	0	2897	5.9	15.9	21.8	-
		8	1	2797	6.9	18.1	25.0	15
		10	2	3197	8.8	21.7	30.5	40
536	♀	4	0	1529	8.2	23.0	31.2	-
		10	2	3493	9.6	22.3	31.9	2
		10	4	3527	10.7	25.3	36.0	15
537	♂	9	0	4560	11.3	26.8	38.1	-
		8	1	4505	15.1	31.8	46.9	23
		10	2	5814	17.7	34.6	52.3	37
536	♂	5	0	2764	13.4	26.0	39.4	-
		10	2	4812	18.1	35.8	53.9	37
		10	4	4829	23.6	37.6	61.2	55

The recombination values for the Bt₁-Pr region were obtained from the A₂ kernels only, since a₂ kernels lack color.

Although the data have not yet been statistically analyzed, both of the above families and the one mentioned last year showed increased

recombination for the $\underline{A_2-Bt_1-Pr}$ region in the megasporocytes when B chromosomes were present. Furthermore, it appears that the B chromosomes had a dosage effect. The increase in crossing over and the dosage effect in both the $\underline{A_2-Bt_1}$ and $\underline{Bt_1-Pr}$ regions were more marked in the microsporocytes than in the megasporocytes and are similar to those obtained by Rhoades for the $\underline{C-Wx}$ region of Tp9 plants. In the latter case, however, there was a corresponding decrease in the recombination value for the adjacent $\underline{Yg_2-C}$ region of chromosome 9, which would indicate a shift in the distribution of crossovers along the chromosome arm (cf. "Replication and Recombination of Genetic Material", pp. 229-241. Eds. W. J. Peacock & R. D. Brock. Austral. Acad. Sci., Canberra, 1968). Ayonoadu & Rees (Genetica 39:75) have reported indications of an altered distribution of chiasmata and have found an increase in the total number of chiasmata, due to B chromosomes in Black Mexican Sweet Corn. The increased recombination in the $\underline{A_2-Bt_1-Pr}$ region of chromosome 5 could thus be the result of a shift in the distribution of crossovers, an increase in the total amount of crossing over, or a combination of both.

Paul Nel

5. Further studies on chromosome elimination induced by supernumerary B chromosomes.

In the 1967 Maize News Letter and in a paper appearing the same year in the Proc. Nat. Acad. Sci., data were presented showing a correlation between the number of B chromosomes and the rate of loss of the $\underline{A_1}$ allele in chromosome 3 at the second division of the microspore. In plants with low numbers of B's there was little or no loss of the \underline{A} marker while in individuals with higher numbers of B's this locus was eliminated in 10% or more of the sperm cells. The earlier data did not provide a good estimate of the dosage effect of B's on loss of the \underline{A} locus. Not all of the classes were represented and the data were fragmentary in some cases. Rather extensive data have since been obtained from a set of closely related plants in which the numbers of B chromosomes ranged from none to eight. The frequencies of F_1 endosperms exhibiting the recessive \underline{a} phenotype in crosses of $\underline{a a} \varphi \times \underline{A A} \sigma$ where the pollen parents differed in numbers of B's are given below:

No. of B's in pollen parent	% kernels with recessive phenotype	Population size
0	0.1	1568
1	0.2	2412
2	0.2	5784
3	4.9	8490
4	11.1	10680
5	12.5	5493
6	13.3	3520
7	11.4	5280
8	13.7	2393

These data suggest that there is no significant increase in loss of the A locus when the number of B's is greater than four. The data further suggest that in this material loss of the A gene frequently takes place in microspores with two or more B's and that it rarely occurs in microspores with one or no B's. If loss of the A locus is limited to spores with two or more B's then the frequency of loss in 3 B plants should be about one-half of that in 4 B plants since in 3 B plants approximately 50% of the spores would have 2 B chromosomes and 50% would possess 1 B. It is assumed that the rate of elimination of A in the 2 B spores from a 3 B plant is the same as that in 2 B spores from a 4 B individual. Disjunction at meiosis in a 3 B plant is not invariably 2 by 1 since trivalents are not always formed and the number of 2 B spores would be somewhat less than that of 1 B microspores. If the loss of A takes place in 2 B microspores and not in 1 B spores, the rate of loss in 3 B plants should be slightly less than one half of that in plants with 4 B's where 2 by 2 disjunction at meiosis is believed to occur regularly. The observed loss rates of 4.9% in 3 B plants and of 11.1% for 4 B plants are in accord with the above assumptions.

Since the A locus is near the distal end of the long arm of chromosome 3, its loss could signify that only the distal portion of the long arm is missing. In order to test for the extent of the deletions, crosses were made in 1966 of gl lg a x G1 Lg A pollen from plants known to give a high frequency of loss for the A locus. We reported (PNAS 57: 1626-1632) that 4.7% of the seedlings from kernels with colored aleurone

were gl lg. The A allele could not be scored in these exceptional F_1 seedlings but it was assumed that most, or all, were hemizygous for the a allele. Since the G1 locus lies in 3L close to the centromere and Lg is between G1 and A, the exceptional gl lg seedlings, which were assumed to be a, came from the loss of most or all of the long arm. Cytological studies of somatic prophase of the exceptional gl lg plants disclosed that 27 individuals had 19 chromosomes of the regular complement plus a short fragment. The fragment chromosomes were apparently telocentric and almost certainly consisted of the short arm of 3, the long arm with the dominant marker genes having been eliminated. However, five exceptional gl lg plants had 19 chromosomes and no fragment, indicating the complete loss of chromosome 3. In short, these limited data indicated that sperm may be deficient for all of chromosome 3, but much more frequently are deficient for only the long arm.

In addition to the gl lg exceptions described above there were five exceptional gl lg plants with 20 chromosomes (no fragment). The suggestion was made that these were compensating types in which chromosome 3 was monosomic and another chromosome of the complement was trisomic. This is almost surely not the case; not only would such an explanation demand a high frequency of newly originating trisomes but they would have to arise in the same spore division where elimination of chromosome 3 occurred. Furthermore, segregation at anaphase must be nonrandom in that the pole disomic for one chromosome is deficient for chromosome 3.

The following hypothesis provides a more reasonable explanation, one that is in accord with all available data. We have demonstrated that the G1 locus lies in the .1-.25 segment of 3L. Breaks in the long arm proximal to G1 result in deficient chromosomes 3 consisting of 3S and portions of 3L of varying lengths. In somatic prophase it would be difficult to distinguish these modified chromosomes 3, which are not telocentric, from the shorter members of the regular complement. Consequently, these gl lg exceptions would be scored as possessing 20 A chromosomes and only examination of the meiotic prophase would disclose their true constitution.

Our published conclusion that breakage of knobbed chromosomes is restricted to the centric region is at variance with the above hypothesis

to account for the gl lg plants with 20 A chromosomes and no recognizable fragment. This conclusion is undoubtedly in error. Although the centric region is a weak spot that is susceptible to rupture by tension, it is now apparent that breaks can occur throughout the length of the long arm of a knobbed chromosome 3.

This past summer information of a genetic nature was obtained about the types and frequencies of chromatin loss induced by supernumerary B chromosomes in chromosome 3. Pollen from plants of a high loss line homozygous for dominant alleles at the D₁ Lg₂ and A₁ loci was applied to silks of d₁ lg₂ a₁ testers. The D locus is in the short arm of 3 and the Lg and A loci are in the long arm. The kernels with colored aleurone and the exceptional kernels with the recessive a phenotype that arise from sperm cells deficient for the A locus were planted in the field and the ensuing plants scored for pollen sterility and the dwarf and liguleless phenotypes. A plant that is d Lg A represents loss of the D allele in 3S, a D lg a plant loss of the Lg and A alleles in 3L, and d lg a individuals arise from loss of D in 3S and of Lg and A in 3L. Data from the cross of d lg a x D Lg A pollen from high loss plants are as follows:

<u>colored kernels</u>				
<u>D Lg N</u>	<u>D Lg 4</u>	<u>D lg 4</u>	<u>D Lg 3N</u>	<u>d lg</u>
899	30	87	9	4

Forty-four of the D lg 4 plants were testcrossed. Forty-three gave only a progeny, indicating loss of both Lg and A. One gave a low A ratio, indicating an internal deficiency in 3L including Lg but not A. Twenty D Lg 4 plants were testcrossed. Twelve gave 1:1 A:a ratios, indicating a normal chromosome 3 from the pollen parent. Six gave only colorless kernels, indicating loss of A but not of Lg. Two gave low A ratios, indicating deficiency not including Lg or A.

<u>colorless kernels</u>		
<u>D Lg N</u>	<u>D Lg 4</u>	<u>D Lg 3N</u>
129	14	1

Six D Lg 4 plants were testcrossed; all gave 1:1 A:a ratios.

Of the 91 lg plants, 87 were D and only 4 were d. These data confirm the conclusion reached in our earlier studies that loss of both arms

occurs much less frequently than does chromatin elimination from only the long arm. The $43 \underline{D} \underline{lg} \underline{a} \uparrow$ plants do not necessarily contain telocentric 3S chromosomes. The ratio of $\underline{D}:\underline{d}$ in testcross populations will provide information on the amount of proximal 3L chromatin in the modified chromosomes deficient for \underline{Lg} and \underline{A} . The percentage of \underline{D} plants in the testcross progenies of individuals with a normal chromosome 3 and a telocentric 3S is a measure of the recombination between \underline{D} and the centromere. Higher percentages of \underline{D} will occur in the progeny of those individuals heterozygous for a modified chromosome 3 possessing a proximal segment of 3L.

The $6 \underline{D} \underline{Lg} \uparrow$ plants deficient for \underline{A} , the $2 \underline{D} \underline{Lg} \uparrow$ individuals giving low \underline{A} ratios in testcrosses, and the $\underline{D} \underline{lg} \uparrow$ plant with the \underline{A} locus present in the deficient chromosome provide unequivocal evidence that breaks in chromosome 3 are not restricted to the centric region although they occur there in a disproportionately high frequency.

A reexamination of the 1966 data lends further support to this conclusion. Among the hypoploid plants of \underline{lg} phenotype were 13 offtype individuals which did not appear to be \underline{gl} but classification was uncertain. These were not included in the data reported in the 1967 paper. It now appears likely that these plants were actually $\underline{Gl} \underline{lg}$ and had lost only the distal portion of 3L.

In 1967 we advanced the hypothesis that knobs may be incompletely replicated at the second microspore division if B chromosomes are present and that anaphase separation is prevented by the conjoined knobs, leading to bridge formation and subsequent rupture at late anaphase. However, our earlier data were interpreted to indicate that bridge breakage was adjacent to the centric region and it was not clear why breakage should be so restricted in dicentric bridges resulting from faulty knob replication while no such restriction occurs in bridges coming from inversion crossing over. This argued against the validity of our hypothesis. We now know, however, that rupture is not restricted to the centric region and the hypothesis becomes more plausible.

All of the data presented above came from the original high loss strain which was relatively homozygous since it had been maintained by inbreeding for several generations before the loss phenomenon was

detected. Although our data clearly show that the knobbed chromosome 3 is subject to elimination at the second microspore division and that no increase in rate of loss of knobbed 3 occurs when more than four B's are present, a number of problems remain to be resolved. One is the effect of the genetic background on rate of loss, a second is whether B chromosomes from other strains are as effective in inducing loss as are the B's found in the original high loss line, and a third is whether the K3L knob in the high loss line differs in its response to B chromosomes from K3 knobs in unrelated strains.

In order to provide at least a partial answer to some of these questions a cross was made using a Black Mexican plant with nine B's onto silks of an individual from the high loss line which had 1 B chromosome and which gave no loss of the A gene in appropriate tests. All of the F₁ plants were heterozygous for the K3 knob contributed by the high loss parent and carried varying numbers of B chromosomes, of which only one at most would be a B from the high loss line. Less than half of the F₁ plants would possess the single B of maternal origin. The number of B's present in the tested F₁ plants varied from none to eight. The A allele was homozygous. The F₁ plants were used as the pollen parent in crosses with a testers and the percentage of A loss determined. The data are as follows:

No. of B's	% <u>A</u> loss	Population
0	0	913
1	0.08	2407
2	0.1	1450
5	1.1	3341
6	2.8	1799
7	3.6	1461
8	2.1	413

If there were no effect of the genetic background contributed by the Black Mexican parent and if all B's were equally potent in inducing loss of knobbed chromosome 3, the percentage of A loss in, for example, the 5 B class should be one-half that found for 5 B plants of the high loss strain. The former are K3 k3 and the latter K3 K3; loss of A in homozygous K3 K3 plants should be twice that in K3 k3. However, only 1.1%

of the kernels from crosses of the Black Mexican-high loss F_1 were colorless while 12.5% of the kernels from 5 B plants of the original high loss line were colorless. Since the K3 knob was identical in both types of crosses, the low rate of A loss of the F_1 plants cannot be attributed to a knob difference in susceptibility to loss. The Black Mexican line may have contributed a set of genetic modifiers for low loss rate that were partially dominant to the genome of the high loss line or else the B chromosomes derived from Black Mexican are not very effective in inducing loss of A chromatin even when the number of B's is as high as seven or eight. No distinction can be made as yet between these two explanations of the low loss rate produced by the F_1 individuals. Any confusion arising from the fact that some of the F_1 plants had a single B from the high loss line will be eliminated this summer when F_1 individuals from the cross of Black Mexican with B's by a no-B plant extracted from the high loss line will be tested.

In the experiments described in the preceding paragraphs, the F_1 plants had 50% of their genes from the high loss line and hence would possess modifiers favoring high rate of loss. A further test of the possible existence of genetic modifiers came from testing a plant with 4 B's which arose from the cross of a B-containing plant of Black Mexican with an F_1 heterozygote of Kys and a Nicaraguan strain with high knob number. The tested plant with 4 B's was heterozygous for K3 knobs and might be expected to exhibit loss of the A marker. However, only one A loss was found in a population of 1883 and self contamination has not been excluded. Here again we cannot distinguish between an inhibiting effect of the genetic background and impotency of the B's from Black Mexican to induce loss of the knobbed chromosome 3. It is also true that the K3 knob from the Nicaraguan strain may differ from the K3 knob of the high loss strain in response to induced loss by B chromosomes.

M. M. Rhoades
Ellen Dempsey

6. Preliminary studies of the effect on crossing over of the gene ameiotic.

Bianchi (MNL 1959) reported an "asynaptic" condition which, when heterozygous, apparently resulted in a slight increase in crossing over

Table 1
Recombination in Chromosome 9 Heterozygotes

Genotype of C Sh Wx c sh wx	Total	C Sh Wx	c sh wx	C sh wx	c Sh Wx	C Sh wx	c sh Wx	C sh Wx	c Sh wx	% Recombination C-Sh Sh-Wx	
Male parent											
Am Am	2755	966	972	69	65	313	346	14	10	5.7	24.8
Am am	3580	1268	1226	102	86	393	471	19	15	6.2	25.1

Table 2
Recombination in Chromosome 2 Heterozygotes

Genotype of Ws Lg Gl ws lg gl	Total	Ws Lg Gl	ws lg gl	Ws lg gl	ws Lg Gl	Ws Lg gl	ws lg Gl	Ws lg Gl	ws Lg gl	% Recombination Ws-Lg Lg-Gl	
Male parent											
Am Am	2914	1118	1064	98	96	240	238	34	26	8.7	18.5
Am am	2596	984	947	100	79	222	220	28	16	8.6	18.7

for the yg - sh region on chromosome 9. It was thought that other meiotic mutants might have an influence on crossing over when heterozygous; therefore the gene ameiotic was tested.

Sibling plants of the constitution Am/Am, C Sh Wx/c sh wx or Am/am, C Sh Wx/c sh wx were used as male parents and crossed to c sh wx testers. Similarly the Ws Lg Gl region of chromosome 2 was studied. The male parents were also self-pollinated and the progeny were scored for the presence or absence of ameiotic plants in order to distinguish Am/Am from Am/am.

Tests of individual backcross progenies for a 1:1 segregation of C:c, Sh:sh, Wx:wx and Ws:ws, Lg:lg, Gl:gl within each of the genotypes Am Am and Am am gave small X^2 values for heterogeneity. Therefore they were considered homogeneous. Tests for heterogeneity within the genotypes Am Am and Am am were also made for the eight classes of chromosome 9 markers and for the chromosome 2 markers. The slight differences that were observed are well within the range expected from sampling as was shown by the relatively small X^2 values for heterogeneity. The pooled results are shown in Tables 1 and 2.

A method outlined by Serra (1) was used to determine the significance of the different crossover values obtained. Crossing over in the marked regions of chromosome 2 and chromosome 9 did not differ statistically in Am/Am vs. Am/am plants. Studies are in progress to test the effect of the ameiotic gene on crossing over on the female side.

1. Serra, J.A. 1965. Modern Genetics. Vol. 1, ch. 17. Academic Press. New York.

Reid G. Palmer

7. Biochemical effects of the gene ameiotic.

The gene ameiotic was first reported by Rhoades (MNL 1956) and is inherited as a single Mendelian recessive, which in the homozygous condition results in plants which are male and female sterile. Sinha (MNL 1959, 1960) attempted to find a biochemical basis for the failure of ameiotic plants to undergo meiosis. Using a modification of the Ogur and Rosen method, Sinha examined perchloric acid extracts of plant tissues for their nucleic acid content and nucleic acid precursors. He found that

the RNA/DNA ratio was higher in ameiotic ears, ovules, anthers and root tips than in normal structures. To investigate this apparent difference between the ameiotic and normal tissues, the RNA was fractionated. He found some differences in the ratio of RNA-1, RNA-2, and RNA-3, to DNA, but the differences between different parts of the same ear were greater than those between the two genotypes. The quantity of DNA was the same in both normal and ameiotic plants.

In normal ears precursors of nucleic acids were found only transiently in the premeiotic stage, whereas in ameiotic ears large quantities of precursors were found at all times except at a very early stage. The accumulation of precursors in ameiotic anthers at the premeiotic stage was also much greater than that in normal anthers. Sinha postulated that the am gene stimulated the production of a ribose containing precursor which was rapidly converted to RNA and that this accounted for the greater accumulation of precursor and RNA in ameiotic plants.

He found biochemical differences in vegetative tissue of normal and ameiotic plants although there are no detectable morphological changes. In view of Sinha's results, it seemed pertinent to further investigate this reported difference in nucleic acid metabolism.

Plants from families known to be segregating for ameiotic were sampled throughout the course of development by taking leaves, root tips (5 mm.), and anthers. In the case of the anthers, every third floret was fixed in 3:1 (ethanol: glacial acetic) and was examined cytologically in order to determine the meiotic stage of the remaining two florets. The fresh tissue was washed, chopped, lyophilized, ground under liquid nitrogen and stored in a vacuum desiccator at -20°C. After anthesis, when the plants could be identified as normal or ameiotic, samples taken from the same genotype on the same day were pooled when necessary to obtain 500 mg. dry weight samples for nucleic acid extraction and analysis.

Two methods of nucleic acid extraction were used to determine which procedure resulted in the greatest quantitative yield of RNA and DNA from the same sample (Table 1). The method of Kirby (Biochem. J. 96, 1965) as modified by Williams was chosen. Not only did this method give higher yields of RNA and DNA from the same sample, but reproducibility

was also better with this modified phenol extraction. Protein contamination was considerably higher in the hot NaCl method than in the phenol extraction. UV absorption was determined on an Hitachi-Perkins-Elmer Model (139) spectrophotometer for hydrolyzed RNA and DNA. Some fractions were assayed for protein by the Lowry determination (Lowry et al. 1951, J.B.C. 193), for RNA by the orcinol determination (Ceriotti 1955, J.B.C. 214) and for DNA by the p-nitrophenylhydrazine determination (Webb & Levy 1955, J.B.C. 213).

Table 1

A Comparison of Two Methods for the Extraction of Nucleic Acids from Maize Leaves and Roots

Age (days)	Guinn* (hot NaCl extraction)				Kirby & Williams (modified phenol extraction)			
	RNA**		DNA**		RNA**		DNA**	
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
62	9.5		1.93		11.3		2.47	
62	10.3		2.17		10.9		2.51	
62	9.7	12.9	1.86	4.75	11.8	13.3	2.43	5.10
62	10.1	12.3	2.25	4.80	11.3	12.9	2.62	4.96
71	11.0	12.0	1.92	4.68	11.7	13.8	2.39	5.01
71	9.2	11.7	2.01	4.78	11.0	13.4	2.44	4.98

*Guinn, G. 1965. Extraction of Nucleic Acids from Lyophilized Plant Material. *Plant Physiology* 41:689-695.

**values expressed as mg/g dry weight.

The results of the nucleic acid determination are shown in Tables 2 and 3. The differences between normal and ameiotic plants in their RNA and DNA contents are quite small. This is true for all developmental stages in the leaves, roots and anthers, with the possible exception of the comparison of anthers at the quartet stage versus those in a "late" stage from ameiotic plants. As is evident from cytological observations at this stage, ameiotic cells are broken down and no cellular organization exists. Such differences could therefore be the result of this degeneration. Both the orcinol and p-nitrophenylhydrazine colorimetric

Table 2

Nucleic acids extracted from maize leaves and roots as indicated by UV absorption at 260 mμ

Age (days)	Phenotype	RNA (mg/g dry weight)		DNA (mg/g dry weight)	
		Leaves	Roots	Leaves	Roots
34	N	10.4	12.8	2.40	5.41
	N	10.5	12.2	2.48	5.15
	am	10.7	11.9	2.30	5.22
	am	10.2	12.8	2.42	5.37
41	N	--	13.0	--	5.25
	N	--	13.3	--	5.11
49	am	10.6	--	2.64	--
	am	11.0	--	2.52	--
50	N	10.8	--	2.50	--
	N	10.6	--	2.60	--
62	N	11.1	13.2	2.42	5.06
	N	11.5	13.7	2.48	4.90
	N	11.3	--	2.50	--
	N	11.6	--	2.60	--
	am	11.2	13.8	2.58	5.05
	am	11.5	13.3	2.48	4.83
71	N	11.3	12.9	2.40	4.70
	N	11.7	13.2	2.34	4.55
	am	11.5	13.2	2.38	4.53
	am	11.6	13.5	2.31	4.77

Table 3
Nucleic Acids Extracted from Maize Anthers

Stage	Phenotype	RNA Extract			DNA Extract		
		Protein (ug/g dry weight)	RNA (mg/g dry weight)		Protein (ug/g dry weight)	DNA (mg/g dry weight)	
			UV absorption (260 mu)	orcinol test		UV absorption (260 mu)	p-nitro- phenylhydrazine test
Very young whole tassel	N	72.6	7.8	8.1	72.9	1.42	1.45
	N	76.4	8.1	8.3	47.5	1.47	1.53
	am	91.0	8.3	8.9	54.6	1.54	1.50
Premeiotic	N	83.1	9.6	9.5	80.3	1.48	1.51
	N	95.7	9.9	10.3	75.7	1.40	1.47
	am	100.2	10.1	10.7	68.9	1.39	1.42
	am	87.3	9.6	9.7	79.2	1.42	1.39
Prophase	N	87.2	9.6	10.8	91.0	1.33	1.27
	N	103.1	10.0	11.1	76.4	1.25	1.33
Early	am	96.3	9.4	9.8	80.5	1.20	1.28
	am	110.1	9.6	10.4	65.4	1.28	1.37
Quartets	N	53.4	9.1	10.0	53.4	0.71	0.65
	N	80.0	9.5	10.4	64.0	0.62	0.69
	N	96.4	10.1	11.1	80.0	0.57	0.60
Late	am	83.4	9.0	9.7	96.4	1.05	0.90
	am	72.1	8.8	9.1	79.3	0.89	0.98

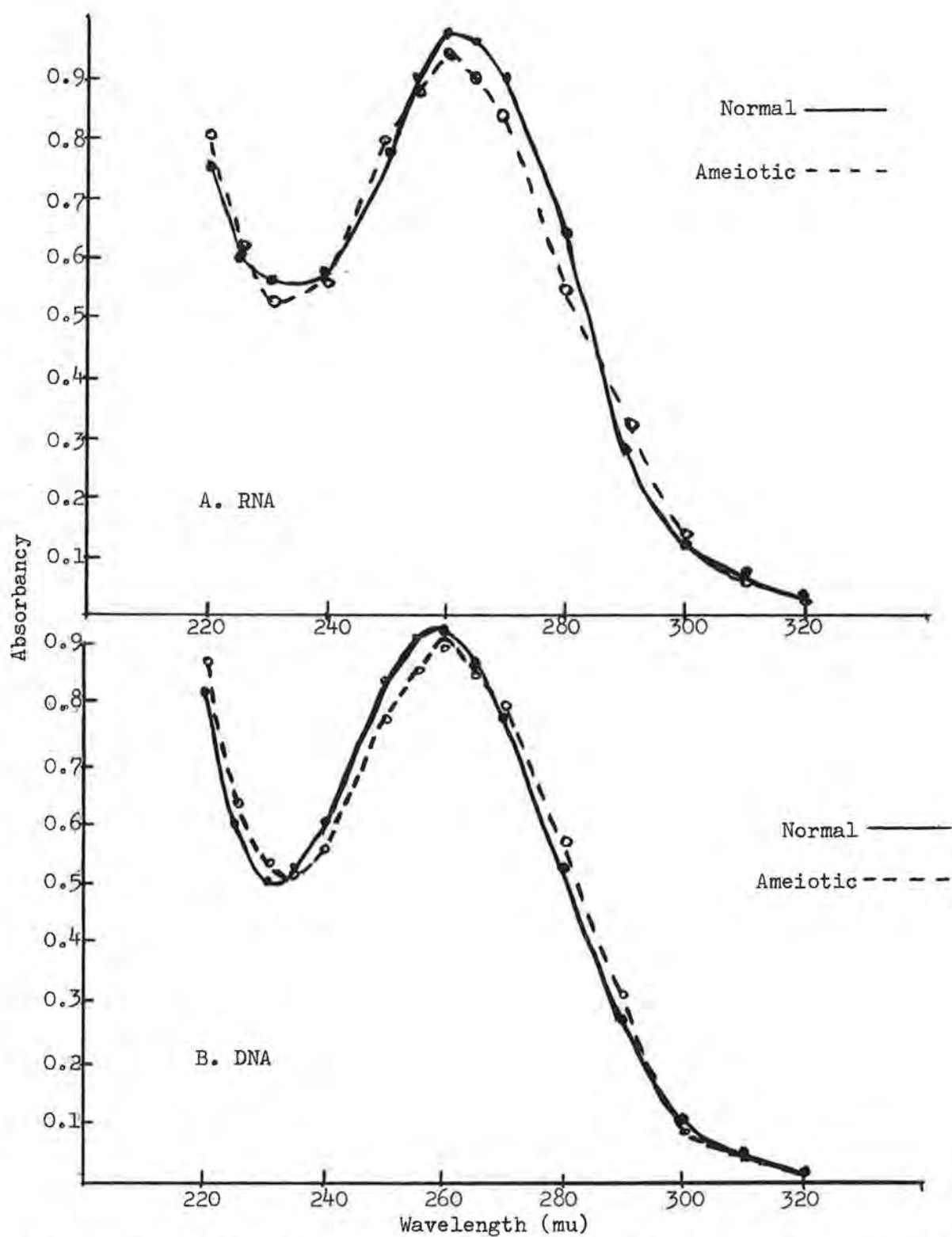


Figure 1. UV absorption spectra of nucleic acids extracted from normal and ameiotic maize anthers in the premeiotic stage. A. RNA. B. DNA.

tests result in slightly higher values for RNA and DNA, respectively. The orcinol test for pentose is a measure only of the sugar which was associated with the purine moieties and therefore non-RNA ribose will also be measured and would contribute to the absorption. Similarly, the p-nitrophenylhydrazine test for pentose is a measure only of the sugar which was associated with the purine moieties and non-DNA deoxyribose would also be measured. Also like the orcinol method for RNA, significant deviations in base composition will affect the accuracy of this method. However, since the differences between UV absorption results and the colorimetric test were small, it was concluded the deviations in base composition were non-existent or small. The RNA samples were tested for contamination by DNA and the DNA samples were tested for contamination by RNA. In both cases contamination was found to be negligible. While there is some variability in the protein content within samples as well as between samples, at present no significance can be attributed to this variability. Since the protein content in the RNA and DNA samples is low, it did not significantly interfere with the UV absorption measurements.

UV absorption curves of RNA and DNA extracted from anthers are shown in Figure 1. The curves are typical of standard nucleic acid samples in that the maximum and minimum optical density values occur at the expected wavelengths.

The results described above do not confirm the work of Sinha but indicate that there is no difference in the RNA or DNA content of normal and ameiotic sibs. In contrast to Sinha's procedure, dry weight samples were used instead of wet weight and a totally different extraction method was employed. It is uncertain at present whether the discrepancy between the results reported here and those of Sinha should be attributed to these factors. Extraction of DNA and RNA from anthers will be carried out by the Ogur-Rosen method in an effort to duplicate the results of Sinha and determine whether or not a difference between normal and ameiotic plants can be detected.

Reid G. Palmer

IOWA STATE UNIVERSITY
Ames, Iowa
Department of Agronomy

1. Spontaneous losses of chromosome 3 markers associated with a translocation.

On kernels expected to be colored and fully round from the cross of $\underline{a_1sh_2}/\underline{a_1sh_2}$ (female) by $\underline{A_1Sh_2}/\underline{A_1Sh_2}$ (male), colorless-shrunken sectors ($\underline{a_1sh_2}$ phenotype) appeared with a high frequency. The male parent originated from a cross of the many-knobbed Maiz Chapolote strain to a knobless flint, and consequently was heterozygous for a number of knobs. The female parent represents a standard tester line. In three successive tests using selected sectorized kernels as male parents on the $\underline{a_1sh_2}$ tester, sectorized kernels were observed among the progeny.

Sector size (i.e. that of the $\underline{a_1sh_2}$ phenotype) was found to be uniform and relatively large, indicative of an early event in endosperm development. (Small sectors, indicating a late event, were found to be non-heritable).

The frequency of kernels showing sectors varied in the different crosses. In $\underline{a_1sh_2}/\underline{a_1sh_2}$ (female) \times $\underline{A_1Sh_2}/\underline{a_1sh_2}$ (male) crosses, where only one-half of the progeny (the $\underline{A_1Sh_2}$ types) could show losses, the frequency of sectorized kernels varied from 0% to $> 25\%$. In the examinations of various tested selections the frequencies were not homogeneous. Frequency peaks were found at 40% and 49%. It appears therefore that the sectoring is heritable.

Cytological observations of PMC's taken from plants used as the male parent show eight typical bivalents and a chain configuration in diakinesis. The latter (chain-of-four) is also observed in Metaphase I and indicates that two chromosomes are involved in a translocation present in heterozygous condition. A very distal breakage point in one of the chromosomes involved would account for the chain-of-four configuration instead of a ring-of-four. After examining sporocytes of a large number of progeny from the colored-round kernels from the cross given above, a correlation was found to exist between the presence of the chain in diakinesis and the appearance of sectorized kernels among the progeny of the same plant. It appears, therefore, that the translocation is related to the sectoring behavior.

From pachytene analysis the translocation was shown to involve chromosomes 1 and 3 with the break in chromosome 1L at approximately .95 and the break in 3L at approximately .35. The detection of a break at .95 is in agreement with the open configuration (chain-of-four) observed in diakinesis. An extremely long chromosome (1^3) results from the translocation described, and a medium-sized knob is present in 1S while a large knob is carried by the segment of chromosome 3L translocated to 1L. The markers, A_1Sh_2 , are located in the knobbed 3L segment. It is suggested that the abnormal length and structure of chromosome 1^3 interfere with its normal replication and are therefore the cause of correlated phenotypic changes (A_1Sh_2 losses) observed in the endosperm.

Peter A. Peterson*
Achille Ghidoni**

*On leave: Department of Microbial Genetics, Karolinska Institutet, Stockholm, Sweden

**On leave from Department of Genetics, University of Milan, Milan, Italy

2. Evidence for instability of a tetrasomic condition.

In last year's Maize News Letter, data were reported on the transmission of the chromosome B^4 when it is present as a single supernumerary chromosome in the plant. This chromosome, which carries the dominant marker Su, was originally extracted from TB-4a and transferred into normal stocks (su₁ testers) where its transmission can be observed by the Su marker.

Plants with two B^4 chromosomes were obtained in the field (summer 1967) and in the greenhouse (spring 1968) after selfing plants with the genotype: $4su$, $4su$, B^4Su . The question then was raised as to whether the transmission of the chromosome B^4 would be affected by the presence of two such chromosomes, since a more regular pairing and disjunction is expected to result when the B^4 has an identical partner as in the genotype: $4su$, $4su$, B^4Su , B^4Su .

Reciprocal crosses as well as selfings of the given genotype were then made to a su₁ tester. The results are reported in Table 1. Nine ears only were obtained in one series of crosses (top row), because most of these short plants (due to the tetrasomic condition of 75% of the short arm of chromosome 4) were destroyed by muskrats during the milk-stage.

Table 1

Genotypes	No. of ears examined	<u>Su</u>	<u>su</u>	Total
(<u>4su</u> , <u>4su</u> , B ⁴ <u>Su</u> , B ⁴ <u>Su</u>) ♀ x <u>su</u> ♂	9	585	224	809
<u>su</u> ♀ x (<u>4su</u> , <u>4su</u> , B ⁴ <u>Su</u> , B ⁴ <u>Su</u>) ♂	25	3,721	1,979	5,700
(<u>4su</u> , <u>4su</u> , B ⁴ <u>Su</u> , B ⁴ <u>Su</u>) selfed	28	1,752	171	1,923

From an examination of the su class, it appears that the chromosome B⁴ is lost during meiosis in a number of cells. This means that the tetrasomic condition is unstable and that the supernumerary chromosome would tend to be eliminated very rapidly from the population if it were not selected for. These data show that there is practically no possibility of fixing this supernumerary chromosome. The following points could be discussed as possible explanations of the data given above:

1. The two B⁴ chromosomes do not always pair as a bivalent. Irregular pairing between B⁴ and chromosome 4 may result in tetravalents, loose pairing and irregular disjunction of the B⁴.

2. The two B⁴ chromosomes do pair with each other most of the time, but these chromosomes are by themselves unstable (as far as co-orientation is concerned) having a sub-telocentric structure derived from the B-chromosome. Their length and structure may be responsible for the absence of regular crossing over.

Obviously, a cytological analysis of PMC's will be of substantial help.

The comparison of the data given above (from plants with two B⁴'s) with those obtained last year (from plants with one B⁴) has led to some observations, emerging from Table 2:

1. The average percent of Su recovered is quite typical of each of the crosses made with one or two B⁴'s present in the plants.

Table 2

Genotypes	% <u>Su</u>	Ratio <u>Su</u> : <u>su</u>	Gamete recovery of B ⁴ (%)	Loss of B ⁴ (%)	Differential loss of B ⁴ (%)
(1967) <u>One B⁴ present:</u>					
(4 <u>su</u> , 4 <u>su</u> , B ⁴ <u>Su</u>) x <u>su</u>	28.7	0.4 : 1	57.4	42.6	25.4
<u>su</u> x (4 <u>su</u> , 4 <u>su</u> , B ⁴ <u>Su</u>)	16.0	0.2 : 1	32.0	68.0	
(4 <u>su</u> , 4 <u>su</u> , B ⁴ <u>Su</u>) selfed	42.1	0.7 : 1			
(1968) <u>Two B⁴'s present:</u>					
(4 <u>su</u> , 4 <u>su</u> , B ⁴ <u>Su</u> , B ⁴ <u>Su</u>) x <u>su</u>	72.0	2.6 : 1	72.0	28.0	7.0
<u>su</u> x (4 <u>su</u> , 4 <u>su</u> , B ⁴ <u>Su</u> , B ⁴ <u>Su</u>)	65.0	1.9 : 1	65.0	35.0	
(4 <u>su</u> , 4 <u>su</u> , B ⁴ <u>Su</u> , B ⁴ <u>Su</u>) selfed	91.0	10.0 : 1			

2. Because of the presence of two B^4 's in the plant, the meiotic loss of this chromosome is cut down from 42.6% to 28.0% through the female. The loss through the pollen is cut down from 68.0% to 35.0%. It therefore appears that the B^4 suffered heavier losses when present in the univalent condition.

3. The difference between the results obtained in reciprocal crosses is less striking when two B^4 's are present in the plant. Gametophyte competition (of normal pollen grains versus B^4 -hyperploid ones), is therefore less important, as a loss factor, when the majority of spores in the pollen pool is carrying one B^4 .

Achille Ghidoni

IOWA STATE UNIVERSITY
Ames, Iowa
Department of Genetics

1. New albino alleles and a mutable allele at the w_3 locus.

Two alleles have been reported for the w_3 locus located in the long arm of chromosome two. The original mutant, w_3 , is a viviparous white-albino mutant (white endosperm-albino seedling). A second allele pas₈₆₈₆ (white endosperm-pale green seedlings) has also been described. Four additional viviparous white-albino alleles have been found, three of these among stocks of albino mutants sent to Iowa State University for testing and one was found in a Tama flint line grown here. The alleles have been given the temporary symbols $w_{\text{Everett}\#1}$, w_{Illinois} , $w_{\text{Chase}\#8}$ and w_{Tama} .

More interesting alleles of w_3 were found in a stock supplied by Dr. J. L. Kermicle. Some years ago he sent me a pale yellow endosperm mutant that produced pale green zebra plants which could be grown to maturity. This mutant was given the symbol $w_{\text{Kermicle}\#3}$ and has been placed on chromosome ten.

One of the pale yellow seeds from the original ear supplied by Dr. Kermicle was planted and the resulting plant selfpollinated. The ear from this plant was homozygous for pale yellow seeds, but no seedling test was made of it. Progeny of this selfed ear was grown and one plant

was used as a male parent on a translocation stock T3-9c and a sibling plant was pollinated by the inbred N25.

Progeny of the cross with T3-9c were grown and self pollinated. Of the eight segregating ears, seven segregated for $\underline{w}_{\text{Kermicle \#3}}$ and one segregated for $\underline{w}_{\text{Kermicle \#3}}$ and a white endosperm-albino seedling mutant. In future generations it was possible to separate the white endosperm-albino seedling from $\underline{w}_{\text{Kermicle \#3}}$ and establish a line that only segregated for the new albino mutant. This mutant was given the symbol

$\underline{w}_{\text{Kermicle \#2}}^{\circ}$

Progeny of the cross between the homozygous $\underline{w}_{\text{Kermicle \#3}}$ and the inbred N25, described above, were grown and self pollinated. Of the nine segregating ears, eight segregated for $\underline{w}_{\text{Kermicle \#3}}$ and one segregated for $\underline{w}_{\text{Kermicle \#3}}$ seedlings, pastel, albescent-like and white seedlings. Progeny from this self were grown. Upon self pollinating the progeny plants, 1/4 of them segregated only for $\underline{w}_{\text{Kermicle \#3}}$, 1/2 segregated for both $\underline{w}_{\text{Kermicle \#3}}$ and the new mutant and 1/4 segregated only for the new mutant, in which various combinations of mutant phenotypes were observed among the seedlings coming from the white seeds. These phenotypes included seedlings that were pastel (pale green), albescent, or white (albino). Some ears segregated for only albino seedlings, others for only pastel, while still others segregated for various combinations of albino, pastel and/or albescent seedlings. This mutant was given the symbol $\underline{w}_{\text{Kermicle \#1}}^{\circ}$

Allele tests of $\underline{w}_{\text{Kermicle \#1}}$ and $\underline{w}_{\text{Kermicle \#2}}$ with \underline{w}_3 established that both of these new mutants were allelic to \underline{w}_3 .

Plants of $\underline{w}_{\text{Kermicle \#1}}$ that segregated only for pastel seedlings were selected for further testing since they resembled the \underline{pas}_{8686} allele of \underline{w}_3 . However, the pastel $\underline{w}_{\text{Kermicle \#1}}$ allele did not prove to be stable like \underline{pas}_{8686} . Self pollination of progeny from homozygous pastel ears, besides producing homozygous pastel ears, would frequently have ears that segregated for pastel and albescent seedlings. Some of these ears also had mutable pastel (pale green with dark green stripes) or mutable albescent seedlings (albescent seedlings with dark green stripes). Most of the progeny of outcrosses of homozygous pastel $\underline{w}_{\text{Kermicle \#1}}$ plants to standard lines upon being self-pollinated would produce ears segregating

for pastel seedlings and albescent seedlings with occasional pastel mutable and albescent mutable seedlings. An occasional plant was found that, besides the pastel and albescent seedlings, segregates for some white seedlings and one plant had a white mutable seedling.

At the same time the selfs described in the above paragraph were made, the selfed plants were crossed as male parents to w_3 plants. These outcrosses to an albino allele resulted in the segregation of the same classes of seedlings described above. However, white and white mutable seedlings were more frequent and they were found in some outcrosses in which the self pollinated male parent did not segregate for white seedlings. In two instances selfed plants which did not segregate for any white seedlings gave outcross plants that were homozygous for white seedlings.

The data suggest that the $w_{\text{Kermicle \#1}}$ allele is a mutable allele of the w_3 locus that can mutate to various levels of expression (e.g. pastel, albescent or albino). The instability of this gene in transmission from one generation to the next and the occurrence of mutable phenotypes support this conclusion. The nature of the mutable system can not be determined from the present data.

The fact that $w_{\text{Kermicle \#1}}$ and $w_{\text{Kermicle \#2}}$ are descended from the same self pollinated ear would certainly suggest that they might have had their origin in the same mutational event.

Donald S. Robertson

2. A pseudoallele test at the y_1 locus.

Since several alleles are known at the y_1 locus, a pseudoallele test was undertaken involving two of them. One of the alleles was the standard y_1 (white-endosperm-green plant) found in genetic stocks. The second allele was w^{mut} , (white mutable). This is a mutable allele of y_1 that originated as a spontaneous mutant in one of Dr. E. G. Anderson's stocks. The original mutant had white endosperm with small areas of yellow tissue and seedlings which when grown at high temperatures were pale green with streaks of green tissue. Stable lines have been derived from this original mutant in which there is no mutability in the endosperm or seedling. These lines have white endosperm and pale green seedlings

(at high temperatures). The stable w^{mut} gene was used in the test described here.

For outside markers in this test, the translocation 6-9e and ms_1 were used. The break point of T6-9e is known to be proximal and very close to the y_1 locus (see MGCNL 41:93-94, 1967). In a two point test, about 3% recombination was measured between y_1 and ms_1 . Although the direction of ms_1 with respect to y_1 is not known it was used as one of the markers, since it is close to the y_1 locus and since w^{mut} and ms_1 had been obtained in coupling as the result of an earlier linkage test.

The F_1 had the genotype $\frac{T y_1 +}{+ w^{mut} ms_1}$ and was homozygous for wx (used as a contamination marker). The F_1 plants and homozygous $y_1 wx$ pollen parents were planted in an isolation plot. All F_1 plants were detasseled. The ears of F_1 and male plants (controls) were harvested and checked for the presence of waxy yellow seeds that would be expected as the result of a crossover within the y_1 locus or back mutation to dominant Y_1 . The ears were shelled and the resulting seeds weighed. A 1000 gram sample of seeds was counted and from this the number of seeds harvested was calculated based on the total weight of seeds obtained. The F_1 yielded 901,494 seeds which represent the number of F_1 gametes tested. The male parents yielded 347,165 seeds, but since each is the result of two y_1 gametes, this represents a total of 694,330 gametes tested from the male plants. No yellow waxy seeds were observed on either the F_1 ears or those of the male parents. Thus no evidence was obtained that y_1 and w^{mut} are pseudoalleles.

There are several possible explanations for these negative results. If the mutation sites for y_1 and w^{mut} were very close together in the y_1 cistron, then crossing over necessary to demonstrate pseudoalleles might be extremely rare. If one or the other or both of the alleles were the result of large deficiencies within the locus, the crossover necessary for the production of a normal allele may be impossible. Another possibility for failure may involve the w^{mut} allele which was derived from a mutable system. There may be some attribute of this mutable system that interferes with crossing over. The use of translocation 6-9e as an outside marker may have reduced the probability of crossing over. Since this translocation is very close to y_1 , there may have been considerable

distortion of pairing in this region resulting in little or no crossing over. Anderson, Kramer and Longley (1955, Genetics 40:531-538) found that heterozygous translocations involving the long arm of chromosome 6 frequently produced a marked suppression of crossing over in the $\underline{Y_1}$ - $\underline{P1}$ region.

The actual reason for the negative results may have been a combination of two or more of the above explanations. However, there is evidence that the second alternative might have been one of the factors involved. In this experiment a total of 2,051,571 $\underline{y_1}$ bearing gametes was tested (694,330 from the male rows, 901,494 male gametes that fertilized the F_1 plants and 455,747 $\underline{y_1}$ female gametes produced by the F_1 plants) and no back mutations to $\underline{Y_1}$ were observed. If the mutation causing $\underline{y_1}$ was due to a simple base substitution back mutations might be expected, yet none was observed in a reasonably large sample. However, no back mutations would be expected if the mutation was the result of a deletion.

Donald S. Robertson

UNIVERSITY OF IOWA
Iowa City, Iowa
Botany Department

1. Unstable derivatives of the B^9 chromosome.

The B^9 chromosome of the translocation, B-9b, can be separated from the reciprocal 9^B chromosome and maintained as a supernumerary in a stock with two chromosomes 9. When crosses of the type $\underline{c} \underline{sh} \underline{wx} \times 9^c \underline{sh} \underline{wx} \ 9^c \underline{sh} \underline{wx} \ B^9C \underline{Sh}$ are made, about 10% of the progeny are phenotypically $\underline{C} \underline{Sh}$ and result from functioning of $9^c \underline{sh} \underline{wx} \ B^9C \underline{Sh}$ pollen. (Cross-over $9^c \underline{Sh} \underline{wx}$ chromosomes also give rise to $\underline{C} \underline{Sh}$ kernels, but can be distinguished in the next generation by testcross data--Robertson, 1967). A relatively large percentage of the $\underline{C} \underline{Sh}$ progeny of $9 \ 9 \ B^9$ plants exhibit $\underline{C-c}$ mosaicism (1-2%) and such kernels have been investigated. One might expect the great majority of mosaics to have arisen from a single break in the B^9 , followed by a breakage-fusion-bridge cycle. However, a number of the mosaic kernels investigated showed a heritable mosaicism, and, in this respect, resembled ring chromosomes. Given below are the results of

test-crosses of 31 plants derived from mosaic kernels.

Segregate solid- colored kernels	Segregate kernels with C-c mosaicism	All kernels colorless
12 ears	7 ears	12 ears

Seven independent cases of heritable mosaicism were found, among 31 individuals tested. One B^9 derivative, discovered before the seven cases above, has been tested for C-c mosaicism and Yg-yg mosaicism and found to exhibit both.

A number of explanations can be given for the unstable B^9 derivatives. They could simply be ring chromosomes. On the other hand, a mutation of the timing mechanism, which normally restricts nondisjunction of the B^9 to the second pollen mitosis, could account for their behavior. The fact that nondisjunction of the B^9 requires the presence of 9^B (Carlson), which is absent in these crosses, makes this idea less likely. A third explanation for the derivative B^9 's is loss of the minute short arm of the chromosomes (Randolph) and formation of an unstable telocentric. A cytological analysis should help decide between the alternatives.

Wayne Carlson

2. Regulation of nondisjunction of the B chromosome.

The B chromosome of maize undergoes nondisjunction at the second pollen mitosis, and rarely at other divisions (Roman, 1947). It has been shown, with TB-4a, that nondisjunction of the B^4 chromosome requires the presence of 4^B , even though the 4^B chromosome itself divides properly at the second pollen mitosis (Roman, 1949). The analogous situation was found with TB-10a (Longley, 1956) and TB-9b (Carlson, 1968). One can infer, therefore, that a gene(s) in the distal heterochromatin of the intact B (present on 4^B , 10^B , and 9^B) causes a "stickiness" or incomplete replication in the proximal part of the B, resulting in nondisjunction. The restriction of nondisjunction to the second pollen mitosis might result from repression of this gene(s) during all divisions except the second pollen mitosis. However, Battaglia proposed (1964) a different explanation for the localization of B chromosome nondisjunction to the second pollen mitosis. According to Battaglia, the B chromosomes of

maize and of other species may undergo nondisjunction only during especially rapid divisions. The rapidity of the second pollen mitosis would account for nondisjunction, rather than differential activity of a gene(s) in the distal heterochromatin.

The findings of Bianchi et al. (1961), together with the evidence presented here, indicate that a differential activity of the gene(s) controlling nondisjunction does occur. Bianchi showed, with A-B translocations, that losses of BA chromosomes occur at a low but significant rate during early development of the endosperm and embryo. This was reflected in endosperms and embryos with tissue sectors lacking dominant genes on the B^A . The sectors very frequently covered one-half of the endosperm or embryo. Small sectors were found much less frequently than would be expected if the B^A was equally susceptible to loss at all somatic divisions. Bianchi considered the losses he observed cases of somatic nondisjunction, although other phenomena could also explain the sectors. If somatic nondisjunction did occur, Bianchi's data suggest that the gene(s) regulating nondisjunction is differentially active in early divisions of the endosperm and embryo and relatively inactive at later divisions. The activity during early development can be explained if the gene(s) regulating nondisjunction is active primarily during the second pollen mitosis and inactive at all other divisions. A carry-over of residual gene activity or gene product to the egg and polar nuclei may occur, via the sperm, and account for nondisjunction in early somatic divisions.

Several tests have been made to determine whether early somatic sectoring is controlled by the distal region of the B, and whether a carry-over of gene activity from the second pollen mitosis does occur. Sectoring losses at early divisions of the endosperm and embryo were studied with TB-9b, using the markers C and Yg. The influence of the 9^B chromosome on somatic loss of the B^9 was studied in the endosperm. Since the 9^B chromosome carries a gene(s) that is required for nondisjunction, absence of the chromosome should prevent the appearance of large colorless sectors in the endosperm. Crosses of the following type were made:

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

The major products of meiosis in the male parent are spores with 9^C sh wx B^{9C} Sh or $9^{B^{9C}}$ B^{9C} Sh. Both spore types function, although the unbalanced $9 B^9$ type does so at a reduced rate. Among the progeny, C Sh kernels were selected, divided into wx and Wx classes, and examined for colorless (c) sectors. Selection of wx progeny allows study of the $9 B^9$ spore type, and selection of Wx progeny insures that the $9^B B^9$ spore type is being examined. (Crossing over between Wx and the translocation breakpoint occurs less than 0.5% of the time--Robertson). Since all the kernels examined were C Sh in phenotype, they each contained at least one B^{9C} Sh in the endosperm. Nondisjunction does not occur in $9 B^9$ pollen, and all C Sh wx kernels carried one B^9 in the endosperm. However, nondisjunction occurs at a high rate in $9^B B^9$ pollen, and the endosperms of many C Sh Wx kernels contained two B^9 's. Consequently, somatic nondisjunction of the B^9 in C Sh Wx kernels can be hidden by the presence of a second B^9 . For this reason, a comparison of the absolute rates of sectoring between C Sh wx and C Sh Wx kernels cannot be accurately made. One can, however, compare sector sizes for the wx and Wx classes and determine whether early somatic loss is characteristic of the Wx kernels. Classification of progeny from three male parents was made. Colorless sectors which occupied 1/16 or more of the endosperm were designated "large" and lesser sectors "small". The results were as follows:

Male parent	Kernel type examined	Solid colored	Large colorless sector	Small colorless sector
748-2	C Sh Wx	451	6	1
	C Sh wx	188	1	3
748-10	C Sh Wx	530	7	3
	C Sh wx	100	0	4
713-3	C Sh Wx	968	9	11
	C Sh wx	<u>114</u>	<u>1</u>	<u>3</u>
TOTALS				
	C Sh Wx	1949	22	15
	C Sh wx	402	2	10

The distribution of sectors as to large vs. small is biased toward large sectors in the Wx class and toward small sectors in the wx class. The experiment was repeated in a different genetic background. Since more

data were available in the second experiment, the classification of large vs. small sectors was changed. Losses smaller than 1/16 were not recorded. Sectors covering 1/4 to 1/2 of the endosperm were designated large, and those 1/16 to 1/4 in size small. Only one male parent was used in this cross. The data are given below:

Male parent	Kernel type examined	Solid colored	Large colorless sector	Small colorless sector
1295-34	C Sh Wx	3100	22	3
	C Sh wx	873	2	12

Again, large sectors predominate among the C Sh Wx kernels, but not in the C Sh wx group. Kernels with sectors covering more than one-half of the endosperm were also found in the above experiments. These were not included in the tables. Such kernels probably result from nondisjunction of the B^9 at the first division, followed by irregular development of the endosperm (Stadler). As expected, sectors larger than 1/2 were found exclusively among Wx kernels. The data indicate that the $9^{B^{Wx}}$ chromosome is responsible for early somatic loss of the B^9 .

A second test was made comparing losses of the B^9 chromosome in the absence of 9^B with losses of the regular chromosome 9. Crosses of the following types were made:

1. c sh wx X $9^c sh wx$ $9^c sh wx$ $B^{9^C Sh}$
2. c sh wx X $9^c sh wx$ $9^C Sh wx$

The male parents were sibs, and the $9^C Sh wx$ chromosome was derived from $9^c sh wx$ and $B^{9^C Sh}$ by crossing over. The female parent was an inbred line. C Sh wx kernels were selected from each cross and examined for large vs. small sectors. (Large sectors were 1/16-1/2 in size, while small sectors were less than 1/16). The pooled data from several crosses of each type are given:

Male parent	Solid colored	Large colorless sector	Small colorless sector
9 ^c sh wx 9 ^c sh wx B9 ^C Sh	2060	15	88
9 ^C Sh wx 9 ^c sh wx	1626	2	13

While loss of the B⁹ chromosome seems to occur at a higher rate than loss of the normal 9, the distribution of losses, as to large or small sector sizes, is similar. Therefore, the prevalence of early losses of the B⁹ chromosome in the presence of 9^B may be considered unusual, with regard to the normal pattern of chromosome loss.

A further test was made to determine whether somatic nondisjunction results from the carry-over of gene product (or gene activity) from the second pollen mitosis. Since B^A's undergo nondisjunction in the pollen but not in the female inflorescences, somatic nondisjunction should only occur when the B^A is introduced through the male parent. Reciprocal crosses were, therefore, made between a chromosome 9 tester stock and plants containing TB-9b. To avoid differences in chromosome constitution of the endosperm in these crosses, a seedling trait, Yg, was used to identify B⁹ losses. The crosses are given below:

1. yg bz wx X 9^{Yg} Bz wx 9^{B^{Wx}} B9^{Yg} Bz B9^{Yg} Bz
2. 9^{Yg} Bz wx 9^{B^{Wx}} B9^{Yg} Bz B9^{Yg} Bz X yg bz wx

The yg bz wx stock is an inbred line and the TB-9b line was backcrossed for three generations to an inbred line. Crosses (1) and (2) were not precisely reciprocal, but they were made at the same time and between the same families. The TB-9b parents were all from the same family and appeared uniform. The 9^{B^{Wx}} B⁹ spore type was selected in the progeny of both crosses, using the Wx marker. In the first cross, bz Wx as well as Bz Wx kernels were found, as a result of nondisjunction. Only the Bz Wx kernels were selected for germination, and the seedlings were expected to be either yg (0 B⁹) or Yg (1 B⁹). The Yg plants that germinated were examined for yg sectors. In the second cross only Bz Wx kernels were

found, since nondisjunction of the B^9 did not occur, and all the seedlings were Yg (1 B^9). The chromosomal constitution of seedlings examined from both crosses was the same: $9^{Yg} bz wx 9^{B^{Wx}} B^9 Yg Bz$. Only the source of the 9^B and B^9 chromosomes differed as to the male or female parent. Sectors for yg were classified as large if they occupied 1/4 or more of the first two leaves of the seedling. Sectors smaller than this were also classified, regardless of size, if they extended the length of one leaf. The latter were designated small sectors. Results are given below:

Cross	Totally Yg	Large yg sector	Small yg sector
$yg bz wx$ X TB-9b	738	10 (1.3%)	8 (1.1%)
TB-9b X $yg bz wx$	1152	0 (0.0%)	12 (1.0%)

Since the chromosome constitutions of the individuals classified in both crosses were identical, the absolute rates of sector formation can be compared. As expected, large sectors appeared only when the 9^B was transmitted through the male parent, while small sectors formed at equal rates in the two crosses.

Another experiment suggests that the distal heterochromatin of the B may be relatively inactive at the first division of meiosis. It was shown (Carlson, thesis) that nondisjunction of the B^9 chromosome occurs at a high rate in pollen carrying 9^B and B^9 , but probably never in $9 B^9$ pollen. This agrees with the results of Roman on TB-4a and Longley on TB-10a. In addition, the presence of the 9^B chromosome in the meiotic cells that give rise to $9 B^9$ pollen does not induce nondisjunction of the B^9 . There is no carry-over of activity of the 9^B from meiosis to the second pollen mitosis. The results are, therefore, consistent with a genetic inactivity of the distal heterochromatin of the B during the first meiotic division. (Alternative explanations are, of course, quite possible, and the sensitivity of the experiment to low levels of nondisjunction is questionable).

In conclusion, the data support the idea that a gene(s) controlling nondisjunction is differentially active in the second pollen mitosis, and relatively inactive at other divisions. The restricted activity of the gene(s) acts as a timing device for nondisjunction. A similar method for regulating nondisjunction is probably also present in the B's of rye

and Festuca, since genes required for nondisjunction have been found in these plants. However, it remains to be determined whether somatic losses observed in the present experiments resulted from nondisjunction. This requires cytological examination of fractional seedlings, which is currently underway. For preliminary results, see the following article.

Wayne Carlson

3. Isochromosome formation and the process of nondisjunction.

In the preceding article, sectorial loss of the B^9 chromosome in endosperm and sporophyte tissue is reported and attributed to somatic nondisjunction. Cytological examination of root tips of the fractional seedlings has been made for three plants. Surprisingly, the losses of the B^9 in these plants may be attributed to isochromosome formation, rather than nondisjunction. In one plant, two root tips gave chromosome counts of 20 and 21. The root tip with 21 chromosomes contained no telocentrics (as seen in metaphase) but did contain one chromosome with a large heterochromatic knob at each end (observable in prophase). In addition, two smaller knobs were seen halfway between the center of the chromosome and the distal knobs. The normal B^9 chromosome carries a large terminal knob ($K9^L$) and a second smaller knob (derived from the heterochromatic region of the B) in a median position. The extra chromosome found, therefore, was probably a B^9 isochromosome. In a second plant, only one root tip has been examined, but again metaphase and prophase cells indicate the presence of a B^9 isochromosome. A third plant was examined which showed multiple sectoring for loss of Yg, rather than single sector formation. Of four root tips that were examined, two contained 21 chromosomes, with one telocentric. One B^9 was apparently present in these roots. The other two root tips carried 22 chromosomes, only one of which was telocentric. Prophase cells showed one chromosome with the isochromosome knob pattern and another with one-half this pattern. One B^9 and one isochromosome B^9 were likely present. Somatic nondisjunction of the B^9 , followed by isochromosome formation could account for the findings in this plant.

In the previous article, evidence suggested that somatic sectoring is controlled by the same gene(s) that induces nondisjunction at the

second pollen mitosis. The finding of isochromosomes in the fractional seedlings suggests that isochromosome formation may be a normal step in nondisjunction. The B chromosome may carry a gene(s) responsible for splitting the centromere of the B isochromosome following the second pollen mitosis. This gene(s) may be primarily active during or immediately after the second pollen mitosis and relatively inactive at other cell divisions. In this respect it would follow the activity cycle of the gene(s) responsible for initiating nondisjunction (see previous article). Evidence from maize and other organisms allows the following explanation for nondisjunction of the maize B chromosome:

1. A gene(s) located distally in the B chromosome becomes active during the second pollen mitosis and induces a "stickiness" in the heterochromatic knob adjacent to the B centromere.
2. The inability of the chromosome to disjoin results in mis-division of the centromere and isochromosome formation. (Whether this step is essential to nondisjunction or simply a by-product of nondisjunction is not known).
3. The chromosome migrates to one pole.
4. During anaphase or later another gene(s) on the B chromosome becomes active and causes a splitting of the centromere of the isochromosome.
5. The heterochromatic knob adjacent to the B centromere also splits following nondisjunction. This step may or may not be controlled by genes on the B chromosome.

The sequence given above accounts for isochromosome formation. Residual activity of the gene(s) that induces heterochromatin stickiness may cause formation of isochromosomes in early somatic divisions of the embryo (see previous article). Activity of the gene(s) controlling centromere splitting may often be inadequate in somatic divisions to complete nondisjunction. The hypothesis also accounts for results of Rhoades, Dempsey, and Ghidoni (1967). They reported an abortive type of nondisjunction of A chromosomes that is induced by large numbers of B's. Rhoades et al. found that chromosomes carrying heterochromatic knobs are subject to loss of the knobbed arm at the second pollen mitosis. The best explanation of the data was that the knobs on the A chromosomes become sticky at the second pollen mitosis and this prevents disjunction. After anaphase bridge formation, one of the centromeres separates from its knobbed chromatid arm, and a telocentric and acentric are formed.

The acentric is lost. The findings are unusual in that (1) the primary site of nondisjunction is the heterochromatic knob and not the centromere in the A chromosomes, and (2) the anaphase bridge does not break in a conventional manner but splits adjacent to one of the two centromeres. The results can be explained, however, if the gene(s) that induces nondisjunction is specific for heterochromatin (the knob adjacent to the B centromere) and if another gene(s) produces an enzyme that attacks centromeres (splitting the B isochromosome; producing an A telocentric). The proposal is also consistent with the findings of Crouse (1960) on X chromosome nondisjunction in Sciara. She used translocations to demonstrate that a heterochromatic region close to the centromere is responsible for nondisjunction.

Finally, it has often been suggested that the telocentric nature of the B chromosome is a contributing factor in nondisjunction. Yet the B chromosome of rye, which undergoes nondisjunction in a manner similar to the B of maize, is a metacentric. In rye, two "sticky" regions appear on either side of the B centromere and prevent disjunction. The sticky regions are induced by a separate gene(s) on the B chromosome. In maize, the induction of a sticky region in one arm of a metacentric results in an unusual type of disjunction (Rhoades, Dempsey, and Ghidoni 1967). The possibility that nondisjunction might occur in maize chromosomes with heterochromatic knobs in both arms has not been tested. However, if the isochromosome B⁹'s are recovered in the greenhouse crop, they can be tested for nondisjunction. These chromosomes should have sticky regions in both arms. Nondisjunction by these chromosomes would indicate that the telocentric nature of the B is not essential for its nondisjunction.

Wayne Carlson

Characters	$\bar{y}_{ij} / \bar{y}_{Ij}$			$\bar{y}_{Ij} / \bar{y}_{Ij}$			Significance of differences	
	Plants	Mean	Standard error	Plants	Mean	Standard error	"t" values	Probability level
Plant height (cm)	82	110.73	1.83	26	105.92	3.40	1.27	0.3-0.2
Ear height (cm)	82	52.70	1.19	26	53.50	1.97	0.36	0.8-0.7
Stem diameter (mm)	82	13.78	0.18	26	13.00	0.41	1.97	0.1-0.05
Internode number	82	11.12	0.11	26	10.92	0.18	2.72	0.01-0.001
Leaf length (cm)	82	64.40	0.79	26	61.15	1.90	1.84	0.1-0.05
Leaf width (mm)	82	77.89	0.85	26	73.53	2.10	2.27	0.05-0.02
Pollen shedding time*	82	13.17	0.37	26	15.46	0.75	2.93	0.01-0.001
Silking time*	82	17.41	0.38	26	20.34	0.80	3.60	> 0.001
Shedding - silking period (days)	82	4.24	0.18	26	4.88	0.28	1.74	0.1-0.05
Ear length (mm)	84	109.13	2.79	24	103.75	5.36	0.90	0.4-0.3
Middle ear diameter (mm)	84	38.44	0.31	24	36.12	0.78	3.25	0.01-0.001
Row number	84	15.58	0.18	24	15.16	0.42	1.03	0.4-0.3
Kernels per row	84	26.38	0.76	24	24.12	1.30	1.42	0.2-0.1
Weight of 100 kernels (g)	84	18.17	0.28	24	17.25	0.66	1.45	0.2-0.1
Kernels per ear	84	411.35	13.25	24	372.00	26.37	1.38	0.2-0.1
Total kernels weight (g)	84	75.04	2.85	24	64.64	5.18	1.72	0.1-0.05

*Days from August 1st.

ISTITUTO DI GENETICA VEGETALE
Università Cattolica Del S. Cuore
Piacenza, Italy

1. Effect on quantitative characters of a permanent heterozygosis system.

Several quantitative plant and ear traits have been studied in the offspring of one selfed F_2 ear derived from crossing two lines, possessing respectively the well known y factor and an ij type semilethal mutant. The recombination frequency between these factors, both located on chromosome 6, is very low (near 0.02); moreover, the y ij/y ij recombinants, when found, behave as lethals.

The aim of this work was to determine whether the double heterozygote (Y ij/y Ij) has some morpho-physiological advantage compared with the other viable genotypes involving the same alleles. Through self-pollination, the genotype of the tested plants (with the exception of two Y Ij/Y ij and one y Ij/y ij) proved to be Y ij/y Ij or y Ij/y Ij; the comparison, then, has been possible between these two classes only. The data obtained are reported in the table.

The lack of information about the behaviour of the other viable genotypes prevents a decision as to whether the higher performance of the double heterozygote depends on a negative action of the y factor, when homozygous, or on a heterotic effect due to the factors under study or to some others closely linked with them. In any case, the y and ij factors seem to constitute a permanent heterozygosis system, less drastic than previously studied systems based on balanced lethal factors, but possibly more efficient, at least in some environmental conditions, in improving the fitness of a population.

C. Lorenzoni
M. Pozzi

ISTITUTO SPERIMENTALE PER L'ORTICOLTURA
Sezione di Ascoli Piceno, Italy

1. Further data on the Ga factor of chromosome 9.

In 1968 further data have been obtained in order to locate Ga₈ on the linkage map and to test its rate of transmission. Plants of Ga wx/

ga Wx constitution pollinated by Ga Ga individuals produced, out of 176 plants, 21 recombinants. This corresponds to 11.93% crossing over, a value unaffected by the possible competition between ga and Ga pollen since the heterozygous parent was used as the female. Since this value is not lower than that obtained on the hypothesis of non functioning of ga making use of self-pollinated progenies (as reported in a previous MNL), it should follow that ga pollen is practically incapable of functioning at least in the presence of Ga. This actually appears to be the case. Among the progeny of plants of Ga wx/ ga Wx and Ga Wx/ ga wx constitutions appear individuals segregating about 25% wx kernels. If ga pollen functioned to some degree, some of these individuals should be Wx wx ga ga; actually, out of 46 tests, not a single case of such a genotype has been detected. All the ears showing 25% wx kernels turned out to be Wx wx Ga Ga. Apparently either ga does not function or the ga ga combination is lethal.

The very poor functioning of ga pollen is also indicated by the results of pollinating Ga Ga plants with tassels of Ga Wx/ ga wx and Ga wx/ ga Wx individuals. The latter combination is especially convincing; if a value of 12% crossing over is considered, the comparison is as follows:

No. of ears	Non crossover	Crossover
Actual	3	108
Expected	97.7	13.3

Obviously it should be concluded that the functioning of ga pollen is negligible (from these data ga fertilizes 264 times less than Ga:

$$\frac{97.7}{13.3} \times \frac{108}{3}.$$

These results indicate that in calculating linkage relationships between Ga_g and chromosome 9 markers it is practically correct to assume that ga pollen is unable to perform fertilization in the presence of the Ga grain.

A. Bianchi
M. R. Parlavecchio

2. Concomitant occurrence of different controlling elements.

Further testing of the unstable factor reported in previous News Letters (1967, pages 100-101; 1968, page 91) has led to the following conclusions:

(a) The pale bronze unstable factor, controlled by an Activator factor in chromosome 9, is confirmed not allelic to any of the color factors-- \underline{a}_1 , \underline{a}_2 , \underline{c}_1 , \underline{c}_2 , and \underline{r} . Confirmed is also the linkage with markers of chromosome 1. F_2 data from four plants having the unstable factor in repulsion phase with \underline{f}_1 or \underline{br}_1 are as follows:

	X Y	X y	x Y	x y
\underline{f}_1	721	366	320	10
\underline{br}_1	882	360	159	16

They give $16.9 \pm 1.7\%$ and $31.6 \pm 1.6\%$ crossing over between the factor and the markers \underline{f}_1 and \underline{br}_1 , respectively. The indication that the factor is \underline{bz}_2 has been definitely proven by testcrosses with the Neuffer \underline{bz}_2 stocks.

(b) Crossing of our material with the stocks provided by Dr. Neuffer revealed that actually we are dealing with a situation similar or identical with that described by this author.

(c) The Spm test has been repeated and, on the basis of the \underline{wx}^{m-8} and \underline{a}_1^{m-1} response, we have to assume that an Spm element is present.

(d) Since the material, in which we now recognize the presence of both an Ac element and an Spm element, had been under control for several years without manifesting signs of possessing controlling elements, there is an indication that the breakage-fusion-bridge cycle which occurred in the short arm of chromosome 9 as a consequence of radiation treatment (Bianchi and Tomassini, 1965, Mutation Research 2:352-365) gave rise to a controlling element located on the same chromosome as well as to another one possibly located elsewhere.

A. Bianchi
F. Salamini
F. Restaino

UNIVERSITY OF MASSACHUSETTS
Waltham, Massachusetts

and

HARVARD UNIVERSITY
Cambridge, Massachusetts

1. Further studies on the inheritance of cob-rachis diameter.

The initial experiment conducted with cobs alone, as reported in last year's MNL, was repeated in part in 1968 to include tassel measurements together with those of the cob. This has led to the characterization of the two recessive genes involved in the thick cob of Iowa 5125 in terms of tassel morphology. One of the recessives results in high condensation in the tassel branches followed by reduced branching while the other recessive produces profuse tassel branching. An interaction between these two genes in the double recessive condition produces the thick cob and normal tassel of 5125.

Another genetic system produces thick cob in the northern flint derived races. This involves a third recessive gene which operates independently of the high "condensation--ramosa" system. It is suspected that the derived southern dents, like the Corn Belt dents, have acquired a combination of these two systems which would require the homozygous recessive condition at the three major loci concerned.

W. C. Galinat

2. Cytogenetic correspondence of corn chromosome 9 and its *Tripsacum* homeolog.

The assemblage of loci on a *Tripsacum* chromosome (no. 7 or 8, see item 8) is closely similar to that on corn chromosome 9. Of the 8 loci tested with markers yg_2 , c_1 , sh_1 , bz_1 , $wx:gl_{15}$, bk_2 , bm_4 , all are common to the two homeologs. Many other loci are probably also common between these chromosomes because the *Tripsacum* chromosome may substitute for its homeolog in the corn genome. Pachytene analyses of addition disomics ($2n = 20 + 2$) have shown that this *Tripsacum* chromosome has a total length of 34.0 microns with an arm ratio of 4.0:1.0. Its long arm is terminated by a large knob. Both in its length and arm ratio, this chromosome differs from chromosome 9 of corn which is about 43.0 microns long and has an arm ratio of 1.8:1.0.

Corn-Tripsacum crossovers have been obtained with some regularity (ca 1%) involving yg₂ on the short arm and bk₂ and bm₄ on the long arm of corn 9. Recombinants for the five other loci in the interstitial region of corn 9 have not been observed so far.

Because of preferential pairing at pachytene of the concerned corn and Tripsacum homologs observed in the addition disomics, it is not known yet whether the gene sequence or their relative distances are the same in the two chromosomes. The differences in their lengths and arm ratios could mean differences in both. Heterozygous substitution stocks (2n = 20) carrying all 8 dominants are under study and may provide this information.

W. C. Galinat
B. G. S. Rao

3. "Overdominance" between corn and Tripsacum alleles at the Bz locus.

When the dominant Bz gene borne on Tripsacum chromosome 7 or 8 (see item 8) is combined as a heterozygous substitution with its recessive allele bz on corn chromosome 9, the aleurone coloration is a deeper purple than when the Tripsacum chromosome is present as a homozygous substitution. The genotypes and phenotypes of kernels (aleurones) borne on a self-pollinated ear heterozygous for this corn-Tripsacum substitution are as follows:-

<u>Genotype*</u>	<u>Phenotype Color</u>	<u>Ratio</u>
<u>Bz^T/Bz^T/Bz^T</u>	pale purple	1
<u>Bz^T/Bz^T/bz</u> } <u>bz/bz/Bz^T</u> }	dark purple	2
<u>bz/bz/bz</u>	bronze	1

*Both the corn and Tripsacum chromosomes were dominant C. The presence of the Tripsacum allele is shown as Bz^T.

If this were a single allelic interaction, it would be overdominance. An alternate explanation could be the presence of a recessive modifier on the Tripsacum chromosome.

W. C. Galinat
B. G. S. Rao

4. Gene exchanges between corn and *Tripsacum*.

Genetic crossing over is observed at regular frequencies between certain common loci shared by the homeologous chromosomes of corn and *Tripsacum*. Both crossover products between the lg_1 and gl_2 loci on the short arm of corn chromosome 2 and the long arm of a *Tripsacum* chromosome have been obtained. All possible crossover classes have been recorded for the known common loci v_5 , ra_1 , gl_1 , and ij on chromosome 7 of corn and 4 of *Tripsacum*. Exchanges between corn chromosome 9 and *Tripsacum* 7 or 8 are confined to yg_2 at the distal end of the short arm and bk_2 , bm_4 on the long arm (of corn 9). The frequency of these observed gene exchanges is in the order of 1%. It has not been possible yet to use the rate of crossing over to ascertain the order of the different genes on the *Tripsacum* chromosomes.

Cytological studies have shown that the concerned chromosomes of corn and *Tripsacum* are different in their lengths and arm ratios. In the addition disomics, they show preferential pairing at pachytene to their respective homologs and because of the apparent lack of meiotic pairing between the corn and *Tripsacum* chromosomes, the regions involved in the exchanges could not be determined in the materials examined so far. The morphological differences in the pachytene chromosomes of corn and their *Tripsacum* homeologs, as well as the probable premeiotic exchanges involving regions of different lengths, indicate differences in the arrangement of the common loci on the chromosomes of the two genera.

W. C. Galinat

B. G. S. Rao

5. A possible elimination of corn chromosome 4 in the hybrid origin of *Tripsacum*.

Genetic comparisons of six linked loci on corn chromosome 4 to the genome of *Tripsacum* do not reveal a corresponding assemblage of these loci on any single *Tripsacum* chromosome. The position of the Su_1 locus in both corn and *Tripsacum* is close to the centromere but the similarities seem to stop there. The Su_1 chromosome of *Tripsacum* does not include La to which Su is linked by 11 crossover units on the short arm of corn chromosome 4 nor does it include 4 other loci tested on the long arm. A

different Tripsacum chromosome bears Gl₃ but like the Su-marked Tripsacum chromosome, it does not include La nor three other loci (Bm₃, Ra₃, J₂) on the long arm of corn chromosome 4. Possibly these loci are distributed among the different chromosomes in the Tripsacum genome.

In connection with our hypothesis that Tripsacum is an ancient amphidiploid of wild corn and Manisuris with genomes of 9 pairs derived from each parent, we suggested that corn chromosome 8 could be the one that is eliminated in the genome of Tripsacum. This was based on the apparent deficiency in known functional loci on corn chromosome 8. The lack of a Tripsacum linkage group corresponding to that of corn chromosome 4 is in contrast to observations with loci on corn chromosomes 7 and 9 and the loci on the short arm of corn 2. This suggests that the "lost" chromosome for Tripsacum is more likely to be corn chromosome 4 rather than chromosome 8.

Further studies on the identity of chromosomes showing haploid pairing in maize (Chaganti, 1965) might be revealing.

W. C. Galinat
B. G. S. Rao

6. Numerical and structural variations of the Tripsacum homeolog for corn chromosome 9 in different derivatives.

Among the progenies being grown to study the linkage groups of dominants contributed by the Tripsacum homeolog for corn chromosome 9, individuals are encountered with variable chromosome number and structure of the Tripsacum chromosome. All of these were derived from the descendants of one addition monosomic plant ($2n = 20 + 1$). After isolation they were either selfed or backcrossed to the recessive corn parent. The observed meiotic behavior in the different families is briefly reported here.

1. Numerical Variation:

(a) Addition monosomics ($2n = 20 + 1$):

The Tripsacum chromosome can always be recognized in pachytenes by the presence of a large terminal knob on one of its arms. Usually it does not pair with any of the corn chromosomes and remains a univalent. All such univalents show inside pairing (nonhomologous pairing) to variable extents. The centromere and its other morphological features are

not distinct. A low percentage of PMC's from the same source showed two or three of these chromosomes within a nucleus; these, however, remained univalents without any synaptic affinities. The reason for the apparent loss of pairing amongst them is not known. Such exceptional PMC's are possibly derived from irregular segregation of the division products of the extra chromosome in the mitoses immediately preceding the differentiation of PMC's. Except in such cases, 10 bivalents and a univalent are observed at Diakinesis and Metaphase I. Occasionally $9_{II} + 1_{III}$ are seen. The univalents at anaphase I show precocious divisions and one or both the chromatids move to one of the poles.

(b) Addition disomics ($2n = 20 + 2$):

The extra pair shows complete and normal pairing at pachytene. Its morphology is described in item 2. In certain exceptional PMC's heteromorphic bivalents involving chromosomes of unequal lengths are seen (see item 7). Associations other than 11_{II} or $10_{II} + 2_I$ have not been observed in the later stages. Orientation of the extra bivalent at metaphase I is irregular. Numerical nondisjunction or delayed disjunction, probably related to disturbed centric activity of the extra bivalent is frequent at anaphase I.

(c) Aneuploids with $20 + 3$ and $20 + 4$ chromosomes are also recorded. Obviously these are the products of a sporadic fertilization of gametes with more than the expected $10 + 1$ chromosomes. The more frequent chromosome associations at Diakinesis are: $10_{II} + 3_I$ or $11_{II} + 1_I$ and $2_{III} + 9_{II}$ or $1_{III} + 10_{II} + 1_I$ respectively. The expected irregularities in orientation on the metaphase I plate and segregation at anaphase I are observed.

(d) Substitution stocks:

Plants with $2n = 20$ chromosomes and showing all the 8 known dominants of Tripsacum have been recognized. Though normally 10_{II} are observed at diakinesis and metaphase I, PMC's showing $9_{II} + 2_I$ or $8_{II} + 4_I$ and occasionally $1_{IV} + 8_{II}$ or $1_{III} + 8_{II} + 1_I$ have been observed in different individuals. It has not yet been possible to ascertain the nature of substitution, whether by the elimination of the entire corn chromosome 9, its function being taken over by the Tripsacum homeolog, or by a segmental interchange involving the Tripsacum segment carrying

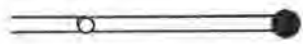

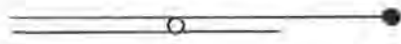

	<u>Plant No.</u>	<u>2n No.</u>
	68-286-1	20+2
	68-271-10	20+0
	68-270-2	20+0
	68-270-9	20+0

Fig. 1. Variability in the structure of the Tripsacum homeolog for corn chromosome 9 in different progenies.

the dominants. Similarly the corn chromosomes involved in the higher associations could not be identified at pachytene. The occurrence of these higher associations, however, suggests that the Tripsacum chromosomes are probably homeologous to at least one chromosome pair other than 9 within the corn complement; a spontaneous reciprocal translocation unrelated to the Tripsacum chromosome pair is also a possibility, which needs to be confirmed.

II. Structural Variations:

The terminal knob on the long arm and the relatively short short arm of the Tripsacum chromosome are so distinctive that its presence, intact or recombined, can be readily recognized in the corn complement. Some of the chromosome types observed in different plants are compared with the original form in Fig. 1. These forms apparently represent the Tripsacum or corn homeologs which have undergone mutual interchanges (primary, secondary, or tertiary) during the previous generations. It is not yet known if and how far they are stable and would be inherited. Considering the relatively high sterility observed in these plants some of them at least are lethal and are likely to be eliminated.

B. G. S. Rao
W. C. Galinat
P. Chandravada

7. Possible premeiotic gene exchanges between corn and *Tripsacum* homeologs.

A higher rate of gene transfer than would be suspected on the basis of chromosome associations at meiosis has been occasionally observed in some addition disomics. Our studies on the synaptic behavior of the *Tripsacum* homeologs for corn chromosomes 7 and 9 indicate that at least part of this crossing over occurs at some stage prior to pachytene.

In 33 out of 46 PMC's in which the extra pair could be identified, the *Tripsacum* bivalents show normal, regular and complete pairing at pachytene. In the exceptional PMC's these two chromosomes are of unequal lengths and the extra segment of the longer partner shows nonhomologous pairing within itself and appears as a buckle. The buckle, showing the pairing configuration characteristic for duplication/deficiency chromosomes, is always internal but variable in its extent and position from cell to cell. In two cases a corn bivalent showing a similar buckle is recorded within the genome. In two other nuclei the two heteromorphic bivalents of corn and *Tripsacum* were seen to be paired for short segments at the region of the buckle.

Apparently these two bivalents represent the reciprocal interchange products. Since both the normal and the abnormal (heteromorphic) bivalents occur in different cells of the same plant (sometimes within the same anther) it appears that the concerned chromosome segments were exchanged earlier than pachytene, possibly in some premeiotic stage.

B. G. S. Rao
W. C. Galinat

8. Progress in the cytogenetic comparison of the genomes of corn and *Tripsacum*.

The present state of knowledge in our comparison of extracted chromosomes of *Tripsacum* on marker gene stocks of corn is summarized in Table 1.

A cytological map of the eighteen chromosome pairs in the Kansas form of *T. dactyloides* ($2n = 36$) from which the different homeologs have been extracted is not yet available. However, Tantravahi (Bussey Inst., Harvard Univ., 1968) reports complete and regular pachytene pairing in the *T. floridanum* x *T. dactyloides* hybrid and it may therefore be assumed

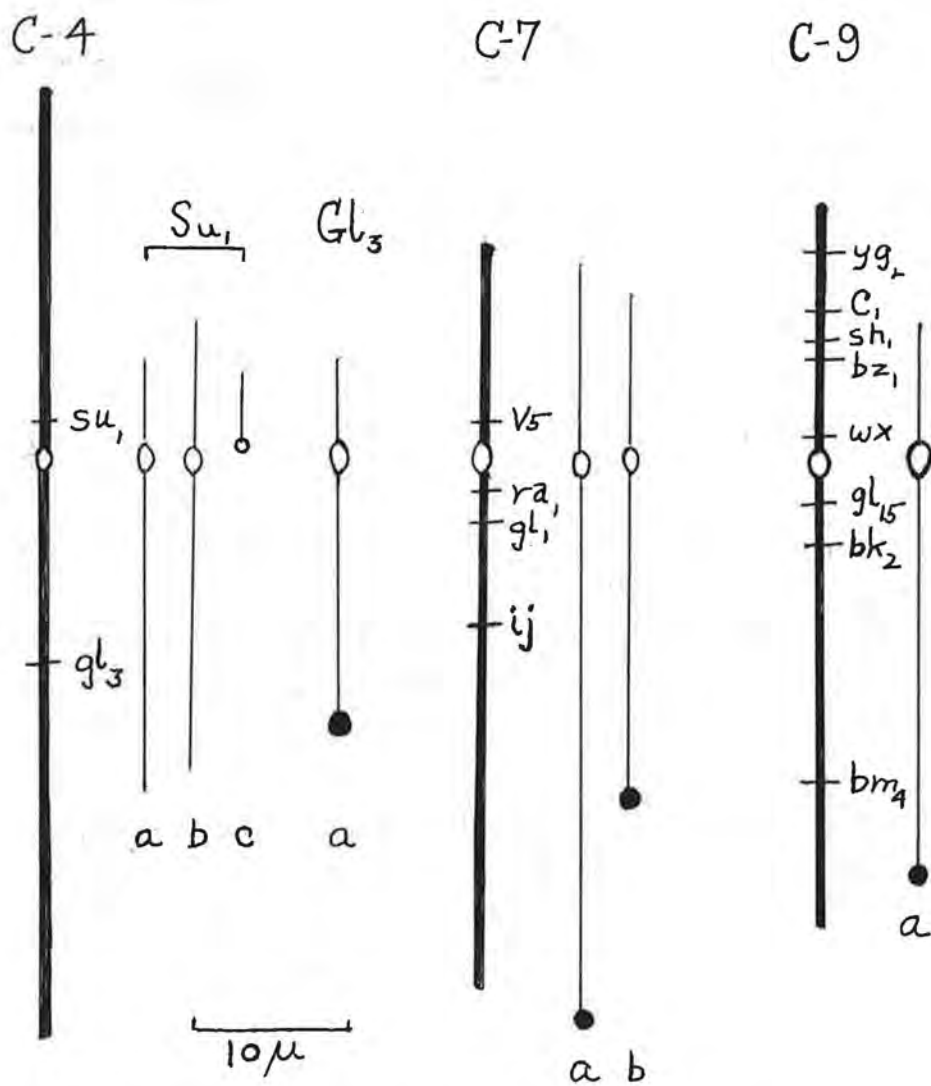


Fig. 1: Comparative idiograms of the corn and *Tripsacum* chromosomes and their known common Loci (Original forms shown as (a) while (b) and (c) are derived).

that the chromosomes of the two species are morphologically similar. To identify the extracted chromosomes of T. dactyloides, the relative positions accorded to similar chromosomes in the complement of T. floridanum (Tantravahi, 1968) are given tentatively.

Table 1

Cytogenetic correspondence of some corn and Tripsacum chromosomes

Known loci common between corn and <u>Tripsacum</u>	Chromosome no. in the complement of		Morphology of the <u>Tripsacum</u> chromosome		
	Corn	<u>Tripsacum</u>	Length u	Arm ratio	Remarks
<u>Bm</u> ₂	1	*	-	-	
<u>Ws</u> <u>Lg</u> ₁ <u>G1</u> ₂ <u>Sk</u> <u>Fl</u> ₁	2S	-	34.0 ^M	3.8:1 ^M	TKL
<u>V</u> ₄	2L	14 to 18	-	-	
<u>A</u> ₁	3	*	-	-	
<u>Su</u> ₁ (but not <u>La</u>)	4S	12	29.3	2.8:1	
<u>G1</u> ₃ (but not <u>Bm</u> ₃ <u>Ra</u> ₃ <u>J</u> ₂)	4L	13	22.4	3.5:1	TKL
<u>V</u> ₅ : <u>Ra</u> ₁ <u>G1</u> ₁ <u>I</u> _j	7S&L	4	45.0	2.8:1	TKL
<u>J</u> ₁	8	*	-	-	
<u>Yg</u> ₂ <u>C</u> <u>Sh</u> ₁ <u>Bz</u> ₁ <u>Wx</u> : <u>G1</u> ₁₅ <u>Bk</u> ₂ <u>Bm</u> ₄	9S&L	7 or 8	34.0	4.0:1	TKL
<u>G</u>	10	*	-	-	

S - Short arm; L - Long arm; * - Not yet identified

M - data of Maguire (1961); TKL - Terminal knob

The morphological features at pachytene of the concerned corn and Tripsacum homeologs are compared in Fig. 1.

W. C. Galinat
B. G. S. Rao

MACDONALD COLLEGE OF MCGILL UNIVERSITY
Province of Quebec, Canada

1. golden-2.

The location of golden-2 is still in doubt (MGCNL 36:49 and 39:118). Further evidence that it is not near Bn on chromosome 7 where it is placed in some publications comes from the following data:

F ₁ genotype	Parental combina- tions	Recombinations						Total
		Region 1		Region 2		Regions 1 & 2		
Tp ij g2	173 118	43	50	134	120	41	38	717
+ + +	291	93		254		79		
	40.6%	13.0%		35.4%		11.0%		

The accepted map is $\frac{Tp \quad ij \quad Bn}{46 \quad 52 \quad 71}$ whereas these data show golden-2 to be 46 crossover units from iojap and this may well indicate independent assortment. On the other hand the amount of crossing over between Teopod and iojap in these data (24%) is three times the accepted map distance.

R. I. Brawn

UNIVERSITÀ DI MILANO
Milan, Italy
Istituto Di Genetica

1. Recombination in the long arm of abnormal chromosome 10.

The abnormal chromosome 10, carrying a large knob in its distal end (referred to as K10), is preferentially segregated during megasporogenesis. Rhoades (1942) found that it is the knob which is responsible for preferential segregation leading to the recovery of more than 70% of the ovules with the abnormal chromosome 10. He also reported that in K10 heterozygous stocks the percentage of recombination between R and the distal end is strongly reduced while in the R proximal region, marked with g, no corresponding decrease is observed.

The data here presented refer to:

1. The effect of K10 upon crossing over in the long arm of chromosome 10. In this study \underline{g} has been used as \underline{R} proximal marker and \underline{M}^{st} as \underline{R} distal marker. The latter, lying 6 units to the right of \underline{R} , appears a more suitable marker than K10 which was employed in previous studies.
2. The effect of the alkylating agent E.M.S. (ethyl methane sulphonate) on the recombination in plants heterozygous for the abnormal chromosome 10 and in sibs carrying two normal chromosomes 10.

Before analyzing the effects described in points 1 and 2 we have established the amount of recombination taking place between \underline{g} \underline{R}^{st} and \underline{M}^{st} in the stocks used in this experiment. This has been done by crossing plants genotypically \underline{G} \underline{R}^{st} $\underline{M}^{st} / \underline{g}$ \underline{r}^r \underline{m}^{st} with a homozygous \underline{g} \underline{r}^g $\underline{m}^{st} / \underline{g}$ \underline{r}^g \underline{m}^{st} line.

This cross gave 7471 colorless, 7037 stippled and 283 light stippled kernels. Upon germination the seedlings obtained from these kernels were classified for their golden constitution (\underline{g}) with the following results:

Constitution of chromosomes:	\underline{G} \underline{R}^{st}	\underline{g} \underline{R}^{st}	\underline{g} \underline{r}^r	\underline{G} \underline{r}^r	Total
# of seedlings:	5427	1285	5726	1327	13,765

Among the light stippled seedlings 7 were classified as double recombinants (\underline{g} \underline{R}^{st} \underline{m}^{st}) and 6 of them survived. After progeny tests only 3 of them proved to be double recombinants and 3 were \underline{g} \underline{R}^{st} \underline{M}^{st} . From the above data it appears that:

1. The \underline{R}^{st} - \underline{M}^{st} recombination value amounts to 3.86% (283/7320).
2. The \underline{g} - \underline{R}^{st} recombination value amounts to 18.97% (2612/13765).
3. The coefficient of coincidence is 0.07 (0.051/0.710).

To study the effect of K10 and E.M.S. upon crossing over in the long arm of chromosome 10, individuals with the following genotypic constitution were prepared:

1.
$$\frac{\underline{g} \underline{R}^{st} \underline{M}^{st}}{\underline{G} \underline{r}^r \underline{m}^{st} \underline{K}}$$
2.
$$\frac{\underline{G} \underline{R}^{st} \underline{M}^{st}}{\underline{G} \underline{r}^r \underline{m}^{st} \underline{K}}$$
3.
$$\frac{\underline{g} \underline{R}^{st} \underline{M}^{st}}{\underline{G} \underline{r}^g \underline{m}^{st}}$$
4.
$$\frac{\underline{G} \underline{R}^{st} \underline{M}^{st}}{\underline{G} \underline{r}^g \underline{m}^{st}}$$

Plants with the above genotypes were crossed as pistillate parent with a $\underline{g} \underline{r}^g \underline{m}^{st} / \underline{g} \underline{r}^g \underline{m}^{st}$ line. Kernels obtained from the testcross ears were grouped into three phenotypic classes - stippled, light stippled, and colorless. Upon germination they were scored for their golden constitution.

In testcross ears produced on plants of genotype 1 and 2, the $\underline{R} - \underline{M}^{st}$ recombination value cannot be determined on the basis of one strand analysis (i.e. from the parental stippled and the light stippled recombinant seeds). In fact, in these plants the light stippled recombinants, carrying the distal knob, undergo preferential segregation. It thus appears necessary to extend the recombinational analysis to both strands. With the knowledge that normal chromosome 10, marked with \underline{R}^{st} , and the abnormal 10, marked with \underline{r}^r , are recovered in a ratio of 70:30 in the egg cells and assuming that crossing over is reciprocal we can infer the percentage of recombinants among the colorless seeds ($\underline{r}^r \underline{r}^g$) produced on the testcross ears and extend the recombinational analysis to both strands (see footnote (1) of Table 1). The results so obtained are reported in the following table:

Table 1
Recombination data between \underline{R} and \underline{M}^{st} from the testcrosses of plants of genotype 1, 2, 3, and 4 with a $\underline{g} \underline{r}^g \underline{m}^{st} / \underline{g} \underline{r}^g \underline{m}^{st}$ line

Pistillate parent genotype	Treatment	Constitution of chromosomes				Total $\frac{p}{\text{(rec.\%)}}$	
		$\underline{R}^{st} \underline{M}^{st}$	$\underline{R}^{st} \underline{m}^{st}$	$\underline{r} \underline{M}^{st}$	$\underline{r} \underline{m}^{st}$		
A. $\underline{R}^{st} \underline{M}^{st} / \underline{r}^g \underline{m}^{st}$	None	1284	58	58	1242	2642	4.4
B. idem	E.M.S. (2)	874	51	51	823	1799	5.7
C. $\underline{R}^{st} \underline{M}^{st} / \underline{r}^r \underline{m}^{st} \underline{K}$	None	2547	31	13	5923	8514	.5
D. idem	E.M.S.	762	14	6 ⁽¹⁾	353	1135	1.8
χ^2 (A vs B) = 3.7 ns.		χ^2 (A vs C) = 241.3**					
χ^2 (C vs D) = 23.6**		χ^2 (B vs D) = 26.6**					

(1) 14:0.70 = x:0.30 x = 6

(2) In this and following treatments a buffered solution of E.M.S. (1 or 2 x 10⁻²M) was injected in the stem of plants in the premeiotic stage.

Table 2
Recombination data between \underline{g} and \underline{R}^{st} from the testcrosses of plants
of genotype 1 and 3 with a $\underline{g} \underline{r}^{\underline{g}} \underline{m}^{st} / \underline{g} \underline{r}^{\underline{g}} \underline{m}^{st}$ line

Pistillate parent genotype	Treat- ment	Constitution of chromosomes				Total \underline{p} (rec.%)	
		$\underline{g} \underline{R}^{st}$	$\underline{G} \underline{R}^{st}$	$\underline{G} \underline{r}$	$\underline{g} \underline{r}$		
A. $\underline{g} \underline{R}^{st} \underline{M}^{st} / \underline{G} \underline{r}^{\underline{g}} \underline{m}^{st}$	None	277	70	322	75	744	19.5
B. idem	E.M.S.	128	23	138	30	319	16.6
C. $\underline{g} \underline{R}^{st} \underline{M}^{st} / \underline{G} \underline{r}^{\underline{r}} \underline{m}^{st} \underline{K}$	None	274	31	401	50	756	10.7
D. idem	E.M.S.	55	5	110	14	184	10.6

$\chi^2(A \text{ vs } B) = 1.21 \text{ ns.}$
 $\chi^2(A+B \text{ vs } C+D) = 25.13^{**}$
 $\chi^2(C \text{ vs } D) = 0.02 \text{ ns.}$

The data presented in Table 1 and 2 indicate that:

1. In stocks heterozygous for the abnormal chromosome 10 there is a strong reduction of recombination in the \underline{R} distal region and a less intense but still significant reduction in the \underline{R} proximal region. The latter observation is at variance with previous reports.
2. E.M.S. treatment leads to a partial suppression of the K10 effect upon crossing over. This effect, however, is confined to the \underline{R} distal region.

Even though more data on this point are required, the possibility exists that the alkylating agent induces specific breakages of the heterochromatic knob.

G. Gavazzi
G. Avila

2. Evidence on the compound nature of \underline{R}^{st} and \underline{R}^{sk} .

Genetic analysis of the main \underline{R} alleles, i.e., \underline{R}^r , $\underline{R}^{\underline{g}}$, \underline{r}^r and $\underline{r}^{\underline{g}}$, (Stadler 1951, and Emmerling 1958) has shown that \underline{R} is a compound locus, subdivisible in two components, \underline{P} and \underline{S} , conditioning plant and seed color respectively. On the other hand the structural analysis of \underline{R}^{st}

and other pattern alleles has not yet been performed. \underline{R}^{st} could be envisaged, by analogy with other \underline{R} complexes, as a dual structure consisting of a recessive form of the gene conditioning anthocyanin biosynthesis in the plant (\underline{p}) and a second gene controlling pigment production in the seed (\underline{S}). The appropriate symbolism to designate the stippled complex would thus be $\underline{p} \underline{S}^{st}$, the "st" superscript standing for the stippled pattern determined by \underline{S} . The experimental proof of the validity of this hypothesis rests on the isolation of $\underline{P} \underline{S}^{st} / \underline{p} \underline{s}$ recombinants from the cross of heterozygous $\underline{p} \underline{S}^{st} / \underline{P} \underline{s}$ individuals with a homozygous $\underline{p} \underline{s} / \underline{p} \underline{s}$ line. The $\underline{P} \underline{S}^{st} / \underline{p} \underline{s}$ recombinants should be easily recognizable on the basis of the association of their stippled phenotype in the aleurone with the anthocyanin production in their sporophytic tissues.

In this note we present data from the recombinational analysis of the \underline{R}^{st} and \underline{R}^{sk} alleles heterozygous with \underline{r}^r . The following heterozygous combinations were employed:

- $$\begin{array}{lll} (1) & \underline{G} \underline{R}^{st} \underline{M}^{st} / \underline{G} \underline{r}^r \underline{m}^{st} \underline{K} & \underline{G} \underline{R}^{sk} / \underline{G} \underline{r}^r \underline{K} \\ (2) & \underline{G} \underline{R}^{st} \underline{M}^{st} / \underline{g} \underline{r}^r \underline{m}^{st} & \underline{G} \underline{R}^{sk} / \underline{g} \underline{r}^r \quad \underline{G} \underline{R}^{sc} / \underline{g} \underline{r}^r \end{array}$$

Individuals of group (1) carry only \underline{R} distal markers (except a few cases in which \underline{R}^{st} and \underline{R}^{sk} have \underline{g} as a proximal marker) while those of group (2) are marked on both sides of \underline{R} . Plants with these genotypes were testcrossed as pistillate parents with a homozygous $\underline{g} \underline{r}^g \underline{m}^{st} / \underline{g} \underline{r}^g \underline{m}^{st}$ line.

Kernels produced on the testcross ears are colorless ($\underline{r}^r \underline{r}^g$) and variegated (stippled $\underline{R}^{st} \underline{r}^g$ or smoky $\underline{R}^{sk} \underline{r}^g$) or colored ($\underline{R}^{sc} \underline{r}^g$). Variegated and colored seeds were transferred to germinating pans. Upon germination those exceptional seedlings with red roots and coleoptiles were isolated as putative intralocus recombinants, transferred to the field and progeny tested by crossing them with a homozygous $\underline{g} \underline{r}^g \underline{m}^{st} / \underline{g} \underline{r}^g \underline{m}^{st}$ line.

The results of the progeny test are reported in Table 1. In this table and in the following lines the various \underline{R} alleles will be referred to in terms of their \underline{p} and \underline{s} components. Only 27 of the 37 individuals originally isolated could be tested. 15 of them proved to be contaminants, ten showed segregation ratios suggestive of trisomy for chromosome 10 ($\underline{p} \underline{S}^{st} / \underline{Ps} / \underline{p} \underline{s}$), one was genotypically $\underline{G} \underline{P} \underline{S}^{st} \underline{M}^{st} / \underline{g} \underline{p} \underline{s} \underline{m}^{st}$ and one

Table 1
Progeny test of putative $\underline{P} \underline{S}^{st}$, $\underline{P} \underline{S}^{sk}$ and $\underline{P} \underline{S}^{sc}$ intralocus recombinants

Pistillate parent genotype	No seedlings examined	Putative recombinants		Progeny test results			$P^{(1)}$
		a. isolated	b. tested	1. contam.	2. recomb.	3. trisomics	
$\underline{G} \underline{Ps} \underline{m}^{st} \underline{K} / \underline{G} \underline{pS}^{st} \underline{M}^{st}$	2442	9	7	1	1	4+1 ⁽²⁾	0.52
$\underline{G} \underline{pS}^{st} \underline{M}^{st} / \underline{g} \underline{Ps} \underline{m}^{st}$	6713	9	6	5	0	1 ⁽²⁾	0.00
$\underline{G} \underline{Ps} \underline{K} / \underline{G} \underline{pS}^{sk}$	1578	8	7	2	1	4	0.79
$\underline{G} \underline{pS}^{sk} / \underline{g} \underline{Ps}$	986	4	2	2	0	0	0.00
$\underline{G} \underline{pS}^{sc} / \underline{g} \underline{Ps}$	1126	1	1	1	0	0	0.00

(1) Frequency of recombination ($\times 10^{-3}$): adjusted value on the number of individuals tested.

(2) Not sufficiently tested.

$\underline{g} \underline{P} \underline{S}^{sk} / \underline{g} \underline{p} \underline{s}$.

The last two individuals were produced from the cross of plants $\underline{G} \underline{p} \underline{S}^{st} \underline{M}^{st} / \underline{G} \underline{P} \underline{s} \underline{m}^{st} \underline{K}$ and $\underline{g} \underline{p} \underline{S}^{sk} / \underline{G} \underline{P} \underline{s} \underline{K}$, respectively, with a $\underline{g} \underline{p} \underline{s} \underline{m}^{st} / \underline{g} \underline{p} \underline{s} \underline{m}^{st}$ line. Plants with a similar genotype could occur following \underline{p} back mutation, intralocus recombination and gene conversion. While a distinction between the last two possibilities seems not feasible at the moment, the first possibility can be discarded on the ground that no \underline{P} back mutants were previously observed in extensive experiments (Stadler 1952). In addition, we analyzed more than 14,000 gametes produced from homozygous $\underline{p}/\underline{p}$ plants without recovering a single case of \underline{P} back mutation.

Whatever the mechanism leading to a $\underline{P} \underline{S}^{st}$ (or $\underline{P} \underline{S}^{sk}$) recombinant, its occurrence is here considered sufficient positive evidence in favour of the hypothesis of the compound nature of \underline{R}^{st} and \underline{R}^{sk} .

G. Gavazzi

G. Avila

3. In vitro growth rate of maize root tips: heterozygote superiority and environmental variations.

One of the features of heterozygote advantage is that its degree might vary according to several environmental factors. In the case of temperature it has been shown that an increase of temperature above the optimal value may result in an increase of heterosis. This effect is interpretable in terms of the higher developmental stability of the heterozygous versus corresponding homozygous individuals.

Langridge (1962) interpreted this effect (in Drosophila and Arabidopsis) as the result of a complementation among temperature non-sensitive alleles of different genes.

In this experiment growth rate of maize root tips at different temperatures was used as a means to analyze the heterotic advantage. The validity of this system has been discussed in previous papers (Ottaviano e Zannini, 1965; Ottaviano 1966). In this research two inbred lines, W 22 and 33-16, and their reciprocal crosses were employed. In the context they will be referred to as A, B, A x B, and B x A, respectively. W 22 is a monoploid-derived line, kindly furnished by Dr. S. Chase. Four levels of temperature were used, namely, 20, 25, 30 and 35° C. Pilot experiments proved that the optimal temperature for growth is around 25° C.

Fig. 1a

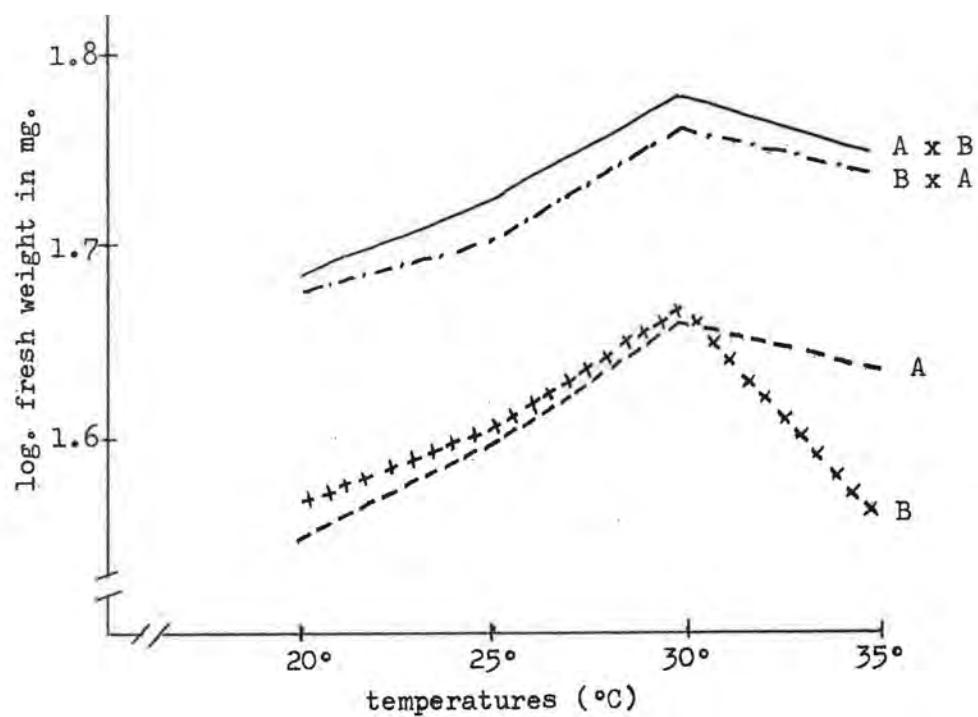
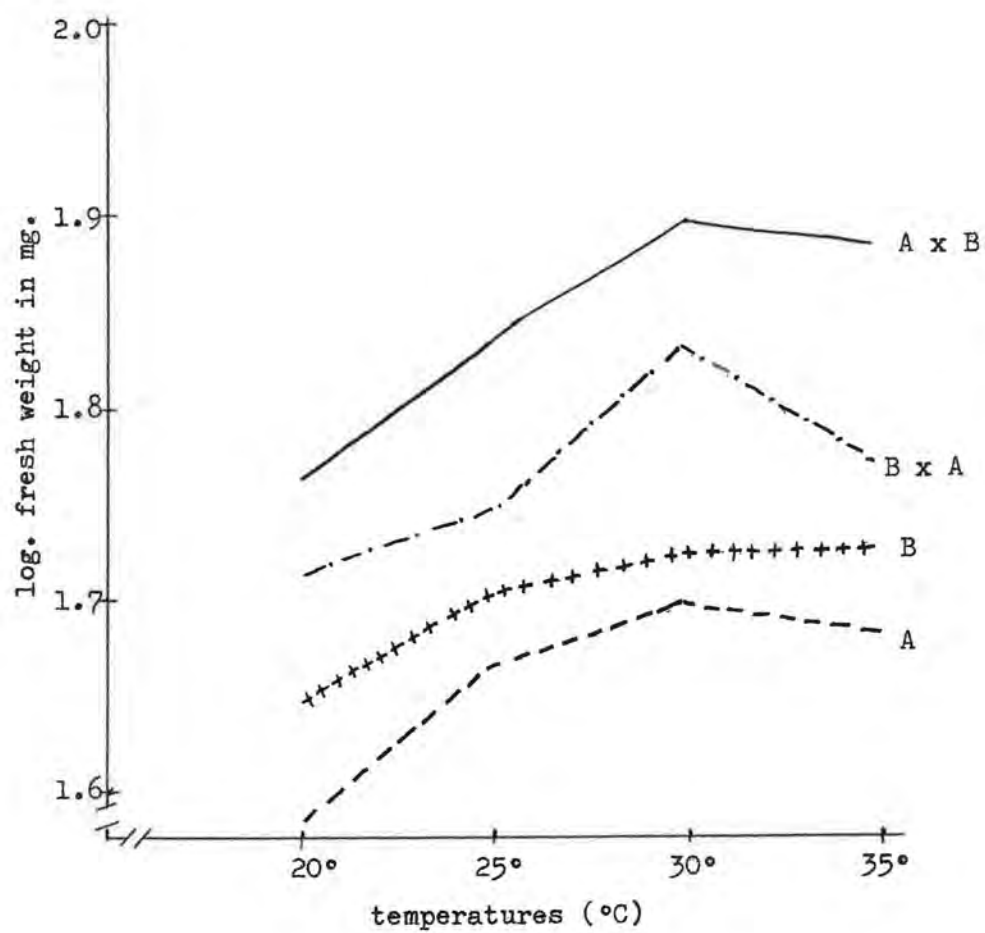


Fig. 1b



The basic medium (M₁) consisted of mineral salts, agar, sugar and vitamin B₁ (Ottaviano e Zannini, 1965). The possibility exists that the high temperature damage is due to the inhibition of synthesis of some important cellular metabolite. Accordingly the experiment was performed also in an enriched medium (M₂) carrying as additional components casein hydrolysate, pyridoxine and nicotinamide.

Single root tips were grown in testtubes. The experiment was arranged in randomized blocks, with ten replications for each genotype-temperature-medium combination. Growth was allowed to proceed for ten days. In each case the temperature was kept at 25° C for the first two days. Growth was measured as fresh weight at the end of this period.

Table 1

Genotypes	Medium	Temperatures			
		20°	25°	30°	35°
A	M ₁	1.5536	1.6010	1.6681	1.6407
	M ₂	1.5971	1.6711	1.7067	1.6907
B	M ₁	1.5707	1.6052	1.6755	1.5642
	M ₂	1.6575	1.7106	1.7304	1.7351
A x B	M ₁	1.6894	1.7263	1.7820	1.7544
	M ₂	1.7731	1.8497	1.9065	1.8990
B x A	M ₁	1.6815	1.7097	1.7698	1.7431
	M ₂	1.7200	1.7598	1.8444	1.7816

The results obtained, expressed in log mg. of fresh weight (Table 1, Figures 1a and 1b), have been analyzed statistically (factorial analysis of variance). The main findings can be summarized as follows: the growth response to temperature variations is not the same for all the material considered. Above 30° C and on minimal medium (M₁), W 22 shows a reduction in growth, while the other line and the two hybrids have constant growth. The addition of casein, pyridoxine, and nicotinamide (M₂)

repairs completely the temperature effect observed on the thermosensitive line (W 22) and brings out differences between reciprocals which are not observable with the minimal medium.

On the whole these results fit the model of the contribution of temperature-sensitive alleles to the heterotic advantage exhibited at high temperatures. However, in this case the role played by the cytoplasm should also be considered. Furthermore, it is not possible at the moment to exclude the involvement of ontogenetic processes that are temperature sensitive in this phenomenon.

E. Ottaviano

UNIVERSITY OF MINNESOTA
St. Paul, Minnesota
Department of Agronomy and Plant Genetics

1. A ring-of-20 chromosomes.

An association of 20 chromosomes at diakinesis was observed by John Stout in F_1 plants of a cross between two multiple interchange stocks. One multiple interchange parent was a 6-3-2-4-8 homozygote, the other was from semisterile plants selected from (5-7-1-9-10 x 8-10) backcrossed to 5-7-1-9-10. Plants with the crossover in the differential segment of chromosome 10 were expected to be semisterile, i.e. 5-7-1-9-10-8/5-7-1-9-10 heterozygotes. These were crossed on 6-3-2-4-8 and also self-pollinated for increase to establish the 5-7-1-9-10-8 homozygote. Half the plants from the cross were expected to have the ring-of-20. The selfs were grown and the fertiles increased and the test-crosses will be grown this summer to identify the 5-7-1-9-10-8 homozygote.

The Inman scheme (Burnham "Discussions in Cytogenetics," p. 113) will be used to combine the interchanges in one pure stock. A (6-3-2-4-8 x 8-10) F_1 was backcrossed to 6-3-2-4-8, and will be grown this summer. Semisterile plants, which should carry the 6-3-2-4-8-10 crossover, will be increased and also crossed with 5-7-1-9-10-8. Since the 8-10 interchange is common to both parents, one combination from random segregation should combine the two for a 6-3-2-4-8-10-9-1-5-7 multiple interchange stock.

There are several possible uses for hybrids with such a stock:

(1) in tests of exotic stocks or mixtures for genes that may induce apomixis. (2) The absence of seeds on the ears should result in an increase of sugars and carbohydrates in stalks and leaves (J. Amer. Soc. Agron. 28:85-91, 1936). Since female and male sterility are very high, this would be more effective than male sterility against stray wind pollination. Under open pollination in the genetics field, plants with two rings of 10 had 0 to six seeds.

C. R. Burnham
J. T. Stout

2. Tests for non-random segregation in unequal chromatid pairs in interchange heterozygotes.

Data presented by Zimmering (Genetics 40:809-825, 1955) show that in Drosophila there is non-random disjunction when crossing over in an interstitial segment produces unequal chromatid pairs. The shorter chromatid is the one preferentially recovered.

If the shorter chromatid is the normal one, fertile progeny will be in excess; if the shorter one is the interchange chromosome, interchange heterozygotes will be in excess.

In corn, data from T2-3a (an interchange from Dr. R. A. Emerson's cultures) with breaks at about 2S.9 and 3L.6 and from T1-5 (8041) with breaks at 1L.80 and 5L.10 furnish information on this point.

For T2-3a, the interchange arm of the 2^3 chromosome would be about 50% longer than the normal short arm of 2, and the interchange arm of the 3^2 chromosome would be about 50% shorter than the normal long arm of chromosome 3. Crossing over in either interstitial segment would produce a pair of unequal chromatids, but it is probable that most of such cross-overs would have been in the interstitial segment in chromosome 2. The shorter chromatid in that case would be the normal one from chromosome 2.

For T1-5 (8041), the new interchange chromosome 1^5 is 44% longer than the normal long arm of 1. Again, the shorter of the two chromatids resulting from crossing over in the interstitial segment would be the normal chromatid of chromosome 1. Preferential recovery of the shorter chromatid would lead to an excess of fertile plants among the progeny from interchange heterozygotes crossed with normal stocks. The data from

backcrosses are as follows:

	<u>S.S.</u>	<u>F</u>	<u>Lg</u>	<u>lg</u>
+ 2-3a/lg + ^o X lg/lg (group 1)	149	109	178	186
" X " (group 2)	259	252	301	265
lg/lg X + 2-3a/lg +	388	346	324	294
Bm 1-5 (8041)/bm + X bm/bm	446	409	<u>Bm₁</u>	<u>bm₁</u>
			<u>314</u>	<u>323</u>

In no case, was there an excess of fertile progeny. Certain of the differences in the other direction were significant, but were not consistent, either in different tests or for similar deviations in the segregation for closely linked alleles.

It is obvious that in corn in this type of material, segregation of unequal chromatid pairs at anaphase 2 is at random in the female parent. In species in which this segregation is not random, segregation ratios for alleles linked with the breakpoints would be different in reciprocal backcrosses.

C. R. Burnham

Supported in part by N.S.F. Grant G.B 5543.

3. Recombination in single and multiple interchange heterozygotes in maize.

The single interchanges used were: T1-7 (4405) = 1S.43, 7S.46; T1-9b = 1L.50, 9L.60; T5-7 (5179) = 5L.55, 7L.73; and T9-10b = 9S.13, 10S.40. The multiple interchange stocks that had been synthesized from these stocks were: T1-7-5, T7-1-9, T1-9-10, T5-7-1-9, and T5-7-1-9-10. The order of genes and breakpoints and recombination values with genes nearest the single interchange breakpoints were:

Chromosome 1: Sr-10-(T1-7)-4-P- ad-1-(T1-9)-35-bm₂

Chromosome 5: Pr-2-(T5-7)-4-ys

Chromosome 7: O₂-2-(T1-7)-1-v₅--ra gl-23-(T5-7)

Chromosome 9: wx-6-(T9-10)-6-gl₁₅ bk₂-6-(T1-9)

Chromosome 10: nl-1-(T9-10)-11-g₁

Recombination values in regions adjacent to the breakpoints were reduced in single and multiple interchange heterozygotes. There was no consistent change in recombination in the other regions of the chromosomes

including the differential segments in the bigger rings.

Cytological analysis of backcross progeny from the ring of 8 and the ring of 10 showed that 64% and 76% of the progeny, respectively, were the parental type, either the big ring or 10 pairs. The remainder had smaller rings and were presumably the products of crossing over in differential segments. "The fact that there is no drastic reduction in recombination in the bigger rings must be taken into account in any application of multiple interchanges as a tool in gametic selection."

Helmy Ghobrial (Ph.D.
Thesis)
C. R. Burnham

4. New combinations for genetic marker stocks.

chromosome 1

br segregating ts₂

chromosome 3

Stock segregating ra₂ and d₁

chromosome 4

Stocks homozygous for su, expected to segregate for la gl₁

Linkage tests with a₃

Tests of a₃ with R vs r, sr₂, and g₁ give no satisfactory evidence of linkage.

C. R. Burnham
Richard V. Kowles

5. Albino seedling W7748.

Stocks segregating albino W7748 (originally from Coop stock 60-529-1) failed to show linkage with ba₁ (originally from Coop stock 62F-1116-4), as reported in M.N.L. 41:133, 1967. Ears of this material that were segregating for one to three aleurone color factors were used by a senior undergraduate student, Mr. Robert Kennedy, as a special problem. He made the seedling tests for linkage between aleurone color and albino seedlings. Cultures from ears segregating for three aleurone color factors, and certain of those segregating for two, showed linkage between aleurone color and albino.

The past summer, plants from the colored aleurone classes from ears showing linkage were selfed. An ear segregating 3:1 for aleurone

color and for albino shows linkage in coupling with about 12% recombination based on very small numbers. Crosses will be made this summer to identify the aleurone color factor with which it is linked.

C. R. Burnham

6. White-tipped seedlings.

In 1966, self progeny from one ear from one of Kenneth Michel's cultures of Minnesota A188 inbred segregated for white-striped seedlings. The plants were short, with thin stalks and narrow leaves. All leaves were striped, but the stripes varied in width. Two striped plants produced pollen which was used in crosses with interchange stocks in the "all arms tester series" and with sib plants in the same culture. Remnant seed from the original ear and from sibs of the original ear failed to segregate for striped plants. Also the crosses of green x striped sibs failed to segregate striped plants. The latter did segregate for seedlings with a patch of white radiating back from the leaf tips. Self progeny from the crosses of the A188 interchange stocks x striped also segregated 3 green:1 white tipped.

The character is easily classified in the seedling stage and the plants appear normal in vigor. No linkage has been found in the tests made thus far.

C. R. Burnham

7. Keeping numbered tags in order for field use.

In collecting tassel samples for preservation in 70% alcohol for later pollen abortion determinations, it is advantageous to make out the tags, such as the 37B Dennison string tag, in advance in the laboratory. The first workable item was one seen in sporting goods stores used for display cards of the short leaders used in fishing. These come in different widths, 1" or 1 1/2" across the side, and are bent in this form:



The tags can be strung on this holder in order. We held the strings in one direction as they were added, and experienced no difficulty removing them in order.

Mr. John Mead who was helping found that a paper clip bent in the same manner worked fully as well.

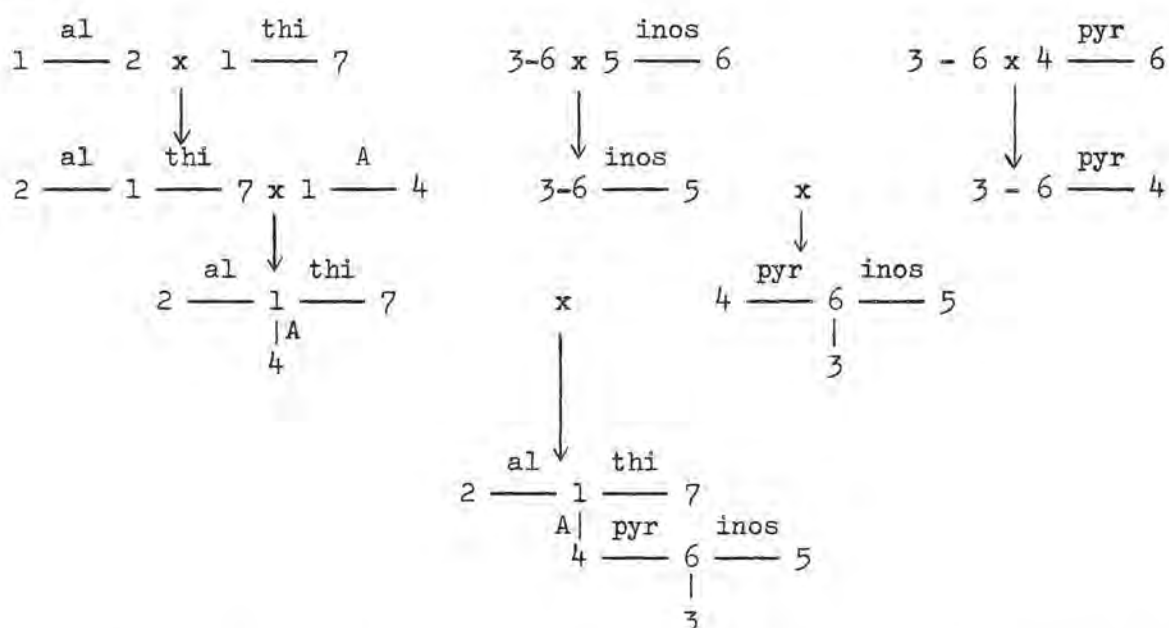
If only a few florets or single tassel branches are being collected, perforated sheets of tagboard can be used. These can be perforated to any desired size. One we use is $1 \frac{3}{4}'' \times 2 \frac{3}{4}''$. These can be folded and stapled tightly close to the branch or so as to enclose the florets. It is best to use aluminum staples, since steel ones rust in alcohol.

C. R. Burnham

8. Synthesis of a 7-chromosome (complete) interchange in *Neurospora crassa*: a comparative study with maize.

The cytogenetic behavior of interchanges in *Neurospora crassa* is similar to maize. Since the equivalent to maize backcross data can be obtained in 3-4 weeks, we are using *Neurospora* to obtain more immediate answers to questions pertinent to higher plants, specifically maize. The study briefly reported here was prompted by the near development of a maize stock which has every chromosome involved in an interchange such that the heterozygote forms a ring-of-20 chromosomes (Burnham, see note in this News Letter). Burnham (J. Amer. Soc. Agron. 38:702-707, 1946) outlined the potential advantages of such a stock in maize for the rapid establishment of inbred lines. The amount of crossing over in such a complex of chromosomes is of utmost importance to its proposed use. Since several years will be required to obtain this information in maize, it was decided to synthesize and study a multiple-interchange strain of *Neurospora*.

The first stage of this study, the synthesis of a 7-chromosome (complete) interchange of *Neurospora*, was initiated in February, 1968, and is now complete. This strain produces a ring-of-14 chromosomes when crossed with wild type. Each step in the synthesis has been documented genetically and cytologically. The general scheme of synthesis was as follows:



The arabic numerals specifying an interchange denote linkage groups. Four of the seven linkage groups have been assigned to their respective chromosomes. Each single interchange strain, except the 3-6, carried a closely or completely linked gene which marked the presence of the interchange. The interchanges were combined through crossing over in a differential segment in a common chromosome in all cases except for the 4-chromosome interchange involving 3, 4, 5, and 6. In this case, the 3-6-5 and 3-6-4 interchanges were combined through random chromosome segregation, since they form two rings of four chromosomes when crossed. In general, the percent of aborted spores paralleled that expected in maize for comparable interchange complexes.

Information is currently being obtained on the frequency of recombination for the 7-chromosome interchange and its various constituent interchanges.

This work is supported in part by grants from the University of Minnesota Graduate School and the DeKalb Agricultural Association.

R. L. Phillips
Jane Magill

MISSOURI BOTANICAL GARDEN
St. Louis, Missouri

1. Zapalote chico.

The following reference has long been difficult to obtain:
Zapalote Chico: An Important Chapter in the History of Maize and Man.
Congreso de Americanistas, San José, Costa Rica, 1959.

Zapalote Chico has been described and illustrated in Wellhausen, et al., 1952 and in Wellhausen, et al., 1958. The substrain described in this note was collected by Jonathon Sauer in 1947 in a conservative community and exhibits the very distinctive characters of Zapalote Chico more markedly than any other collection. As discussed on page 233, it is virtually independent of length of day. Transferred directly from southern Mexico to Minnesota, it produced normal plants only slightly taller than those grown in Mexico. It therefore might play a unique role in experiments on the basic physiology of maturity in maize or in studies on the genetics of photoperiodic response.

Xeroxed copies of the paper are available from Edgar Anderson, Missouri Botanical Garden, 2315 Tower Grove Avenue, St. Louis, Missouri, 63110.

A limited amount of seed of this strain is in cold storage and can be obtained by writing to: Dr. William L. Brown, Vice President and Director of Research, Pioneer Hi-Bred Corn Company, 1206 Mulberry Street, Des Moines, Iowa, 50308.

Edgar Anderson

UNIVERSITY OF MISSOURI
Columbia, Missouri

1. Negative interference.

The aim of this present study was to explore further the occurrence of an apparent negative interference component associated with the long arm of chromosome 1 (see Maize News Letter 42:129-132).

Statistical analyses were done on backcross data from material which was either structurally normal or heterozygous for one of several

translocations. Two inbred backgrounds, 6X3, a Coe standard, and 4Co63, a Brink standard, were used.

The long arm of chromosome one was divided into four regions: $\underline{br-an}_1$ (region 1), $\underline{an}_1-\underline{bz}_2$ (region 2), $\underline{bz}_2-\underline{gs}_1$ (region 3), and $\underline{gs}_1-\underline{bm}_2$ (region 4). From all samples individual recombination values were calculated for the raw data from all four regions. Average recombination values were obtained (\bar{p}_n) by dividing the total number of recombinants by the total number of individuals free from structural aberrations for each interval population. This value was used both in the heterogeneity calculations and as the mean to which individual recombination frequencies were compared to identify those which were higher than the average from those which were lower. In addition, the standard errors for the p_i values were calculated to determine fit, that is, percentage of the time a given recombination frequency higher or lower than the mean would be expected by chance.

Using \bar{p}_n , two different heterogeneity calculations were made. First, determination was made as to whether or not the intervals carried on two structurally normal chromosomes were homogeneous. Second, a similar set of calculations was made using the translocation heterozygotes.

Coincidence calculations were made on all pertinent double exchanges. In addition, the probabilities of these values occurring by chance in a population whose true coincidence was one were determined.

Five graphs were constructed as a result of the preceding statistical analyses. The first relates fit to a coincidence of one, separating those with c less than one from those with c greater than one, to frequency. The remaining four graphs relate fit to \bar{p}_n for each region in a similar manner to the one described directly above. Finally, X^2 tests were made, where fit to the experimentally determined ratio, i.e., ratio of coincidence less than one to that greater than one, was tested with regard to background, translocation bearing samples versus normal sibs and adjacent intervals.

The preceding analyses yielded the following information:

1. Heterogeneity was associated with neither structural configuration.
2. The general tendency of the translocations was to reduce

recombination in this study.

3. The χ^2 tests failed to show significance, that is, to affect distribution when the parameters of structural constitution, background and adjacency were considered.
4. The negative interference effect previously reported to be associated with the whole arm has been found to be a property of region 2 specifically, as indicated by χ^2 tests which tested the distribution for pairs of regions against empirical proportion determined from the first graph described.

Detailed description of the results and their theoretical implications are forthcoming in a paper soon to be published.

S. L. Goldman

2. Structure of \underline{an}_{6923}

An experiment was made in which $\pm \underline{an}_{6923} \underline{bz}_2$ was crossed to $\underline{an}_1, \underline{bz}_2$. Both the bronze and purple seed classes were planted. Of the 4,952 bronze seeds recovered, all gave rise to plants which were anther ear in phenotype. Of the 5,048 plants derived from fully colored seed, no instance of anther ear phenotype was noted.

In a population of 10,000 gametes, ten crossovers are expected between \underline{an}_1 and \underline{bz}_2 , since the map distance separating them in the control amounted to 0.1 of a map unit. The failure to obtain any crossovers when \underline{an}_{6923} was involved is further support for the idea that this may indeed be a pollen transmissible, homozygous viable deletion.

S. L. Goldman

3. Survival kinetics of pollen grains in aqueous medium.

Pollen of ACR stock was mixed with 25 ml of aqueous medium (Newsletter 42:126) and applied sequentially with a #8 brush to silks of a colorless F_1 hybrid, W23/M14. Colored kernels were counted at harvest.

Five experiments were conducted (Table 1). In Expt. A, substantial spilling occurred in the haste of keeping to 5-sec. intervals; these numbers of ears and kernels are adjusted to account for spillage. Estimates indicate 2.5×10^6 pollen grains per cc of dry pollen, so the overload (in thousands of pollen grains per kernel) was both extreme and

surprisingly uniform. A 10-fold dilution made relatively little difference in the overload level, where the averages of two replicates (B+C vs. D+E) yielded one kernel per 5,400 vs. 3,900 pollen grains, respectively.

Table 1
Sequential pollination experiments with pollen in 25 ml
of aqueous medium

Expt.	Pollen cc	Interval sec	Ears no.	Kernels no.	Pollen grains per kernel (10^3)	Av. kernels per ear
A	10	5	64	3883	6.4	61
B	10	10	59	3912	6.4	66
C	10	10	68	5352	4.7 ^{5.4}	79
D	1	10	59	782	3.2	13
E	1	10	53	507	4.9 ^{3.9}	10

The implication that dilution is unimportant is not supported by the time pattern, however (Table 2). The 10-fold richer suspension (B+C) at first yielded only 4 times as many kernels as the other (D+E), but by the time 270 sec. had passed the yield became 20 times greater. Apparently half or more of the potentially effective pollen grains are prevented from functioning (possibly by competition for the same ovules) during the first few minutes. Later the richer mixture develops a double advantage, per pollen grain applied, over the other. Since both appear to level off by the 6th to 7th minute, long-term survival is influenced by a "population effect".

The extreme overload requirement (over 1,000 pollen grains per kernel during the first half minute) is puzzling. Dry pollination of these ears would have yielded 500 to 800 kernels each, but even with extra care in application of the suspensions less than 1/2 of the maximum set was obtained (Expt. C, highest individual set was 232). Notably, the dilute suspensions (D and E), though applied with the same care as the richer ones, gave even less per ear (highest set 82, next higher set 59). Since the same number of silks have been "effectively painted" at both concentration levels, a property of pollen grains must be responsible for the overload requirement.

Thus, for efficient pollen treatment work in aqueous medium, populations should be kept high (e.g., 1 cc pollen in 2.5 ml medium), and

pollination should be delayed for two minutes or longer.

Table 2
Seed sets from sequential pollinations

Time sec	Sliding average of five ears			Ratio B+C/D+E
	A	B+C	D+E	
30	80	169	39	4
60	75	167	38	4
90	57	115	37	3
120	73	112	26	4
150	48	109	16	7
180	48	76	8	9
210		72	6	12
240		77	5	14
270		93	4	25
300		82	4	22
330		57	3	17
360		50	3	17
390		44	2	19
420		39	2	20
450		45	2	19
480		39	2	19
510		37	2	21
540		33	2	22
570		40		
600		50		

E. H. Coe, Jr.

UNIVERSITY OF MISSOURI
and
ARS, UNITED STATES DEPARTMENT OF AGRICULTURE
Crops Research Division and
Northern Utilization Research Division

1. Multiple aleurone layering in maize.

The aleurone layer of corn comprises from 1 to 2 percent of the corn kernel. It contains about 20 percent protein with a large proportion of the amino acids being of the basic type and therefore valuable nutritionally. Unfortunately, less than 5 percent of the kernel protein is found in the aleurone layer. Normally the aleurone layer is made up of

Table 1

Progeny performance for single and double aleurone selections from recurrent selection series A100C.
Grown near Homestead, Florida in the winter of 1967-1968.

67-68 W Row No.	Pedigree	Parental Aleurone Composition	No. Ears	No. Kernels Examined	Progeny Aleurone Layer Composition				
					Single	Partly Double	Double	Partly Multiple	Percent Kernels With More Than 1 Layer
731	793 (X)	Single 100%	8	37	37	-	-	-	0
732	794 (X)	Segregating	5	26	5	10	10	-	81
733	795 Seg (X)	Segregating	8	37	15	8	8	6	59
734	795 Seg (X)	Segregating	10	53	19	14	6	10	64
CROSSES									
735	(795) x (793) (Seg) x (Single)		10	49	28	7	7	7	43
736	(793) x (795) (Single) x (Seg)		9	45	24	13	6	2	47
737	(795) x (687) (Seg) x (Single)		10	50	17	8	25	-	66
738	(687) x (795) (Single) x (Seg)		10	50	42	7	1	-	16

a single layer of cells at the periphery of the endosperm. Doubling the aleurone layer would increase the protein from this source.

From a recurrent selection study for high-amylose starch involving exotic strains into which the ae gene (amylose extender) had been introduced, several selections with high-amylose content and high-protein and several with high-amylose and low protein were found. While examining these selections microscopically for starch and protein characteristics, kernels with double-aleurone layers were found.

A summary of the aleurone composition for the parents and their progenies is given in Table 1. Reciprocal crosses between single and double aleurone layered selections indicate the double-aleurone layer character is dominant. Of additional interest is that some kernels with multiple aleurone layers were also found.

We assumed the source of the multiple aleurone layer characteristic was from the exotic strains. We, therefore, obtained as many of the exotics used in the recurrent selection study as possible. Among these, Peruvian 442 was found to have about 35% of its kernels with more than one layer of aleurone.

Inheritance studies of the multiple aleurone layer characteristic are in progress and we also plan to make comparative biological evaluations of the protein from single, double and multiple aleurone layered selections.

M. J. Wolf
M. S. Zuber
J. L. Helm

2. Tetraploid gene segregation as studied with a repulsion phase marking system.

This system enables one to investigate the many factors which complicate tetraploid gene segregation such as double reduction, numerical non-disjunction, and the viabilities of aneuploid gametes and zygotes. The understanding of these factors is necessary to explain the partial sterility of most autotetraploids.

The system may be described as the following of two genes at the same time in the repulsion phase--each chromosome is marked with a dominant and a recessive gene. For example, plants with the genotypes of

$\underline{A}/\underline{A}/\underline{a}$, $\underline{A}/\underline{A}/\underline{a}/\underline{a}$, and $\underline{A}/\underline{A}/\underline{a}/\underline{a}/\underline{a}$ give phenotypic ratios of $\underline{A} : \underline{a}$ in their progeny that cannot be readily distinguished because they are similar. The frequency of \underline{A} phenotypes is mostly independent of the number of chromosomes carrying \underline{a} . However, if we use plants with the genotypes of $\underline{Ab}/\underline{Ab}/\underline{aB}$, $\underline{Ab}/\underline{Ab}/\underline{aB}/\underline{aB}$, and $\underline{Ab}/\underline{Ab}/\underline{aB}/\underline{aB}/\underline{aB}$, the plants can be easily distinguished by observing the ratio of $\underline{B} : \underline{b}$. Table 1 gives the theoretical gene segregation of fifteen different euploid and aneuploid genotypes.

Table 1

The theoretical gene segregation of euploid and aneuploid types of autotetraploids assuming random chromatid assortment

Genotype	Phenotypic ratio in backcross*			% a	% sh
	<u>Ash</u>	<u>ASh</u>	<u>aSh</u>		
Tetrasomic					
Ash/Ash/aSh/aSh	6	16	6	21.43	21.43
Ash/Ash/Ash/aSh	15	12	1	3.57	53.57
Ash/aSh/aSh/aSh	1	12	15	53.57	3.57
Trisomic					
Ash/Ash/aSh	8	4	3	20.00	58.33
Ash/aSh/aSh	3	4	8	58.33	20.00
Pentasomic					
Ash/Ash/Ash/Ash/aSh	49	40	1	1.11	54.44
Ash/aSh/aSh/aSh/aSh	1	40	49	54.44	1.11
Ash/Ash/Ash/aSh/aSh	3	8	1	8.33	25.00
Ash/Ash/aSh/aSh/aSh	1	8	3	25.00	8.33
Disomic					
Ash/aSh	1	0	1	50.00	50.00
Hexasomic**					
Ash/Ash/Ash/Ash/Ash/aSh	6	5	0	0.00	54.54
Ash/aSh/aSh/aSh/aSh/aSh	0	5	6	54.54	0.00
Ash/Ash/Ash/Ash/aSh/aSh	9.3	26.3	1	2.73	25.45
Ash/Ash/aSh/aSh/aSh/aSh	1	26.3	9.3	25.45	2.73
Ash/Ash/Ash/aSh/aSh/aSh	1	9	1	9.09	9.09

*No ash class is listed as its probability is very low, because of the close linkage between a and sh; also for a gamete to be ash it must have two crossover chromosomes or be monosomic which are rare events.

**Assuming 3 to 3 disjunctions.

Table 2 gives some data for seven different genotypes isolated so far. The data correspond roughly to theoretical expectations. Corrections need to be made for the frequencies of double reduction, numerical non-disjunction, and viability factors of the aneuploid gametes and zygotes. Additional data are needed to make a good estimate of the parameters of tetraploid gene segregation.

Table 2

Tetraploid gene segregation of seven different genotypes, euploid and aneuploid

Genotype	No. of plants tested	No. of gametes tested	Percent <u>Ash</u>	Percent <u>aSh</u>
Ash/Ash/aSh/aSh	155	25,556	19.86	20.68
Ash/Ash/Ash/aSh	14	1,867	48.90	2.52
Ash/aSh/aSh/aSh	21	3,128	2.40	52.17
Ash/Ash/Ash/aSh/aSh	4	468	31.83	8.97
Ash/Ash/aSh/aSh/aSh	5	498	9.84	28.11
Ash/Ash/aSh	7	932	46.03	18.56
Ash/aSh/aSh	5	586	22.69	48.29

The trisomics (Ash/Ash/aSh and Ash/aSh/aSh) and pentasomics (Ash/Ash/Ash/aSh/aSh and Ash/Ash/aSh/aSh/aSh) were isolated from crosses between 4n Ash and 4n aSh plants. The aneuploids are the result of numerical non-disjunction of quadrivalents at meiosis--3 to 1 separations of the chromosomes or from the trivalent-univalent configurations which should result in numerical non-disjunction about half of the time. In case of tivalent-univalent formation, it is possible that the disjunction may be 2 to 1 with one chromosome being lost. This would result in a higher frequency of 2n-1 gametes than 2n+1 gametes. The genotypes of 132 plants from 4n Ash and 4n aSh crosses are given in Table 3.

Table 3
Frequency of aneuploid genotypes in crosses of 4n Ash
by 4n aSh and reciprocal

Cross	4n <u>Ash</u> X 4n <u>aSh</u>	4n <u>aSh</u> X 4n <u>Ash</u>	Total
<u>Genotypes</u>			
Ash/Ash/aSh/aSh	25	97	122
Ash/Ash/Ash/aSh/aSh	1	3	4
Ash/Ash/aSh/aSh/aSh	1	2	3
Ash/Ash/aSh	1	1	2
Ash/aSh/aSh	0	1	<u>1</u>
			132

The 132 plants were formed by 264 gametes, 7 or 2.65% of which were hyperploid for chromosome 3 and 3 or 1.14% of which were hypoploid. The percentage of aneuploid gametes is 3.79%. The data are not adequate for a precise estimate of numerical non-disjunction. If we take the figure as a rough value then if all ten chromosomes behave similarly, the frequency of aneuploid gametes is rather high. It is around $1 - (1 - .04)^{10}$ or 33.5%. The frequency of 40 chromosome eutetraploids in the progeny of 40 chromosome plants should be about 44% $(1 - .33)^2$. The figure observed from the pooled data of Randolph, Kadam, and Catcheside is 60.6% but this probably includes some numerically compensating types (3 of one and 5 of another).

The hexasomic and disomic types of aneuploid may be derived from selfing the pentasomics and trisomics, respectively. One trisomic (Ash/Ash/aSh) was selfed. It gave 7 tetrasomics (5 Ash/Ash/aSh/aSh and 2 Ash/Ash/Ash/aSh) and 9 trisomics (5 Ash/Ash/aSh and 4 Ash/aSh/aSh) and no disomics. We would expect 1/4 of the progeny to be disomic. It is possible that disomic tetraploids are not viable. If this is true, then it would be a big factor in autotetraploid fertility particularly when tetraploids are selfed.

G. G. Doyle

3. Preferential pairing in standard-commercial inbred line trisomic 3 hybrids.

Preferential pairing was studied in eleven different standard-inbred line hybrids. The results are given in Table 4. Individual plants from all of the eleven types of hybrids showed aberrant ratios which are

Table 4
Gene segregation in standard-commercial inbred
line trisomic 3 hybrids

Inbred line	Type of ratio ¹	No. of plants tested	No. of gametes tested	No. of \underline{A} gametes	% \underline{A}	Inter-action χ^2	χ^2 (1A:2a)
M 14	(+)	10	11,280	3,882	34.42	8.67	5.94*
K 55	(n)	3	3,815	1,285	33.68	0.01	0.23
	(+)	3	5,141	1,965	38.22	4.29	55.14**
N 6	(n)	5	5,028	1,668	33.17	5.76	0.06
	(+)	1	1,086	404	37.20	--	7.30**
CI 21E	(n)	7	5,045	1,668	33.06	5.26	0.17
	(-)	3	2,206	532	24.12	1.52	83.28**
C 103	(n)	3	3,453	1,154	33.42	0.14	0.93
	(+)	1	775	326	42.00	--	26.88**
	(-)	1	1,431	435	30.40	--	5.55
CI 7	(n)	2	2,721	890	32.71	0.03	0.48
	(-)	4	5,629	1,580	28.07	15.08**	70.05**
K 6	(n)	3	3,207	1,036	32.30	1.56	1.53
	(-)	2	2,664	614	26.64	0.47	126.82**
Kys	(n)	2	1,304	458	35.12	0.03	1.83
	(-)	33	36,249	10,608	29.26	41.23	270.05**
B 41	(-)	5	6,188	1,535	24.81	18.45**	202.70
38-11	(-)	10	8,473	1,985	23.43	47.41**	373.89**
Hy	(n)	1	709	213	30.04	--	3.36
	(-)	7	6,338	1,241	19.58	65.06**	539.79**

¹
+ > 33%
- < 33%
n = 33%

indicative of preferential pairing. Gross chromosomal structural differences are not present between the chromosomes 3 of the inbred lines and the standard, so the results are probably caused by cryptic structural

rearrangements (small inversions, deletions, and duplications and genetic differences as the result of the differential introgression of Tripsacum via teosinte and spontaneous mutation). Despite the fact that the pollen parent used to form the test trisomics was an inbred line and therefore presumably possessed only one kind of chromosome 3, the data indicate that they were not always homogeneous. In the cases of K 55, CI 21E, CI 7, and K 6 there seems to be a 1 to 1 segregation for normal and aberrant ratios. One explanation is that the parental plants were not homozygous for a structurally similar chromosome 3.* The inbred lines of maize are known to be unstable phenotypically. A preferential pairing test may be very sensitive in detecting small differences.

Another explanation is that there may be a very strong environmental interaction. Anything, for example, temperature, which would change the frequency of trivalent formation would modify the expression of preferential pairing. The influence of the environment will be determined this summer by the use of split planting dates and by serial pollinations. Different planting dates will cause the pollen to be formed under different circumstances. By using the same plant for crosses every day during its pollen shedding period, environmental influences can be assessed. The pollen shed on one day may have formed during a cool period, and that shed on the next day during a warm period.

In general, the data are more homogeneous than those found when trisomic standard-exotic hybrids were used. Note interaction chi squares. The amount of preferential pairing observed is of a similar magnitude. In the cases of Hy and 38-11 it is as strong as that observed when In 3a or In 3b was used. This finding is relevant to the problem of the synthesis of an artificial allotetraploid. At first, it was thought necessary to use inversions; after the results with exotic races the program envisaged collecting all sorts of chromosomes with cryptic structural differences from all parts of the world. Now, it appears to be possible to use native adapted commercial inbred lines. In simple terms, many small structural differences from different inbred lines will be converged together by a breeding system employing recurrent selection for

*Possibly resulting from unequal crossing over in tandem duplications which may be very common in maize; see next note.

preferential pairing. Progress toward allotetraploidy will be monitored by observing the quadrivalent frequency and modifications of gene segregation. The exact details of the breeding system remain to be worked out.

G. G. Doyle

4. X-ray induced duplications from translocations between homologous chromosomes.

Translocations between the same arms of homologous chromosomes lead to the formation of a chromosome with an interstitial deletion and a chromosome with a tandem duplication, if the breakpoints are not identical. If it is assumed that the chromosomes in the interphase nucleus in somatic tissue are arranged at random, then the expected frequency of this event would be very low. Translocations between opposite arms of homologous chromosomes form pseudo-isochromosomes. The frequency of pseudo-isochromosomes has been found by Koo to be $1/4 (n-1)$ times the frequency of translocations between non-homologues, where n equals the haploid chromosome number. However, if there is a tendency for homologous chromosomes to be in a semi-paired state in the interphase nucleus as numerous investigators now believe, then we would expect the frequency of tandem duplication production to be higher than that of pseudo-isochromosomes. The probability of two broken ends uniting to form a new structural rearrangement is a function of the distance between them. A chromosome arm may be just as far away from the opposite arm of its homologue as it is from the arms of non-homologues, but may be much nearer the homologous arm of its homologue, than to any other arm.

The method used to detect tandem duplications has been presented in previous reports. In brief, they are detected by aberrant ratios from plants in the X_2 generation which come about by crossing over in one of the duplicated segments. Kernels which were heterozygous for 10 different markers were given 10,000 r in two different trials. The constitution of these kernels was Kys (with all dominant genes) over Mangelsdorf's tester (bm_2 , lg_1 , a_1 , su , pr , y , gl_1 , j , wx , and g). Only the lg , su , y , gl , wx , and g loci were followed. The plants grown from the irradiated seed were crossed to Kys to form the X_2 generation. There were 1169 X_2 plants which were crossed to Mangelsdorf's tester or to a

hybrid of Mangelsdorf's tester and Kys. The results are given in Table 5 and are summarized in Table 6.

Table 5

The genotypes of X_2 plants with aberrant ratios found in 27 independent cases

Case #	Treat- ment #	Locus	No. of ears from X_1	Genotypes			Pollen parent	Aberrant ratio			Reconstitu- tion test A-a?/A X aa
				AA*	Aa*	Aberr.		A*	a*	%a	
1	1	y	31	13	17	1	a/a	118	6	4.8	negative
2	2	y	10	4	5	1	A/a	166	26	13.5	---
3	2	y	5	1	3	1	A/a	470	1	0.2	---
4	2	y	9	4	4	1	A/a	180	23	11.3	---
5	1	lg	23	9	13	1	a/a	415	3	0.7	---
6	1	lg	16	9	6	1	a/a	95	1	1.0	positive
7	1	lg	18	9	8	1	a/a	145	3	2.1	positive
8	1	lg	16	7	8	1	a/a	91	1	1.1	---
9	1	lg	12	6	5	1	a/a	88	1	1.1	positive
10	2	lg	5	1	3	1	a/a	225	1	0.4	---
11	1	gl	19	12	6	1	a/a	59	1	1.7	negative
12	1	gl	10	4	4	2	a/a	63	1	1.6	negative
							a/a	220	2	0.9	"
13	1	gl	27	10	15	2	A/a	211	27	11.3	---
							A/a	138	29	17.4	---
14	2	gl	5	1	2	2	A/a	224	2	0.9	---
							A/a	230	1	0.4	---
15	1	g	23	7	11	5	a/a	69	1	1.4	positive
							a/a	447	2	0.4	"
							a/a	307	7	2.2	"
							a/a	103	43	29.5	"
							A/a	93	14	13.1	"
16	1	g	20	6	12	2	a/a	34	1	2.9	---
							A/a	191	2	1.0	---
17	1	g	17	9	7	1	a/a	69	12	14.8	positive
18	1	g	11	6	3	2	A/a	148	21	12.4	---
							A/a	99	15	13.2	---
19	1	g	19	7	11	1	A/a	181	22	10.8	---
20	1	g	25	17	7	1	a/a	97	1	1.0	---
21	1	g	22	12	9	1	a/a	65	1	1.5	---
22	1	g	11	3	7	1	A/a	191	3	1.5	---
23	1	g	11	6	4	1	A/a	146	10	6.4	---
24	2	g	9	4	4	1	A/a	264	2	0.8	---
25	2	g	7	4	2	1	A/a	240	5	2.0	---
26	2	g	4	1	2	1	A/a	230	2	0.9	---
27	2	g	5	1	3	1	A/a	292	2	0.7	---

*A and a refer to any of the six genes.

Table 6

Locus	Cross	Genotypes found No. of ears			
		A/A	A/a	a/a	Aberrant
su	#1 X MT	276	257	0	0
	#1 X Kys/MT	155	128	0	0
	#2 X Kys/MT	172	181	0	0
y	#1 X MT	236	296	0	1 (1)*
	#1 X Kys/MT	137	146	0	0
	#2 X Kys/MT	180	170	0	3 (3)
wx	#1 X MT	263	270	0	0
	#1 X Kys/MT	141	141	0	0
	#2 X Kys/MT	164	188	0	0
lg	#1 X MT	282	245	1	5 (5)
	#1 X Kys/MT	133	150	0	0
	#2 X Kys/MT	167	180	0	1 (1)
gl	#1 X MT	242	283	1	3 (2)
	#1 X Kys/MT	134	147	0	2 (1)
	#2 X Kys/MT	168	178	0	2 (1)
g	#1 X MT	265	260	0	8
	#1 X Kys/MT	141	133	0	9 (9)
	#2 X Kys/MT	173	171	0	4 (4)
					36 27

*Figures in parentheses indicate number of independent cases.

There were 27 independent cases of aberrant ratios possibly indicating the presence of a duplication. In six of these cases there was more than one aberrant ratio found in the progeny of an X_1 ear. The presumptive duplication sectors on the X_1 ears seem to be very small. An aberrant ratio in itself is not proof of the existence of a duplication as there are other possibilities, such as the loss of the chromosome segment with the dominant marker which results in a plant hemizygous for the locus--allowing the recessive to be expressed. Another possibility is induction of a gametic lethal or sub-lethal which is linked to the recessive gene and which lowers the transmission frequency of the recessive. The only sure criterion is to restore the original situation ($\underline{A-a/A}$) by crossing the presumptive duplication with the dominant gene

marker and to see if there is a reversion to a when it is backcrossed to the recessive. This has been done with eight of these cases; in five cases the original situation was restored and there were aberrant ratios. In three cases the results were negative. One problem is that the original cross yielded A and A-a gametes in about equal frequencies. These two types cannot be readily distinguished. The duplication shows a lowered transmission rate through the pollen (in most cases around 40%), but this is not adequate to accurately distinguish between them. This difficulty can be surmounted by taking a larger sample so we can be sure the A-a chromosome is represented, or by making the original testcross with the heterozygote (A/a) so that the A-a/a types are formed immediately and there is no chance for the duplication to be lost.

Data on the reconstitution tests will be presented when they are completed.

The expected frequency of tandem duplications for any one gene in this material is $1/36$ or $1/4(n-1)$ times the frequency of translocations between non-homologous chromosomes (observed value 15.4%) times $1/20$ (since there are 20 arms in maize) times $1/2$ (the maximum probability that a gene will have one break proximal and one break distal to it) times total number of ears. This works out to 0.12 per gene in this experiment. Since 6 genes were followed the total frequency should be 0.72.

This calculation is based on the premise that the chromosomes are arranged at random in the interphase nucleus. Since the observed value of 36 is considerably greater (even if some of the cases are spurious), the data may be taken as evidence that there is predilection for translocations between the same arms of two homologous chromosomes probably as a result of a semi-paired condition in the interphase nucleus.

Another method for detecting translocations between homologous chromosomes is to irradiate diploid material which is marked with two very closely linked genes in the repulsion phase. This is the case with A sh/a Sh. A duplication will have the phenotype of A Sh. Genetic tests can be used to separate these kernels from A Sh kernels arising from crossing over. The results of this experiment are given in Table 7 and Table 8.

Table 7

Treatment	No. of ears	Total gametes	No. of gametes				Percent	
			<u>A sh</u>	<u>a Sh</u>	<u>A Sh</u>	<u>a sh</u>	<u>A Sh</u>	<u>a sh</u>
Control	77	25,059	12,241	12,777	20	21	0.08	0.08
5000 r	79	21,926	10,745	11,105	60	16	0.27	0.07
10000 r	71	17,774	8,656	9,065	37	16	0.21	0.09

Table 8

Treatment	Number of ears with n number of <u>A Sh</u> kernels												Total
	n	0	1	2	3	4	5	6	7	8	9	10	
Control		58	18	1	0	0	0	0	0	0	0	0	77
5000 r		49	21	3	3	1	0	0	0	0	0	2	79
10000 r		50	12	5	2	1	1	0	0	0	0	0	71

The excess of kernels with the A Sh phenotype in the irradiated material is highly significant. It cannot be explained by an enhancement of crossing over by irradiation since the reciprocal class remains the same. The corresponding class (the deficient chromosomes) is presumed to be lethal.

Each kernel corresponds to one X_2 plant in the previous experiment. It is a much more efficient way to duplicate endosperm characters. It should be noted that the first method did not uncover any duplications for su or wx. One explanation for this is that these two genes are linked very closely to the centromere. Since the reversion to the recessive phenotype depends on a crossover proximal to the locus any duplications involving su or wx would be difficult to detect. The lg, gl, and g loci have more proximal crossovers since the map distance between these loci and the centromere is large. The y locus is fairly close to the centromere and is also difficult.

Where two closely linked gene loci are not available, an artificial set-up can be used with the aid of translocations. For example, a

homozygous 6-9 translocation with the constitution of $\underline{Y} - T - \underline{wx} / \underline{y} - T - \underline{Wx}$ can be irradiated and kernels with the phenotype $\underline{Y} \underline{Wx}$ can be isolated. The duplication can then be introduced into a normal background by crossing over.

G. G. Doyle

NATIONAL COLONIAL FARM
Accokeek, Maryland

1. Mutations from seed irradiation of B14 maize inbred.

The seed irradiation study, proposed in 1965, has proved to be rather effective in mutation induction. In 1967, seed of B14 was treated with thermal neutrons at the Brookhaven National Laboratory through the courtesy of Robert W. Briggs, whose cooperation is much appreciated. After treatment the seed was planted in an isolated field and allowed to interpollinate. In this way every mutant gamete, even in a small sector of the ear or tassel had the opportunity to participate in fertilization. No self pollinations were made in this field. At harvest time the ears were shelled together. In 1968 a small field was grown and more than 1000 hand pollinations made. After harvest a small sample of seeds was taken from each ear and planted in a seedling bench in the greenhouse.

The results are now complete. Of 1074 seedling rows in the greenhouse, 49 or 4.6% were segregating for some seedling character. These included albinos, luteus, yellow green, virescent and one dwarf. Most, by far, showed segregation for albinos. Of the 49 progenies, 39 were segregating albinos, 6 luteus, 2 virescent, 1 variegated, and the one dwarf previously mentioned. Also many mutations for defective and germless seed were observed. These need further testing to determine how many are true mutations. Also a number of ears presumably heterozygous for translocations were found. Further testing of these is necessary. Limited quantities of seed of stocks heterozygous for the various mutants are available.

2. Old varieties of corn wanted.

The Colonial Farm is interested in antique varieties of corn. Last year Hastings Prolific was obtained from the originator, the Hastings Seed Co., in Atlanta, Georgia. This old variety was obtained from the Indians in the 19th century and has been maintained since by the Hastings Co. Does anyone know of similar old varieties?

3. National Colonial Farm now open.

The National Colonial Farm will be open to the public in the summer of 1969. Besides crop exhibits there will be livestock, characteristic of the colonial period around 1750. There will be Devon Cattle, Quarter Horses, Dartmoor Sheep, as well as hogs and poultry.

W. Ralph Singleton

NORTH CAROLINA STATE UNIVERSITY
Raleigh, North Carolina
Department of Experimental Statistics
and
INSTITUTO DE GENÉTICA, E.S.A.L.Q.
Piracicaba, S.P., Brazil

1. Choice of characters for racial classification.

Analyses of variance for 111 characters from 55 races and sub-races of maize from eastern South America grown at Piracicaba, S.P., Brazil, between 1960 and 1965, indicated that those characters which were least affected by environmental factors and interactions were reproductive characters. In particular, the component of variance due to differences among races for certain ear and kernel characters was much greater than the sum of corresponding components due to differences among years and race by year interactions. The converse was true for all vegetative characters. Tassel characters tended to be intermediate between ear and plant characters.

While some indices had larger components of variance attributable to racial differences than to the effects of environment and/or environmental interaction, some commonly used ones, such as cob/rachis and

rachilla/kernel indices, proved to be quite susceptible to environmental influences. Again, indices based upon solely vegetative characters were consistently influenced more strongly by environmental factors and interaction than were those based on reproductive characters.

Major M. Goodman

OSMANIA UNIVERSITY
Hyderabad-7, India
Department of Genetics

1. Induction of chlorophyll sectors by DES.

Homozygous opaque-2 seeds were treated with various concentrations of DES ranging from 0.0025 M to 0.01M for 8 hrs. by replenishing the solution every hour. Among the 559 surviving plants, 176 were found to have three types of chlorophyll sectors, i.e., yellow, albino and yellow-green. The sectors ranged from 0.1 to 1.5 cm in width and from 1/3 to the whole length of the leaf.

Yellow sectors were more frequent on the 6th, 7th and 8th leaves and albino sectors were more frequent on the 6th and 7th leaves whereas yellow-green sectors were more frequent on the 5th and 7th leaves.

The three types of chlorophyll sectors were examined histologically; the yellow and albino sectors were found to have no chloroplasts, but the yellow sectors retained a yellow pigment in the bundle sheath. The yellow-green sectors showed chloroplasts only in the upper epidermis.

In the treated material it was observed that four plants had one leaf without a mid-rib and two plants had a terminal modified "thread like" leaf.

V. S. Bharathi
G. M. Reddy

2. Some pigment studies in different genotypes of maize.

The husk and aleurone tissue of cherry (r^{ch}) and purple (Pr) and husk tissue of B have been analyzed chromatographically and spectrophotometrically.

The comparison of Rf values and absorption maxima of various

alcoholic tissue extracts with pure sample of cyanidin monoglucoside, aglycone and pelargonidin chloride has shown that all these genotypes have only one type of pigment, i.e., cyanidin monoglucoside. The cherry (r^{ch}) husk tissue extract gave two distinct Rf values, 0.23 and 0.33; however, both have the same absorption maxima (528 mμ).

Since all the three genotypes contain the same pigment, it appears that these genes might play a quantitative role by regulating the availability of the precursor(s) in anthocyanin production.

K. Vaidyanath
G. M. Reddy

3. Opaque-2 gene incorporation studies.

Several inbred and elite lines were selected for the incorporation of the opaque-2 gene to develop a nutritionally superior variety as reported earlier (MNL 1968). This opaque-2 gene was recovered in various backgrounds and it is interesting to note that the seed from inbred line (Eto-25A-F) showed 5% more weight than the original opaque-2 seed (W64 A) from Dr. Nelson.

S. Annapurna
G. M. Reddy

4. Trifurcated leaves in opaque-2 maize.

Among 559 surviving plants, treated with DES, 30 plants were found with one, and eight plants with more than one "trifurcated" leaf blades. The affected leaf terminal was cut into three, each with a distinct outline, the midrib being present in the middle one. Such trifurcated leaves were also found among the controls, their frequency being 30 out of 540 plants examined. These trifurcations were more frequent on the 9th and 10th leaves and the pattern was quite uniform in all the observations.

V. S. Bharathi
G. M. Reddy

THE PENNSYLVANIA STATE UNIVERSITY
University Park, Pennsylvania
Departments of Agronomy and Plant Pathology

1. Inheritance of resistance to strain A and B of maize dwarf mosaic virus.

In Maize Genetics Coop News Letter 42, pp. 149-150, 1968, data were presented in support of the dominant single gene hypothesis for resistance to MDMV strain A in the maize inbreds Pa 11 and Pa 405. In 1968 field inoculated tests of the F_2 of the crosses Pa 11 x W153R and Pa 11 x Pa 54 segregated in a ratio of 3 resistant to 1 susceptible and the backcross to the susceptible parents W153R and Pa 54 respectively, segregated in a 1:1 ratio (Table 1). The F_2 and backcross population of

Table 1

Number of resistant (R) and susceptible (S) seedlings in F_2 and backcross populations from resistant and susceptible crosses when inoculated with MDMV strain A or B

Crosses	MDMV Strain A				MDMV Strain B			
	R	S	χ^2	P	R	S	χ^2	P
(Pa 11 x Pa 54) (X)	234	78	0	100%				
(Pa 11 x Pa 54) Pa 54	163	135	2.61	20-10				
(Pa 11 x W153R) (X)	573	167	2.33	20-10				
(Pa 11 x W153R) W153R	155	141	0.66	50-30				
(Pa 32 x W153R) (X)	270	91	0.01	95-90				
(Pa 32 x W153R) W153R	212	208	0.04	90-80				
(Pa 32 x Pa 33) (X)	199	73	0.49	50-30				
(Pa 32 x Pa 33) Pa 33	131	112	1.5	30-20				
(Pa 32 x Pa 881P) (X)					66	105	0.86	50-30
(WF9 x Pa 32) (X)					39	80	4.15	5-2
(Pa 54 x Pa 32) (X)					53	83	0.48	50-30
(Pa 422P x Pa 881P) (X)					120	132	3.19	10-5
(Pa 422P x Pa 881P) Pa 881P					54	261	4.7	5-2

the resistant inbred Pa 32 crossed with the susceptible parents W153R and Pa 33 also clearly segregated in a ratio of 3 resistant to 1 susceptible and 1:1, respectively. These data strongly support the hypothesis for a monogenic dominant control of resistance to strain A in the maize inbred lines Pa 11, Pa 405, and Pa 32. Additional studies are being conducted with the resistant inbred lines Oh 7B, Pa 422P and Pa 884P. At present the exact nature of gene control for these is not clear.

Recent data support the hypothesis of 3 genes with complementary action controlling resistance to strain B in the resistant inbreds Pa 32 and Pa 422P. The data (Table 1) from F_2 population of Pa 32 with susceptible lines Pa 881P, WF9 and Pa 54 indicate a good fit for a ratio of 27 resistant to 37 susceptible. Another inbred, Pa 422P, also appears to have 3 genes with complementary action for resistance to strain B.

Continued tests are underway to determine: 1. the complexity of inheritance to the control of resistance to maize dwarf mosaic virus strain A and strain B and 2. the relationship, if any, between these two genetic systems controlling resistance to MDMV in a number of resistant maize inbreds.

Gordon L. Scheifele
Department of Plant
Pathology

M. W. Johnson
Department of Agronomy

THE PENNSYLVANIA STATE UNIVERSITY
University Park, Pennsylvania
Department of Horticulture

1. Genetic control of phytoglycogen accumulation.

Investigations on the control of phytoglycogen accumulation in maize endosperm by the genes ae and su₁ were accomplished by quantitating the phytoglycogen from the 16 genotypes resulting from all possible crosses between normal, ae, su₁ and ae su₁. Background effects were minimized by having normal and the mutants in the BC-2 generation of the inbred W64A. The phytoglycogen was extracted in $HgCl_2$ from 20 day old kernels and was quantitated by using glucoamylase from Aspergillus niger

to hydrolyze the polysaccharide to glucose. The glucose was then measured as a reducing sugar. The results of different dosage levels of ae with homozygous su₁ are given below:

Genotype	Phytoglycogen in mg/g dry wt. \pm std. dev.	Absorbancy Maxima (μ)	β -amylolysis limit (%)
+ + + <u>su</u> ₁ <u>su</u> ₁ <u>su</u> ₁	387.6 \pm 7.3	475	40.9
+ + <u>ae</u> <u>su</u> ₁ <u>su</u> ₁ <u>su</u> ₁	343.4 \pm 7.1	475	40.2
+ <u>ae</u> <u>ae</u> <u>su</u> ₁ <u>su</u> ₁ <u>su</u> ₁	232.9 \pm 13.6	475	41.9
<u>ae</u> <u>ae</u> <u>ae</u> <u>su</u> ₁ <u>su</u> ₁ <u>su</u> ₁	42.0 \pm 8.0	475	45.7

Only the endosperms homozygous for su₁ contained phytoglycogen. Increasing doses of ae decreased the amounts of phytoglycogen. The double mutant (ae ae ae su₁ su₁ su₁) contained phytoglycogen in contrast to an earlier report from this laboratory (Black *et al.* 1966, Genetics 53:661-668); however, they were using a different and more heterogeneous genetic background.

Absorbancy maxima in an iodine-potassium iodide and saturated calcium chloride solution indicated the phytoglycogens from each of the genotypes were identical. However, the β -amylolysis limit of the double mutant was higher than the others, suggesting that it may be a more loosely branched phytoglycogen.

Studies are in progress to analyze the starches from these genotypes with regard to the ratio of amylose and amylopectin and the structure of the amylopectin. Studies are planned to survey the genotypes for branching and debranching enzymes.

John E. Ayers
Roy G. Creech

2. Phenotypic dosage effects exhibited by Ae in combination with wx.

It has been observed in this laboratory that Ae exhibits a dosage effect which reflects the genotype of the endosperm. Ae Ae Ae wx wx wx and Ae Ae ae wx wx wx endosperms are full and waxy in phenotype, the two genotypes being indistinguishable. However, Ae ae ae wx wx wx endosperms are tarnished waxy and appear to be smaller in size. The phenotype of

Table 1
Endosperm classes of F₂ families segregating for ae, ae^{B1}, ae^{B3} and ae^{il},
respectively, with homozygous wx

Family no.	<u>Ae</u> alleles in heterozygote ^a (all <u>wx wx</u>)	Total no. kernels	Endosperm classification (all <u>wx wx wx</u>)			χ^2 (2:1:1)	Probability %	χ^2 (1:1) ^b	Probability %
			1. $\frac{\underline{Ae} \ \underline{Ae} \ \underline{Ae}}{\underline{Ae} \ \underline{Ae} \ \underline{ae}}$ (waxy)	2. $\frac{\underline{Ae} \ \underline{ae} \ \underline{ae}}{(\text{tarnished, waxy})}$	3. $\frac{\underline{ae} \ \underline{ae} \ \underline{ae}}{(\text{glassy, wrinkled})}$				
1	$\frac{\underline{Ae} \ \underline{ae}}{\underline{Ae} \ \underline{ae}^{B1}}$	334	176	84	74	1.57	45.7	1.21	28.5
2	$\frac{\underline{Ae} \ \underline{ae}^{B1}}{\underline{Ae} \ \underline{ae}^{B3}}$	376	195	88	93	.65	72.1	.53	47.8
3	$\frac{\underline{Ae} \ \underline{ae}^{B3}}{\underline{Ae} \ \underline{ae}^{il}}$	304	157	76	71	.49	78.2	.33	57.4
4	"	284	142	62	80	2.28	32.0	.004	95.5
5	"	338	153	90	95	3.18	20.5	3.04	10.4
6	"	396	208	99	89	1.52	46.9	1.02	32.7
7	$\frac{\underline{Ae} \ \underline{ae}^{il}}{\underline{Ae} \ \underline{ae}^{B1}}$	304	157	76	71	.49	78.2	.33	57.4
8	"	383	194	93	96	.11	94.5	.07	79.9
9	"	363	187	102	74	4.65	9.9	.34	57.2
10	"	388	183	112	93	3.11	21.3	1.25	27.7
11	"	363	172	105	86	2.98	22.6	1.00	33.0
12	"	254	120	82	52	7.86	2.2	.78	38.9
13	"	158	75	46	37	1.43	48.9	.41	53.2
14	"	453	226	88	139	11.49	.6	.004	94.9
15	"	528	300	108	120	10.36	.8	9.84	3.0
16	"	356	167	99	90	1.81	40.5	1.37	25.8
17	"	307	142	93	72	4.60	10.3	1.73	20.5
18	"	179	89	49	41	.72	69.8	.01	91.8
19	"	310	156	86	68	2.10	35.0	.02	90.2
20	"	287	136	81	70	1.63	44.4	.79	38.5
21	"	277	142	71	64	.53	76.7	.18	67.8
22	"	300	138	87	75	2.88	23.8	1.93	18.3
23	"	239	123	61	55	.51	77.7	.21	65.5
24	"	402	194	113	95	2.10	35.1	.49	49.2
25	"	51	22	16	13	1.31	51.9	1.00	33.0
26	"	96	51	23	22	.40	82.1	.39	54.4

Table 1 (Continued)

Family no.	<u>Ae</u> alleles in heterozygote ^a (all <u>wx wx</u>)	Total no. kernels	Endosperm classification (all <u>wx wx wx</u>)			χ^2 (2:1:1)	Probability %	χ^2 (1:1) ^b	Probability %
			1. <u>Ae Ae Ae</u> <u>Ae Ae ae</u> (waxy)	2. <u>Ae ae ae</u> (tarnished, waxy)	3. <u>ae ae ae</u> (glassy, wrinkled)				
27	<u>Ae ae</u> ^{il}	292	151	75	66	.90	63.9	.35	56.6
28	"	357	175	103	79	3.36	18.7	.14	71.5
29	"	389	179	99	111	3.21	20.2	2.48	13.6
30	"	248	105	88	55	14.60	.4	5.82	2.0
31	"	282	130	80	72	2.17	33.9	1.72	20.0
32	"	369	190	111	68	10.35	.8	.33	57.4
33	"	176	91	39	46	.76	68.4	.20	65.5
10,143			5026	2685	2432				
			Tests of heterogeneity:			df			
			Total			66	106.13	39.32	25.0
			Pooled			2	13.44	.5	.60
			Heterogeneity			64	92.69	1.5	38.72
									20.0
									32

^a ae (amylose-extender); ae^{B1} (amylose-extender, Bear 1); ae^{B3} (amylose-extender, Bear 3);
ae^{il} (amylose-extender, induced 1)

^b Ratio of the value of class 1: value of class 2 + class 3

Table 2
Endosperm classes of testcross families segregating for \underline{ae}^{B1} and \underline{ae}^{B3} ,
respectively, with homozygous \underline{wx}

Family no.	Ae alleles in heterozygote (all $\underline{wx} \underline{wx}$)		Total no. kernels	Endosperm classification			χ^2 (1:1)	Probability %
	♀	♂		$\frac{\underline{Ae}}{\underline{wx}} \frac{\underline{Ae}}{\underline{wx}} \frac{\underline{ae}}{\underline{wx}}$	$\frac{\underline{Ae}}{\underline{wx}} \frac{\underline{ae}}{\underline{wx}} \frac{\underline{ae}}{\underline{wx}}$	$\frac{\underline{ae}}{\underline{wx}} \frac{\underline{ae}}{\underline{wx}} \frac{\underline{ae}}{\underline{wx}}$		
1	$\underline{Ae} \underline{ae}^{B3}$	$\underline{ae} \underline{ae}$	419	202	---	217	.54	47.2
2 ^a	$\underline{Ae} \underline{ae}^{B1}$	$\underline{ae} \underline{ae}$	255	121	---	134	.67	42.4
3 ^a	$\underline{ae} \underline{ae}$	$\underline{Ae} \underline{ae}^{B1}$	362	---	220	142	16.86	< .1
4	$\underline{ae} \underline{ae}$	$\underline{Ae} \underline{ae}^{B3}$	211	---	125	86	7.25	3.2
5	"	"	202	---	112	90	2.41	14.0
6	"	"	279	---	176	103	19.17	< .1
7	"	"	198	---	125	73	13.73	.1
8	$\underline{ae}^{i1} \underline{ae}^{i1}$	"	347	---	207	140	16.86	< .1

^aReciprocal cross - same heterozygote used as female and male, respectively.

ae ae ae wx wx wx endosperms is glassy and wrinkled or partially shrunken.

The purpose of this report is to present the evidence for the dosage effect of Ae with 4 different alleles in a wx background and to present evidence for the lower transmission frequencies of amylose-extender alleles through the male gametophyte. The 4 alleles of Ae as designated by this laboratory group are ae (standard amylose-extender), ae^{B1} (amylose-extender, Bear 1), ae^{B3} (amylose-extender, Bear 3), and ae^{il} (amylose-extender, induced 1).

Kernels from 33 F₂ families that were segregating for Ae and homozygous wx were classified for the 3 phenotypic classes. Each family was tested by the χ^2 test for goodness of fit to a 2:1:1 ratio. A second χ^2 analysis (1:1) was performed by pooling classes 2 and 3. The results of these analyses are shown in Table 1.

All the observed phenotypic ratios fit the expected 2:1:1 ratio except those for 5 families (12, 14, 15, 30 and 32, respectively). However, when classes 2 and 3 were pooled and tested with class 1 for goodness of fit to a 1:1 ratio, only two of these families (15 and 30) failed to fit the expected ratio at the 5% level of significance. In general there appeared to be deficiencies in transmission of ae and ae^{il} through the pollen. These deficiencies are the probable causes for families 12, 30 and 32 not fitting the expected 2:1:1 ratio. However, families 14 and 15 appeared to have an excess of individuals homozygous for ae^{il}. The reason is not known but one possibility may be the gametophyte factor on chromosome 5 that is linked with ae.

The testcross data are shown in Table 2. Five of 8 families did not fit the expected 1:1 ratio, apparently because of deficiencies in transmission of ae^{B1} and ae^{B3} through the pollen. The results with families 1 and 2 indicate that ae^{B1} and ae^{B3} are transmitted in frequencies approximately equal to Ae in the female gametophyte. Families 2 and 3 are reciprocal crosses with the same parents. Family 3 has a lower frequency of ae^{B1} ae ae wx wx wx kernels than family 2, indicating a lower transmission of ae^{B1} than Ae through the pollen but not through the egg.

Carol W. Moore
Roy G. Creech

PURDUE UNIVERSITY
Lafayette, Indiana
Department of Botany and Plant Pathology

1. Recombination between alleles at the sh_2 locus.

In addition to the wx locus, there is also evidence for recombination between mutations at the su_1 and gl_1 loci (Salamini). It seems apparent that this is general. We have been interested in the sh_2 locus because it is implicated in the production of the enzyme adenosine diphosphate glucose pyrophosphorylase.

It has been found both at the Coop and here that the defective seed stock bt^{60-156} is allelic to sh_2 (MNL 41:207). We have used the F_1 stock from the allelism test to test for recombination. The F_1 seed was planted in 1967, and the plants pollinated by a stock that was wx^C/wx^C ; sh_2/sh_2 . A total of 44491 kernels were produced, and 17 kernels were normal in phenotype.

The plants from these kernels were grown in the 1968 greenhouse, and the pollen checked to ascertain if the plants were Wx/wx as would be expected if the seeds arose from fertilization by the wx^C/wx^C ; sh_2/sh_2 stock. Of the 17 plants, 10 were Wx/wx ; 3 were Wx/Wx indicating contamination; and 4 plants did not produce tassel samples that could be checked. This would indicate a recombination rate between sh_2 and bt^{60-156} of 22×10^{-5} on the female side.

Oliver Nelson

2. A lethal ovule factor linked to wx .

In 1965, a plant of the genotype Wx/wx^{B1} ; Sh_2/sh_2 when self-pollinated produced 228 kernels of which 172 (75.4 percent) were wx/wx . The ear was semi-sterile.

In 1966, when the non-waxy kernels were planted and the plants selfed, 9 plants gave high percentages of waxy kernels (ranging from 53.0 to 75.5 percent); 7 plants had percentages varying about 25 percent; one plant was Wx/wx . Six plants were crossed as females times a wx/wx stock; 4 gave high percentages of waxy kernels (91.1-95.3 percent); 2 had approximately 50 percent waxy kernels. All plants with aberrant percentages of waxy kernels were semisterile. The backcross results indicate a

lethal ovule (lo_x) factor linked to Wx (ca. 6 percent recombination), but this could not explain the high percentages (up to 75 percent) of waxy kernels in selfed Wx lo_x/wx Lo plants unless the factor also affects the competitive effectiveness of pollen grains carrying lo_x.

In 1967, normal (non-waxy) kernels from a plant - 34187-8 (X) - that had given 75.2 percent waxy kernels in 1966 were planted. The plants were crossed as females times wx/wx and as males onto wx/wx. The results are given in Table 1. Clearly, the competitive effectiveness of Wx pollen from Wx lo_x/wx Lo plants is reduced. The mean percentage of waxy kernels in the 7 plants with high percentages of waxy kernels when crossed by wx/wx was 93.6. If no lo gametes are capable of functioning, this would indicate recombination approximating 6 percent between wx and lo.

Table 1

The results of crossing plants from non-waxy kernels from (Wx lo_x/wx Lo) (X) by and onto a wx lo_x/wx lo stock

Plant	Percent waxy kernels	
	x <u>wx/wx</u>	onto <u>wx/wx</u>
39141-1	49.7	45.6
" -2	94.3	82.8
" -4	52.1	51.8
" -6	88.6	78.4
" -7	93.2	73.2
" -8	93.9	63.5
" -9	93.0	70.3
" -10	95.4	79.4
" -11	96.8	71.4

In 1968, non-waxy seeds of the crosses of 39141-2, 39141-6, 39141-7 times wx/wx and onto wx/wx were planted. The progenies of the three plants by wx/wx were selfed. In a total of 19 plants, 17 had percentages of waxy seeds distributed around 25 percent. Two plants had high waxy percentages (54.8 and 51.2). The results indicate that the functional megaspores carrying Wx usually result from recombination between Wx and

lo. Apparently, however, megaspores carrying lo are rarely functional as shown by the two plants with high percentages of waxy kernels. The frequency of functional lo megaspores is not high enough to invalidate the conclusion of 6 percent recombination between lo and wx.

The plants from the crosses of 39141-2, -6, and -7 onto wx/wx were selfed and crossed onto a c sh wx gl₁₅/c sh wx gl₁₅ stock. The results are given in Table 2. The percentages of waxy kernels in both (X)'s and crosses are somewhat lower than previously noted indicating that the proportion of lo pollen grains effecting fertilization is probably affected by the genotype of the plants producing them.

Table 2

The results of selfing and crossing onto wx/wx plants of plants from non-waxy kernels of wx lo/wx lo x wx lo/wx lo

Row	Percent <u>waxy</u> kernels	
	(X)	onto <u>wx/wx</u>
41146-2 (<u>wx/wx</u> x 39141-2)	56.4	55.7
-3	48.4	55.9
-4	62.2	58.6
-5	56.9	
-6	63.4	64.9
-8	24.6	46.3
-10	57.1	57.6
-11	29.1	
41147-1 (<u>wx/wx</u> x 39141-6)	69.3	61.6
-3	61.7	60.4
-4	51.5	57.0
-5	44.0	54.3
-7	53.1	58.8
-9	58.9	65.5
-10	100	100
41148-1 (<u>wx/wx</u> x 39141-7)	51.1	61.6
-3	53.4	57.8
-5	60.5	61.9
-6	40.5	
-7	51.8	56.1
-10	100	100
-11	100	100
-12	28.9	53.7

No indication is yet available as to whether lo_x is proximal or distal to wx.

Joseph Van Horn
Oliver Nelson

UNIVERSITY OF RHODE ISLAND
Kingston, Rhode Island

1. Further studies of x-ray-induced mutations at the Sh and Bz loci.

Preliminary data were presented in the 1965 Maize News Letter on the nature of x-ray-induced mutations at the bronze locus. One mutant in particular (bz-x₁), which appeared originally as a possible intragenic change, was studied extensively. Since then, three additional alterations involving bronze which were suspected of being other than gross changes have been subjected to genetic and cytological tests. This report includes data collected on all four mutants.

Pollen from plants homozygous for the Sh, Bz and Wx alleles was x-rayed and applied to silks of sh bz wx tester stocks. (For a complete presentation of the materials and methods employed in these experiments, refer to the 1965 MNL, p. 98.) Three classes of bz mutants were identified--sh bz and Sh bz wx types, showing loss of two dominant markers, and Sh bz Wx mutants, exhibiting loss of Bz only. Table 1 lists the mutants observed in the endosperm and embryo. Of the endosperm mutants, only those of Sh bz phenotype were tested for Wx versus wx. Bronze mutants identified at the seedling stage were scored for Sh and Wx by progeny tests. Putative sh bz embryo mutants may include plants of Sh bz*¹/sh bz constitution in which the Sh bz* gametophytes were non-functional, as well as the expected - -/sh bz class in which both dominant markers have been lost. Simultaneous mutations of Sh and Bz are nearly twice as frequent in the endosperm as in the embryo. Either the viability of the double mutants is lower in the embryo than in the endosperm or selective fertilization occurs in which the sperm nucleus containing the normal chromosome 9 preferentially fertilizes the egg nucleus. Many F₁ seeds

¹The symbol bz* refers to any change at the Bz locus resulting in the bz phenotype.

Table 1

Mutants obtained in crosses of sh bz wx ♀♀ x Sh Bz Wx ♂♂
following irradiation of the male parent

Experiment #	Population	<u>sh bz</u> mutants			<u>Sh bz Wx</u> mutants			<u>Sh bz wx</u> mutants			<u>bz</u> embryo mutants not surviving to maturity	<u>Bz-bz</u> mosaics in endo-sperm
		Endo-sperm	Embryo and endo-sperm	Embryo	Endo-sperm	Embryo and endo-sperm	Embryo	Endo-sperm	Embryo and endo-sperm	Embryo		
1	66,337	601	7	178	2	0	1	0	0	0	160	272
2	66,620	790	42	235	7	0	1	10	0	1	212	166
Total	132,957	1391*	49*	413*	9	0	2	10	0	1	372	438

*The mutants in these classes may range from deletions of the entire chromosome 9 to deficiencies which include only the Bz locus. If functional gametes with the mutant chromosome are not produced, only the sh bz phenotype will be expressed in the testcross progeny.

germinated but did not survive to the stage at which the bronze phenotype could be observed and a large proportion of the lethal seedlings may have been double mutants.

Of the 837 bronze mutants obtained in the embryo, the two exhibiting the Sh bz Wx phenotype were saved for further analysis. These are designated bz-x₁ and bz-x₂. In addition, two sh bz mutants (sh-bz-x₁ and sh-bz-x₂) which had little aborted pollen were maintained. All of the remaining embryo mutants exhibited a high frequency of pollen abortion and, when backcrossed to sh bz wx tester plants, proved to be double mutants of sh* bz* / sh bz or bz* wx* / bz wx constitution. It was assumed that these plants were heterozygous for a chromosomal aberration affecting the short arm of chromosome 9 and that no gene mutation had been induced. Such mutants were not analyzed further. No plants mosaic for Bz and bz sectors were observed in the population of embryo mutants. Such individuals could arise if only a single DNA strand of the bronze gene had been mutated in the sperm nucleus.

A description of the original bz-x₁ heterozygote was presented in the 1965 News Letter. The F₁ plant heterozygous for the induced bz-x₂ mutation was normal in stature. It bore an ear which, when testcrossed, had slightly reduced seed set. The progeny consisted of 78 Sh bz Wx, 17 Sh bz wx, 45 sh bz Wx and 132 sh bz wx kernels. Aborted pollen was lower in frequency than in the majority of the bronze embryo mutants. (Pollen analyses of the original bz-x and sh-bz-x plants were made with a hand microscope on unstained pollen and exact frequencies of abortion were not obtained.)

The F₁ heterozygote carrying sh-bz-x₁ was normal in stature and gave rise to an ear with a low amount of ovule abortion. Testcross progeny of this sh-bz-x₁ Wx/sh bz wx individual included 102 sh bz Wx and 120 sh bz wx kernels. A low amount of aborted pollen was present but the exact percentage was not ascertained.

The original sh-bz-x₂ heterozygote appeared to be completely normal. The ear produced 177 sh bz Wx and 153 sh bz wx kernels when backcrossed to a sh bz wx tester. Seed set was normal and the aborted pollen frequency appeared no greater than that of normal sibs. Except for the shrunken-bronze phenotype, neither the plant nor the ear could be distinguished from normal sibs.

In backcross progenies of all four mutant heterozygotes, the dominant contamination markers from the treated pollen parent were present: hence, the original mutant plants possessed a genome derived from an irradiated sperm and did not arise by fertilization with foreign pollen.

Pollen from individuals heterozygous for each of the mutants and a normal chromosome 9 was stained with IKI and frequencies of aborted grains and grains of sub-normal size were obtained. Control values were obtained from plants of similar background containing two normal chromosomes 9. The results are presented in columns 2 and 3 of Table 2. The frequencies observed in bz-x₁, bz-x₂ and sh-bz-x₁ heterozygotes indicate that the alterations in each case affected gene loci controlling pollen development in addition to affecting Bz and Sh. The 10.7% abortion of sh-bz-x₂ is higher than that observed in control plants; however, the wx:Wx ratio of the normal grains from sh-bz-x₂ Wx/sh bz wx plants was close to unity (1:0.98). If the mutant region was influencing pollen development, Wx grains would be observed less frequently than wx grains. Hence, the greater abortion frequency cannot be ascribed to the presence of sh-bz-x₂.

Transmission of the mutants through the male and female gametophytes was tested in crosses of bz-x/Bz and sh-bz-x/Sh Bz plants with tester stocks. Frequencies were determined by dividing the amount of bz or sh bz kernels by the number of Bz or Sh Bz progeny, respectively, in the following generation. Columns 4 and 5 of Table 2 list the results of these crosses. The data indicate that all mutants except sh-bz-x₂ affect male gametophyte viability drastically and embryo sac development to a lesser degree. In the case of sh-bz-x₂, all plants tested showed full viability of female gametophytes carrying the alteration. Transmission of the mutant chromosome through the pollen ranged from 60% to 82.5% in four individuals but in two additional plants, the values were 109.5% and 116.0%. Tests indicated that, in these two latter cases, a viability factor on the normal homolog closely linked to wx may have caused the deficiency of Sh-Bz kernels. In the progeny from self-pollinations of four Sh Bz/sh-bz-x₂ individuals, the percentages of sh bz kernels were 25.4, 22.8, 22.0 and 21.7. Hence, the viability of pollen carrying the mutant homolog may reach 100% in some cases; however, further tests must be made

Table 2
Effects of the bz-x and sh-bz-x mutants on pollen abortion, gametophyte viability and crossing over

Plant constitution	% aborted pollen	% sub-normal pollen	% gametophyte viability		% recombination		% reduction in crossing over	
			pollen	embryo sac	<u>Sh-Bz</u>	<u>Bz-Wx</u>	<u>Sh-Bz</u>	<u>Bz-Wx</u>
<u>bz-x1</u> normal	9.7	35.2	0	32.0	0.93	16.6	59.4	10.7
<u>bz-x2</u> normal	19.5	39.3	0.001	51.5	0.86	18.6	62.4	0
<u>sh-bz-x1</u> normal	6.2-22.8	2.13	1.05	63.3	0	14.2	100	23.7
<u>sh-bz-x2</u> normal	10.7	0	60-100	100	0	19.9	100	---
<u>normal</u> normal	6.8	0	---	---	2.29	18.6	---	---

with isogenic lines in which the viability of the normal homolog is known. These tests are in progress.

The effects of bz-x and sh-bz-x mutations on crossing over in flanking regions were tested in crosses of sh Bz wx/Sh bz-x Wx and Sh Bz wx/sh-bz-x Wx heterozygotes as pollen parents with sh bz wx testers. The results of these crosses are listed in columns 6-9 of Table 2. Both bz-x₁ and bz-x₂ reduce crossing over in the Sh-Bz interval, but only the bz-x₁ alteration has any effect on the region between Bz and Wx. In heterozygotes containing the sh-bz-x alterations, no exchanges occurred in the Sh-Bz interval. The sh-bz-x₁ mutation reduces crossing over between Bz and Wx to the greatest degree of all the mutants. On the other hand, sh-bz-x₂ appears to increase slightly the frequency of exchange in this region over that found in normal sibs. The difference is small but statistically significant.

Self-pollinations of sh-bz-x/Sh Bz individuals were made to determine whether or not plants homozygous for these alterations are viable. Since these plants were heterozygous for the a₁ allele, the sh phenotype was used as an indication of homozygote viability. No self pollinations were made with the bz-x mutants since functional pollen carrying the mutant allele was either not produced or very rare. Intercrosses of sh-bz-x/Sh Bz and bz-x/Bz individuals were also performed to determine the viability of plants heterozygous for the various mutations. Results of these crosses are listed in Table 3. Progeny from self-pollinations of sh-bz-x₁/Sh Bz plants consisted of less than 1000 kernels, none of which were sh in phenotype. If homozygotes of this mutant are viable, their survival rate is too low to be detected in a population of this size. Data in Table 3 indicate that sh-bz-x₂ has little or no effect on the viability of homozygous sporophytes. In compounds of sh-bz-x₂ with the other mutations, the chromatin present in the sh-bz-x₂ homolog is sufficient to support growth of the heterozygotes to varying degrees.

Extensive cytological examinations of sporophytes heterozygous for each of the mutations and a normal homolog revealed no abnormal pairing in the short arm of chromosome 9; however, known deficiencies such as the a-x mutations reported by Stadler and Roman have no visible effect on synapsis in chromosome 3. Consequently, the cytological observations

Table 3

Tests of viability of sh-bz-X2 homozygotes and of sh-bz-X1/sh-bz-X2, bz-X1/sh-bz-X2 and bz-X2/sh-bz-X2 compounds

Cross	Plant #	Progeny		% <u>sh</u> kernels	χ^2
		<u>Sh</u>	<u>sh</u>		
<u>sh-bz-X2 Wx</u> \otimes <u>Sh Bz wx</u>	1451-1 1457-1 1467-3 1467-4	311 357 411 245	106 105 116 68	25.4 22.8 22.0 21.7	0.04 1.27 2.51 1.79
<u>sh-bz-X1 Wx</u> ♀ ♀ x <u>Sh Bz wx</u>	1486-1	239	45	15.8	
<u>sh-bz-X2 Wx</u> ♂ ♂ <u>Sh Bz wx</u>					
<u>Sh bz-X1 Wx</u> ♀ ♀ x <u>sh Bz wx</u>	1443-1 1443-2 1443-3	242 172 185	34 29 17	12.3	
<u>sh-bz-X2 Wx</u> ♂ ♂ <u>Sh Bz wx</u>				14.4	
				8.4	
<u>Sh bz-X2 Wx</u> ♀ ♀ x <u>sh Bz wx</u>	1447-1 1447-2 1448-1	210 278 265	27 45 36	11.3 13.9 11.9	
<u>sh-bz-X2 Wx</u> ♂ ♂ <u>Sh Bz wx</u>					

of the present study do not rule out deficiencies as the cause of the mutations.

The effects of bz-x₁, bz-x₂ and sh-bz-x₁ on gametophyte viability and crossing over indicate that the three mutations constitute deletions of varying size. On the other hand, since sh-bz-x₂ does not reduce crossing over in the Bz-Wx interval, and since mutant homozygotes are viable, the origin of this mutation remains open to speculation. McClintock (1956) reported four cases of simultaneous loss of Sh and Bz expression induced by Ds. It was demonstrated that three of these mutations represented deletions. The behavior of the fourth, however, was

strikingly similar to that of sh-bz-x₂. McClintock stated that crossing over within the affected segment of some mutant heterozygotes was reduced in varying degrees or completely absent. However, crossover reduction in the fourth mutant was not specifically mentioned.

If sh-bz-x₂ represents suppression of the two alleles by a controlling element, the mechanism by which this element inhibits crossing over may be similar to that of recombination genes.

John Mottinger

UNIVERSIDADE DE SÃO PAULO
Escola Superior de Agricultura "Luiz de Queiroz"
Piracicaba, Brazil
Instituto de Genetica

1. Observed and "expected" heterosis in interracial crosses of maize.

When epistasis is negligible, heterosis of an interpopulational hybrid can be expressed as a function of gene frequencies and dominance effects.

An attempt is made to compare actual heterosis values for yield in 9 interracial crosses of corn with calculated heterosis parameters. Observed heterosis (\hat{h}_{ij}) was measured as the excess in yield of a hybrid over the midpoint between its parents. Experiments conducted in three locations with three rep./location provided the yield averages. Based on knob frequencies obtained by Kato (1964) and Blumenschein (1968) and on hypothetical dominance values, "expected" heterosis parameters were computed as follows:

$$h_{ij}^* = \sum_k \left[(p_{ik} - p_{jk})^2 + 2 \cdot \bar{\Delta}_{ijk} \right] \cdot d_k$$

p_{ik} being the knob frequency [of any type of knob (large, medium or small)] at chromosome position k for population i ; $\bar{\Delta}_{ijk}$ is the average Hardy-Weinberg disequilibrium parameter for populations i and j and chromosome position k , i.e., $\bar{\Delta}_{ijk} = (1/2)(\Delta_{ik} + \Delta_{jk})$, and $\Delta_{ik} =$ the observed frequency of homozygous knobbed plants minus the corresponding expected frequency under Hardy-Weinberg equilibrium, for position k .

Table 1
Observed heterosis and calculated h_{ij}^* values

Cross		Observed heterosis (kg/10 m ²)	Calculated h_{ij}^* values
12	Chapalote x Caingang	0.696	5.142
13	Chapalote x Canario de ocho	1.575	3.132
14	Chapalote x Cristal	0.434	4.936
15	Chapalote x Cateto	1.396	4.206
23	Caingang x Canario de ocho	0.632	0.295
24	Caingang x Cristal	-0.123	1.054
25	Caingang x Cateto	0.686	1.282
34	Canario de ocho x Cristal	1.133	0.153
35	Canario de ocho x Cateto	-----	-----
45	Cristal x Cateto	0.874	0.857

Table 2
Average yield (kg/10 m²) of parental varieties and F_1 's

	CAINGANG	CRISTAL PARAGUAI	CATETO	CHAPALOTE	CANARIO DE OCHO
CAINGANG	3.903	3.656	4.304	3.573	3.590
CRISTAL PARAGUAI		3.654	4.368	3.186	3.867
CATETO			3.334	3.988	-----
CHAPALOTE				1.850	3.407
CANARIO DE OCHO					1.813

σ_k is the dominance value (as defined by Gardner and Eberhart, 1966) for the knob-knobless condition at position k . For comparison with observed heterosis, σ_k values were assumed to be equal to one for all 21 knob positions.

Knob frequencies were obtained by pachytene chromosome examination. The presence or absence of knobs at each knob forming position was recorded. If present, the size (small, medium or large) of each knob was determined and it was noted whether such knobs were present in the homozygous or the heterozygous condition.

To simplify the determination of the heterosis parameters, we considered only the presence or absence of knobs, independent of their sizes, although it is known that knob size is important for characterizing germplasms.

In a certain way, the presence or absence of knobs in these races reflects the absence of a particular knob complex, the Andean Complex. This Complex is basic for South American races of maize and is characterized by the presence of small knobs in the 6 L_3 and 7 L positions and by the absence of knobs in all other knob forming positions.

Thus, the presence of knobs in positions other than 6 L_3 and 7 L can be taken as meaning introgression of other complexes into the Andean Complex.

The results are shown in Tables 1, 2 and 3 and Figure 1. The hybrid Cateto x Canario de Ocho was not included because no data on the yield of this hybrid were available.

The results must be viewed with caution as the samples utilized for knob frequency estimation were not completely comparable to the samples utilized for yield data. In addition, the sizes of some samples were relatively small.

It seems clear, however, that the relationship between observed heterosis and population diversity, as measured by differences in knob frequencies, is not linear. The highest observed heterosis values are associated with intermediate h_{ij}^* values. These results seem to agree with the conclusion reached by Moll et al. (1965): the maximum heterotic expression is observed at an intermediate degree of diversity, and heterosis decreases with very high levels of diversity.

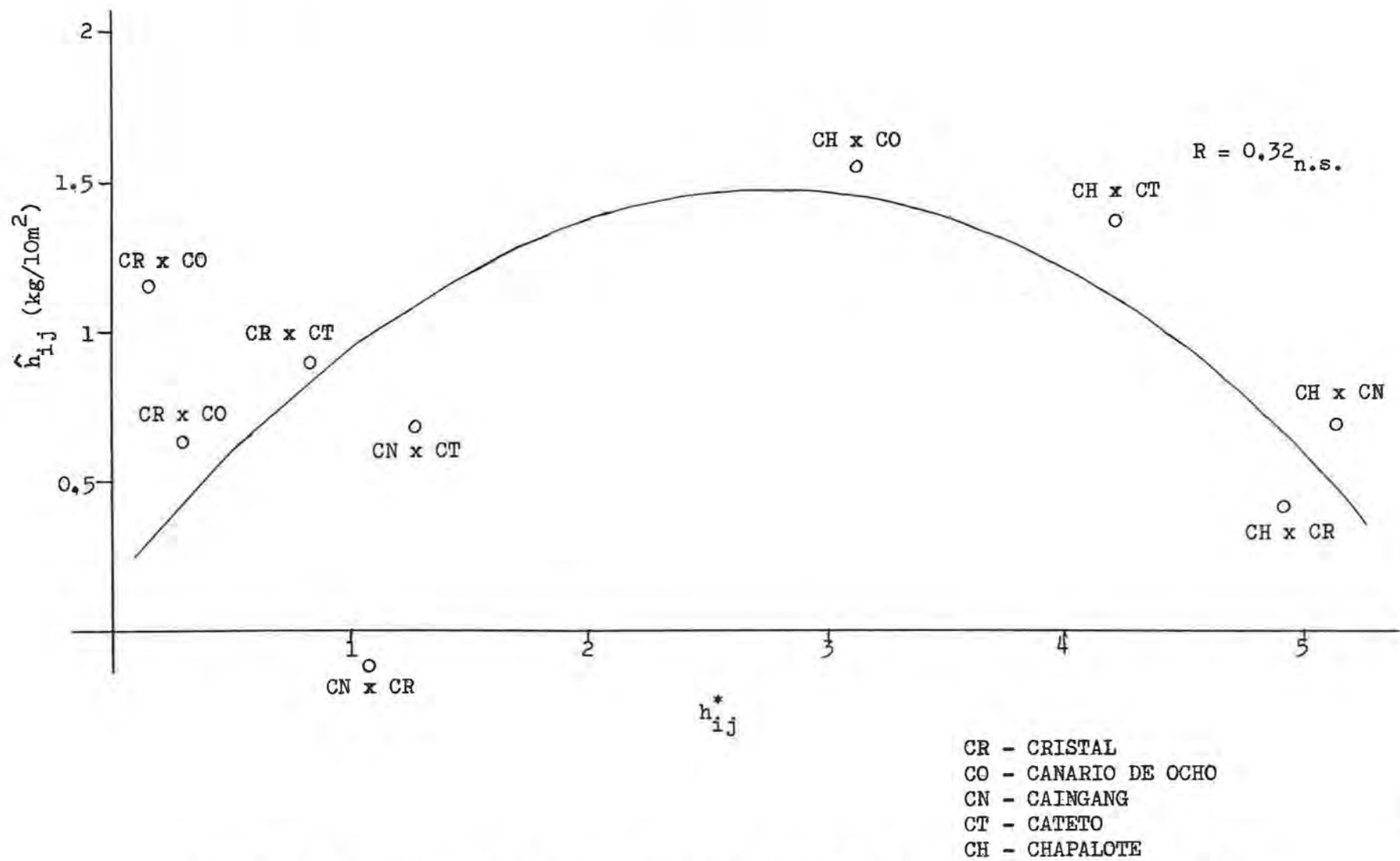


Figure 1. Observed and "expected" heterosis values in interracial crosses of maize

If similar results are obtained in more critical experiments, which we are developing, one could conclude that non-allelic interactions can be important for yield in maize.

Furthermore, it seems clear that, if epistasis exists, it will have a higher probability of expression in crosses between less related races. This is true because in interpopulational hybrids the loss of linkage equilibrium can occur alone as a consequence of differences in genic frequencies between the parental populations. Such a linkage disequilibrium can result in epistasis having a direct effect on the means of racial crosses.

Practically, if heterosis and divergence are curvilinearly related as in the figure, then the use of measures of divergence based upon knob data (or other comparable data) should be useful in the prediction of heterosis values (i.e., yield level) of maize hybrids.

REFERENCES

- Blumenschein, A. 1968. Estudo das Origens e Relações de Parentesco dos Milhos do Leste da América do Sul, a partir dos Knobs Cromossômicos. (In press).
- Gardner, C. O. and S. A. Eberhart. 1966. Analysis and interpretation of the variety cross diallel and related populations. *Biometrics* 22 (3): 439-452.
- Kato, T. A. 1964. Some cytological studies on several Mexican races of maize. M.Sc. Thesis. Raleigh, N.C. USA. North Carolina State of the University of N.C.
- Moll, R. H., J. H. Lonnquist, J. V. Fortuno and E. C. Johnson. 1965. The relationship of heterosis and genetic divergence in maize. *Genetics* 52: 139-144.

R. Vencovsky
A. Blumenschein

2. Preliminary experiment in relation to the effects of gamma-rays on hybrid vigor in corn.

Seeds of two inbred lines of flint and dent corn were gamma-rayed with an approximate dose of 3,700R, and in 1967, the following crosses were carried out as well as selfing of Do, Dr, Fo and Fr:

♂ ♀

Do x Fo

Dr x Fo Fo : non-irradiated seeds of flint corn

Dr x Do Fr : irradiated seeds of flint corn

Do x Fr Do : non-irradiated seeds of dent corn

Dr x Fr Dr : irradiated seeds of dent corn

In 1968, seeds of the above nine combinations were sown according to a balanced lattice design 3x3 with four replications and the following characters were measured on seventy competitive plants on the average per plot:

plant height
survival
width, length and thickness of grain
length and diameter of ear
ear weight

In the statistical analysis, an adjustment for stand was made, when necessary, through a covariance analysis. For those characters where the lattice analysis showed efficiency, the adjustments for block effects were made, otherwise the randomized block analysis was used.

Furthermore, the following contrasts were tested by the "t" test to estimate the effects of gamma-rays on inbred lines and on the amount of heterosis:

$$\begin{aligned} Y_1 &= \overline{Fr} - \overline{Fo} \\ Y_2 &= \overline{Dr} - \overline{Do} \\ Y_3 &= \overline{DoFr} - \overline{DoFo} \\ Y_4 &= \overline{DoFr} - \overline{DrFo} \\ Y_5 &= \overline{DrFo} - \overline{DoFo} \\ Y_6 &= \overline{DrFr} - \overline{DrFo} \\ Y_7 &= \overline{DrFr} - \overline{DoFo} \end{aligned}$$

The effect of radiation on heterosis in a particular cross between two inbred lines was also estimated by the following contrast:

$$Y_8 = (\overline{DrFr} - \frac{\overline{Dr} + \overline{Fr}}{2}) - (\overline{DoFo} - \frac{\overline{Do} + \overline{Fo}}{2})$$

heterosis in a	heterosis in a
cross between two	cross between two
irradiated inbred	non-irradiated
lines	inbred lines

Table 1 shows mean values of the various characters and Table 2 shows the "t" values of the various contrasts. The ear production increased 5.2% significantly in the cross "Dr x Fr" compared with the cross "Do x Fo". The experiment is being repeated this year.

Table 1

Mean of the various characters

Do: non-irradiated dent corn, Dr: irradiated dent corn, Fo: non-irradiated flint corn, Fr: irradiated flint corn.

	plant height (m)	survival number	grain width (mm)	grain thickness (mm)	grain length (mm)	ear diameter (mm)	ear length (mm)	ear weight (gr)
Do	1.40	510	7.05	3.43	11.08	36.80	100.90	31.40
Dr	1.54	580	7.00	3.45	11.13	36.90	105.95	35.00
Fo	1.34	388	7.34	4.68	8.71	31.94	135.88	44.20
Fr	1.44	458	7.30	4.63	8.83	32.05	136.75	42.60
Dr x Do	1.46	537	7.10	3.65	11.00	36.45	107.80	30.82
Do x Fo	2.44	615	7.88	3.38	11.65	41.40	134.60	81.37
Dr x Fo	2.48	538	8.03	3.40	11.55	40.95	144.05	92.75
Do x Fr	2.37	587	7.93	3.35	11.45	40.55	139.70	80.29
Dr x Fr	2.42	610	8.00	3.38	11.60	41.60	139.20	86.41

Table 2

"t" values of the various contrasts

	plant height	survival	grain width	grain thickness	grain length	ear diameter	ear length	ear weight
Y ₁	0.65	3.88**	1.01	0.82	0.56	1.27	0.72	0.18
Y ₂	1.24	3.43**	0.10	0.24	0.38	0.31	1.46	0.42
Y ₃	1.99	1.31	0.10	0.24	1.38	2.04	1.48	2.14*
Y ₄	1.30	2.30*	1.52	0.41	0.19	1.67	1.26	0.90
Y ₅	3.30**	3.25**	1.41	0.16	1.19	0.36	2.74*	3.04**
Y ₆	2.75*	3.39**	0.33	1.41	0.44	0.33	0.00	5.60**
Y ₇	0.54	0.22	1.72	0.00	0.75	0.04	1.33	2.56*
Y ₈	0.33	3.18**	1.76	0.23	1.00	0.46	0.42	2.33*

*significant at the level of 5%

**significant at the level of 1%

Augusto Tulmann Neto
Akihiko Ando

3. Gametophyte factor on the 4th chromosome in South American maize.

Maize collections from the Brazilian Seed Center at Piracicaba were tested in order to get information concerning their constitution with reference to the gametophyte factor on the 4th chromosome. The details of the test are as follows: All possible plants of the variety under test were pollinated by a ga/ga stock, while a pollen mixture from seven plants was used to pollinate a plant of a Ga^s/Ga^s stock. The number of ears with full seed set (more than 90%), intermediary seed set (more than 10% and less than 90%) and no seed set (less than 10%) was scored in the cross ♀ collection x ♂ ga/ga stock. The percentage of seed setting was scored in the cross on Ga^s/Ga^s. The results are shown in Table 1. Our intention is to trace the migration of this gene through the South American maize.

Collections	crossed to <u>ga/ga</u>			crossed on <u>Ga^s/Ga^s</u>		
	Number of ears in relation to seed set			Ears in relation to percent of seed setting*		
	full	interm.	no seed			
1. INDIGENOUS RACES						
1.1 - Moroti						
RGS XIX	46	0	0	20	20	-
PR I	15	9	2	-	-	-
PR II	12	7	3	-	-	-
Pe I	1	1	1	45	-	-
MT II	1	1	0	-	-	-
MT III	2	1	0	-	-	-
MT IV	2	0	0	10	-	-
MT V	3	0	0	90	100	-
PAG VI (1)	5	5	4	0	-	-
(2)	7	9	4	60	-	-
(3)	17	9	0	5	20	50
(4)	-	-	-	40	-	-
(5)	11	0	0	0	5	-
(6)	7	1	0	0	-	-
PAG VII	4	1	1	20	40	100
BOL II	5	3	0	-	-	-
BOL III	14	9	1	-	-	-
1.2 - Caingang						
Par III (1)	22	1	0	0	10	-
(2)	60	0	0	10	-	-
SP XIV	5	2	0	-	-	-

1.3 - Lenha							
RGS XX	22	4	0	30	-	-	
2. OLD COMMERCIAL RACES							
2.1 - Cristal Sulino							
ARG I	45	1	0	-	-	-	
ARG VIII	46	3	0	-	-	-	
URG VIII	49	0	5	30	-	-	
2.2 - Cristal							
SP X	5	2	0	15	15	80	
SP XI	13	3	0	0	0	10	
SP XII	15	1	1	0	0	0	
MG III	24	2	0	5	10	-	
Ba II	2	0	0	-	-	-	
2.2.1 - Semi-dentado							
PAG II	3	0	0	0	50	100	
PAG III	22	1	0	0	10	-	
2.3 - Canario de Ocho							
ARG IV	52	12	0	0	0	-	
ARG VI (1)	-	-	-	40	-	-	
(2)	11	0	0	0	5	-	
(3)	7	1	0	0	-	-	
(4)	41	0	0	-	-	-	
2.4 - Cateto Sulino							
ARG II	22	10	5	0	-	-	
ARG IV	29	5	0	10	10	-	
URG I	11	10	8	0	40	100	
URG II	50	7	0	5	5	10	
URG IV (1)	32	6	0	5	-	-	
(2)	38	4	0	50	70	-	
2.4.1 - Cateto Sulino Escuro							
URG V	40	2	0	30	-	-	
URG VI A	28	4	1	60	60	60	
2.5 - Cateto Sulino							
2.5.1 - Cateto Sulino Grosso							
URG III (1)	54	1	0	-	-	-	
(2)	51	0	0	30	-	-	
(3)	52	0	0	10	-	-	

2.6 - Cateto

SP VII	29	0	0	15	-	-
SP VIII	40	0	0	50	-	-
MG II (1)	26	8	0	0	60	-
MG (2)	35	0	0	50	-	-
BA I	3	2	0	-	-	-
MA I	10	1	0	-	-	-
DESC I	1	1	0	-	-	-
CE I	33	4	0	5	10	-

2.6.1 - Cateto Grande

MT I	15	11	1	20	-	-
------	----	----	---	----	---	---

2.7 - Cateto Nortista

GF II	12	0	0	60	-	-
GF III (1)	1	2	0	0	15	60
(2)	21	3	0	50	-	-
G IN I	35	0	0	50	-	-
G IN II (1)	1	2	0	0	15	50
(2)	21	3	0	5	-	-

2.7.1 - Cateto Nortista Precoce

SUR I	31	0	0	80	-	-
-------	----	---	---	----	---	---

3. RECENT COMMERCIAL RACES

3.1 - Dente Riograndense

3.1.1 - D.R.G. Rugoso

RGS I	25	0	0	0	0	0
RGS II	46	3	0	0	0	0
RGS IV	42	2	0	1	0	0

3.1.2 - Dente Riograndense Liso

RGS V	40	2	0	30	-	-
RGS VI (1)	39	4	0	80	-	-
(2)	32	6	0	10	10	-
SC I	39	0	0	20	20	-

3.2 - Dente Paulista

SP IV	-	-	-	80	-	-
MG I	7	0	0	5	20	-

3.3 - Dente Branco

3.3.1 - Dente Branco Riograndense

RGS X	51	1	0	30	-	-
RGS XI	38	3	2	0	50	-
RGS XII (1)	60	1	0	0	70	-
(2)	20	5	0	0	20	70
RGS XIII (1)	9	3	1	-	-	-
(2)	20	9	3	0	50	-

3.3.2 - Dente Branco Paulista						
SP V (1)	36	9	3	0	-	-
(2)	5	0	0	60	-	-
3.4 - Semi-Dentado						
3.4.1 - Semi-Dentado Riograndense						
RGS XV	18	5	0	10	15	30
RGS XVI	41	5	0	-	-	-
3.4.2 - Semi-Dentado Paulista						
SP IX	19	5	1	0	0	0
MT VII	2	0	0	-	-	-
3.5 - Cravo						
3.5.1 - Cravo Riograndense						
RGS VII	58	3	0	30	-	-
RGS VIII	39	0	0	20	20	-
3.5.2 - Cravo Paulista						
SP I (1)	24	0	0	70	20	-
(2)	18	1	0	80	-	-
SP II	18	0	0	15	-	-
4. EXOTIC COMMERCIAL RACES						
4.1 - Hickory King						
RGS IX	53	0	0	20	20	-
4.2 - Cuba Yellow Dent						
BA III	14	7	4	-	-	-

*) - Subjective classification

Maria Ruth Alleoni

UNIVERSITY OF SASKATCHEWAN
Saskatoon, Saskatchewan, Canada
Department of Biology

1. Ethyl methanesulfonate induced mutations in maize.

To understand the genetic effects of radiations, the frequency of mutations induced by γ -rays and ultraviolet light was reported in our earlier works following treatment of Su pollen grains in maize. The present work is concerned with the mutations induced by ethyl methane-sulfonate (EMS).

Mature pollen grains carrying the dominant gene (Su) were spread in a monolayer on a round plastic plate. Each plate was then placed on a glass supporter inside a petri-dish containing 25 ml of 0.5% EMS solution at $30^{\circ} \pm 2^{\circ}\text{C}$. The EMS solution was prepared in deionized distilled water without buffer before its application. The pollen was exposed to EMS vapor for 0.5, 1.0 and 1.5 hours. The treated pollen was then dusted on ears having the recessive gene (su). After maturity, endosperms were scored for whole or chimera (partial) mutations and data thus obtained are shown in Table 1. Comparison was made of the mutation rate of the treatments with control.

Table 1
Frequency of mutations at the sugary locus from EMS
exposed pollen grains of maize

Treatment	No. of seeds tested	Mutation rate per 100 gametes			Percent of chimeras
		Whole	Chimeras	Total	
Control	2525	.04 \pm .03	.04 \pm .03	.08 \pm .05	50.0
EMS*					
0.5 hr	9684	.05 \pm .02	.32 \pm .06	.37 \pm .06	86.1
1.0	7751	.05 \pm .08	.45 \pm .08	.50 \pm .08	89.7
1.5	8171	.05 \pm .07	.59 \pm .08	.64 \pm .09	92.3

*EMS: 0.5%

Seed setting was not affected by EMS treatment. The dose response curve against mutation rate showed a non-linear relationship. This saturation effect is not due to the reduction of seed fertility. The frequency of whole mutations was not significantly increased by treatments. On the other hand, the frequency of chimera mutations increased significantly with the prolongation of treatment hours. This predominance of chimera mutations was more or less similar to that found by Neuffer and Ficsor (1963) and Chatterjee et al., (1965).

T. Mabuchi
T. J. Arnason

STATION D'AMELIORATION DES PLANTES
Dijon, R.P., France

1. Cytological studies of "necrotic embryos" line MR 077.

Each plant of the inbred line MR 077 produces regularly a number of aborted or partially necrotic embryos (see MNL 34,117). This line is also partially male-sterile, producing a mixture of good and aborted pollen grains. Therefore we attempted a cytological analysis of the line and some of its hybrids.

Mitosis observed in root tip cells appears constant and normal in the line and in hybrid plants.

Meiosis was studied on the different parts of complete tassels. One anther of both flowers of a spikelet cut out on each branch at three levels (base, middle, summit) was squashed in acetocarmine.

Meiosis begins in the central part of the main rachis and gets its extremities which present usually the same stage. As to the branches, meiosis starts mostly at the base but an inversion of this polarity may occur; apical branches may accomplish meiosis prior to basal ones. Inside a spikelet, the time of a complete meiosis separates the two flowers.

Some abnormalities were observed:

On the line plants, before meiosis, some anthers without P.M.C. and some P.M.C. without nuclei have been observed with a very low frequency. During meiosis, no abnormality in chromosome behaviour was seen but we have to note that well spread pachytene cells were difficult to obtain. Some meioses were blocked at early leptotene but with a low frequency. In those cases the small flower completed meiosis while the big one remained at leptotene. This observation suggests that a mechanism prevents meiosis from going on.

After meiosis, young pollen grains were generally normal, full and well coloured, but when the walls and the germ pore appeared, many anthers showed a mixture of good and aborted microspores.

In the hybrid plants, meiosis is perfectly normal and leads to good pollen.

Thus, we may conclude that the partial male-sterility is not due to an abnormal chromosome behaviour at meiosis but to other phenomena

which take place infrequently before or at the beginning of meiosis and to a greater extent after meiosis.

D. Maizonnier
A. Cornu

2. Study of the size of mutated sectors in the corn ear.

Mutagenic treatments of maize seeds were used in order to determine the significance of mutated areas on the ear.

For this purpose, plants derived from seeds of inbred line V7 treated with EMS (1.5 g/l) were detasseled and pollinated by $\underline{sh}_2/\underline{sh}_2$ mutant V7. Observations were made on:

1. \underline{sh}_2 mutated kernels on the ear of treated plants (M_1)
2. Chlorophyll mutants in M_3 progenies obtained by selfing of F_1 plants.

Endosperm mutants.

Two ears of 486 harvested showed the mutation \underline{sh}_2 . In the two cases, about 50% of the total kernels were \underline{sh}_2 . Consequently those two ears are believed to be completely included in periclinal chimaeras (mutated area \geq ear size).

Chlorophyll mutants.

Several types of mutant seedlings were observed in M_3 progenies. Results from families showing 1, 2 or 3 different phenotypic mutants are summarized in Table 1. All progenies segregate with correct mendelian ratios.

The results indicate that:

1. If we consider the destiny of only one mutation, the frequency of segregating progenies per family (frequency of heterozygous embryos in the initial ear) is not significantly different from 50% in about 7 of the 13 studied families. In 5 families this ratio is significantly lower than 50%.

In the first group (including the two \underline{sh}_2/\pm mutated ears found in M_1), the mutated sectors seem to be at least equal to the ear size, whereas in the second group they are smaller.

An aberrant case (H family, ratio $> 50\%$) will be the subject of

Table 1
Segregation of chlorophyll mutants following EMS treatment

Families (one from one ear M ₁)	1 mutation							2 mutations			3 mutations		
	A	B	C	D	E	F	G	H	I	J	K	L	M
M ₃ observed progenies	51	59	57	48	50	50	52	58	35	31	26	24	29
M ₃ segregated progenies	2(av) 5(av) 9(av) 17(av)* 18(av)* 26(x)* 34(v)*							44(av) 17(v)* 11(av)* 2(v) 2(av) 5(a) 1(a,av)	4(xv) 2(ax) 16(x)* 3(v) 1(a) 15(a) 1(a) 2(a,ax) 2(xv) 3(v,a) 1(a,xv) 1(x,av) 1(x,xv)				

Types of mutations: a = albina; x = xantha; v = viridis; av = albo-viridis; ax = albo-xantha; xv = xantha-viridis

* χ^2 ratio 1:1 non significant (P = 0.01)

further investigations.

2. In the "two mutations" cases, it is noted that one of the two mutated sectors is smaller than the other. This suggests that the two mutations did not appear simultaneously, but successively. The possibility of development of two contiguous sectors is not supported by our results.

3. The examples including three mutations show that the same ear can bring three mutations, or one cell can contain two viable mutations (ex: mixed progenies in the J, K, L, M families).

H. Touvin
A. Cornu

UNIVERSITY OF STELLENBOSCH
Stellenbosch, South Africa

1. The identity of South African maize germ plasm.

Most of the maize varieties grown in South Africa before the advent of hybrid maize can be traced to importations made in the first decade of the present century. According to the records given by Burt-Davy (1914) these importations were had from various sources abroad but all seem to have originated in the United States.

With the subsequent expansion of maize production as a commercial crop, the white dents became the dominant type, founded largely on a singularly restricted genetic base. The few varieties concerned were principally Hickory King, Champion White Pearl, Iowa Silver Mine and the Horse Tooth types. The yellow dents were less important but their origin is as clearly to be traced to American importations except that some of the varieties which later became popular showed infusions of flint sources. A number of flints were also imported but of these few became established varieties. In most cases, the early variety trials and farmers' reports referred to flint varieties named White and Yellow Congo, White and Yellow Botman with unspecified origin. Noticeably, both these varieties have both white and yellow variants. Burt-Davy (op cit) also refers to "Kaffir mielies" as being mixed white, red, blue and yellow flints grown in the Native territories. These flints, says

Table 1

Character	Material from					
	Lesotho		Umbazwana		Jozini	
	Av.	S.E.	Av.	S.E.	Av.	S.E.
1. Glume length (mm)	11.2	± 1.61	9.8	± .91	9.6	± .85
2. Number of leaves above ear	4.3	± .60	5.76	± .50	5.93	± .60
3. Number of Prim. Tassel Branch	15.72	± 6.96	26.56	± 4.05	24.6	± 3.76
4. Number of Sec. Tassel Branch	3.30	± 2.18	11.06	± 4.34	14.06	± 6.51
5. Plant height (cms)	192.6	± 17.76	193.3	± 19.35	285.0	± 22.76
6. Tassel exertion (mm)	86.2	± 25.44	47.8	± 22.16	56.16	± 29.52
1. Degree of denting	1.10	± .30	1.96	± .57	3.7	± .75
2. Ear length (mm)	153.5	± 25.83	133.5	± 15.34	187.0	± 21.92
3. Kernel length (mm)	8.02	± .85	8.16	± 2.64	7.3	± .91
4. Kernel row number	9.5	± 1.67	12.0	± 1.38	10.93	± 2.80
5. Shank diameter (mm)	12.5	± 2.37	9.8	± 1.47	9.6	± 1.56
6. Shank length (mm)	141.0	± 62.44	108.5	± 26.89	123.3	± 51.3

Table 1 (Continued)

Character	Material from					
	S.A. Flint Singles		B37 x H49		Non-selected corn belt lines Brown's data	
	Av.	S.E.	Av.	S.E.	Av.	S.E.
1. Glume length (mm)	10.2	± 6.40	10.8	± .63	11.32	± 1.48
2. Number of leaves above ear	5.3	± .42	5.7	± .48	6.00	± 1.09
3. Number of Prim. Tassel Branch	26.5	± 4.61	12.0	± 1.81	17.00	± 6.70
4. Number of Sec. Tassel Branch	7.1	± 3.04	3.0	± .94	2.90	± 3.00
5. Plant height (cms)	231.7	± 11.80	237.5	± 17.96	213.00	± 24.50
6. Tassel exsertion (mm)	57.30	± 9.11	56.5	± 15.28	39.20	± 5.84
1. Degree of denting	1.2	± .7	4.9	± .32	2.24	± 1.09
2. Ear length (mm)	182.2	± 17.4	215.0	± 27.59	152.60	± 28.40
3. Kernel length (mm)	11.00	± .86	11.0	± 1.01	10.26	± 1.00
4. Kernel row number	10.8	± 1.34	16.2	± 6.30	14.40	± 2.36
5. Shank diameter (mm)	13.6	± 2.72	11.3	± 1.64	10.40	± 2.56
6. Shank length (mm)	107.7	± 31.2	138.5	± 36.06	101.00	± 41.70

Burt-Davy, were grown in South Africa long before the introduction of dents and were presumably the types grown by the Bantu farmers whose maize fields European pioneers observed in the first half of the previous century.

Burt-Davy states "Cango and Botman are probably the types introduced by the Portuguese from their Brazilian settlements, into the East Indies and China, and dropped at Mossamedes, Cape Town and on the east coast en route." He goes on to say: "An African grown ear of white flint maize, received from the late Prof. MacOwan, Cape Town, about the year 1884, was classified by Sturtevant as differing only in colour from the Chinese samples exhibited at the Centennial Exposition, and from the Milho dourado grown at Rio Claro, in the Province of Goyaz, on the uplands of Brazil."

Jeffreys (1967a, 1967b) has consistently argued that the Bantu in their southward migration did not get their maize from the Portuguese in East Africa but brought it with them from North West Africa from where their migration supposedly started circa 1400. Jeffreys, therefore, argues that maize was introduced into South Africa by the Bantu before white settlement in 1652 and into Africa before Columbus in 1492. Since the Phoenicians appear to have preceded both Columbus and the Vikings in reaching the new world (see Time, May 24, 1968), the likelihood of the Arabs having done so before Columbus and having brought maize to Africa is not entirely remote.

The matter has genetic and plant breeding significance inasmuch as such South African flints will represent genetic stocks with a separate adaptive history. Their recovery and identification before being completely swamped by exotics and latter-day hybrids should be attempted, therefore.

During the 1967-68 season the author was able to grow a small collection of what is now known as Bantu varieties, and the purpose of this note is to give some data which characterize them allometrically. The samples comprise three varieties from Lesotho supplied by Dr. W. H. Wessels of the Potchefstroom College of Agriculture, a mass collection from Umbazwana in Zululand supplied by Mr. H. O. Gevers of the Natal Agricultural Institute at Pietermaritzburg, and some ear samples obtained

by the author from a Zulu farmer who in his offseason served as a petrol pump attendant at a trading station at Jozini in Zululand.

Table 1 gives average measurements from 30 plants of each of these three sources in comparison to the mean of two local yellow flint single crosses (10 plants of each), the locally grown single cross B37 x H49 from the United States (10 plants), and the measurements given by Brown (1967) for non-selected lines.

The standard errors are high and few significant differences are to be had. As a group the Lesotho varieties have small unbranched, strongly exerted tassels and the ears are borne on relatively long shanks when compared to other flints and semi-flints. One of the Lesotho varieties named Rafolatsane is no different from the White Congo variety agreeing also in having off-white grain colour, early maturity and profuse tillering. This raises the question as to whether White Congo was introduced into Lesotho or was selected there in the first place. If some of its characters are adaptive responses to the short season with cool nights as found in the high altitude of Lesotho, then this might prove to have value in breeding programmes.

The Zululand material has highly branched tassels and the Jozini specimens were rather tall. It is anticipated that these lowland varieties might have some resistance to streak virus.

BURTT-DAVY, J. (1914). Maize, its history, cultivation, handling and uses. Longmans Green & Co., London.

JEFFREYS, M.D.W. (1967a). Who introduced maize into Southern Africa? S.A. Journ. of Sc., Jan., 1967.

JEFFREYS, M.D.W. (1967b). Pre-Columbian maize in Southern Africa. Nature, Aug. 12, 1967.

BROWN, W.L. (1967). Results of non-selective inbreeding in maize. Der Zuchter 37:155-159.

F. X. Laubscher

UNIVERSITY OF SYDNEY
Sydney, Australia
Department of Agricultural Botany

1. Complementary gene determined aleurone variegation involving a mutant R allele.

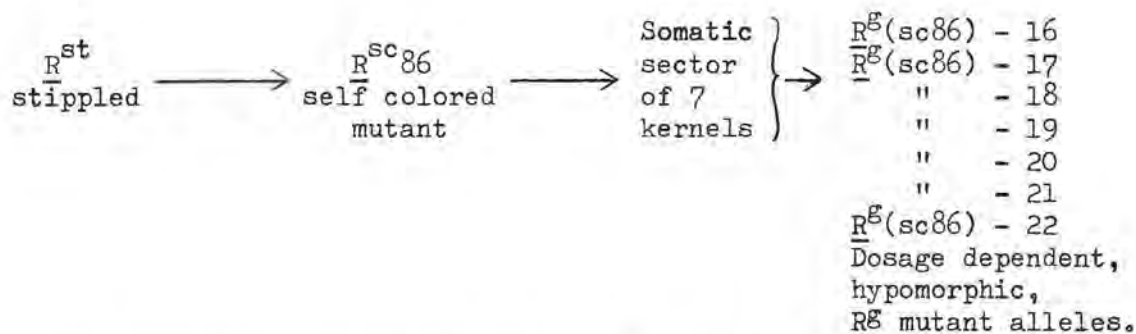
In the course of study of a mutant R allele, a stock in which a variegated aleurone phenotype (colorless areas on a colored background) regularly appears, seemingly by complementary action of independent genetic determinants, has been established.

The variegated phenotype closely resembles that produced by interaction of McClintock's Ac and Ds elements in C Ds/CDs/c aleurone genotypes appropriate for detection of variegation due to chromosome loss in the developing endosperm. In the newly arisen system, variegation might similarly be presumed to be a consequence of genetically induced chromosome breakage events. However, chromosome 10, and the R locus in particular, is clearly implicated in the variegation, since the phenotype was first observed on a single ear, where all kernels were of $\underline{R}^G/\underline{R}^G/\underline{r}^r$ endosperm constitution, resulting from the mating

$$\begin{array}{ccc} A & C & \underline{G}_1 \underline{R}^G \\ - & - & - \\ A & C & \underline{G}_1 \underline{R}^G \end{array} \quad \begin{array}{c} Su & Y \\ - & - \\ Su & Y \end{array} \quad \begin{array}{c} \text{♀} \\ \text{♀} \end{array} \quad \times \quad \begin{array}{ccc} A & C & \underline{g}_1 r^r \\ - & - & - \\ A & C & \underline{g}_1 r^r \end{array} \quad \begin{array}{c} su & y \\ - & - \\ su & y \end{array} \quad \begin{array}{c} \text{♂} \\ \text{♂} \end{array} .$$

On this exceptional ear, there were 91 colored aleurone - not variegated kernels and 76 colored aleurone - variegated kernels. The parental genotypes listed above have been verified by progeny tests of both normal and variegated kernels. The variegated phenotype, therefore, must involve loss of \underline{R}^G allele function in the colorless aleurone areas.

This case of heritable aleurone variegation has an unusual origin. The \underline{R}^G mutant allele involved in the mating was derived from \underline{R}^{st} (stippled) by the sequence of spontaneous mutations:



The dosage dependent, hypomorphic, $\underline{R}^{\underline{B}}$ mutant alleles so derived have been found to show a high frequency of heritable variation in quantitative level of expression of anthocyanin color in the aleurone. A dark aleurone selected line of $\underline{R}^{\underline{B}}(sc86) - 17/\underline{R}^{\underline{B}}(sc86) - 17$ genotype was treated with the chemical mutagen diethyl sulphate, and the exceptional ear under study was derived from a plant grown from a treated kernel.

Determination of the variegated phenotype by complementary action of independent genetic determinants is shown by the segregation ratio 1 colored aleurone - not variegated : 1 colored aleurone - variegated : 2 colorless aleurone kernels from the mating

$$\frac{G_1 \underline{R}^{\underline{B}}}{g_1 r^r} - \text{variegated } \varnothing \varnothing \times \frac{gr^r}{gr^r} \sigma \sigma .$$

Observed numbers of kernels were 126 colored - not variegated : 126 colored-variegated : 247 colorless. This segregation evidence for two determinants, together with the obvious requirement for presence of the $\underline{R}^{\underline{B}}$ allele for detection of aleurone variegation, requires that one determinant of the phenotype must be closely linked with, and possibly is a component of, the $\underline{R}^{\underline{B}}$ mutant allele.

The second determinant of the variegated phenotype was inherited independently of the $\underline{R}^{\underline{B}}$ allele, and yet must have originated in the single exceptional $\underline{R}^{\underline{B}}/\underline{R}^{\underline{B}}$ plant involved in the original mating listed above. This conclusion follows from failure to observe variegation in kernels resulting from the testcross matings,

$$\underline{gr}^r/\underline{gr}^r \varnothing \varnothing \times \underline{GR}^{\underline{B}}\text{-variegated}/\underline{gr}^r \sigma \sigma .$$

Evidently the second determinant induces variegation only when present in at least two doses in the triploid endosperm. Also a proportion of

kernels in which this determinant is present in two doses do not show variegation. Because of these dosage effects, the segregation on ears resulting from self-pollination of plants of \underline{GR}^G -variegated/ \underline{gr}^r genotype do not fit a simple Mendelian ratio. However, such ears invariably segregated a proportion of variegated kernels.

An attempt is being made to isolate the determinants which produce aleurone variegation by complementary action in separate stocks.

The variegation patterns observed with the " \underline{R}^G " system just described strikingly resemble those produced by McClintock's Ac-Ds system. In view of the origin of these materials it is reasonable to postulate that the " \underline{R}^G " system is comprised of a "Ds-like" element located at or near the R locus, and an "Ac-like" element located elsewhere in the complement. Further studies of the origin and interaction of the postulated elements and of their homology with the Ac and Ds elements isolated by McClintock are being conducted.

K. S. McWhirter

UNIVERSITY OF TEXAS
Austin, Texas

1. Distance between K10 heterochromatic regions in several interphase tissues.

In a stock homozygous for K10 these large knobs are thought to be recognizable as the most prominent of the heterochromatic regions found at interphase in acetocarmine squash preparations. Measurements were made in consecutive analyzable cells of systematically scanned slides (at various interphase stages) of the distance between the pair of presumed homologous K10's and of nuclear diameters. Stages studied were: premeiotic interphase and tapetal interphase from very small anthers (about 0.4 mm. in length), premeiotic interphase and tapetal interphase from larger anthers (about 1.0 mm. in length) and tapetal interphase from anthers with sporocytes at pachytene. The ratio of the distance between these heterochromatic regions and the nuclear diameter was calculated for each nucleus observed so that the relative nearness of these knobs could be compared. Mean ratios found for the different stages were as follows:

interphase sporocytes (anther length 0.4 mm) - 0.135

interphase sporocytes (anther length 1.0 mm) - 0.142

interphase tapetals (anther length 0.4 mm) - 0.163

interphase tapetals (anther length 1.0 mm) - 0.192

interphase tapetals (pachytene anthers) - 0.191

The homologous K10's were very significantly (.01 level) nearer than random expectation would predict in both t tests and Kolmogorov-Smirnov tests at all stages studied.

Differences found between average K10 separation were not significant (0.10 level, t test) in the following comparisons:

interphase sporocytes (anther length 0.4 mm) vs interphase
sporocytes (anther length 1.0 mm)

interphase sporocytes (anther length 0.4 mm) vs interphase
tapetals (anther length 0.4 mm)

interphase tapetals (anther length 0.4 mm) vs interphase
tapetals (anther length 1.0 mm)

interphase tapetals (anther length 0.4 mm) vs tapetals
from pachytene anthers

interphase tapetals (anther length 1.0 mm) vs tapetals
from pachytene anthers

The K10's were significantly closer (.01 level) by this test, however, in interphase sporocytes from anthers 1.0 mm. long than in interphase tapetal nuclei from the same anthers.

Marjorie Maguire

TUFTS UNIVERSITY
Medford, Massachusetts
Department of Biology

1. Plants carrying the gene "rootless."

A phenomenon noticed rather strikingly in 1968 field plantings of some 1000 rootless (rt/rt) plants derived from a "nearly rootless" stock was that a portion of the field which was unintentionally left

unirrigated showed no influence of drought, while five parallel rows of W23/L317 F_1 plants which also ran into the unirrigated area showed drastic wilting in these portions within the dry soil area.

Conversation with other maize investigators has revealed that this same drought-hardiness of rootless stocks has been noticed but not explained in fields grown in Ohio and Iowa. The possibility of water conservation by such plants, which may be genetically able to regulate or lower transpiration rates, is the basis for a grant application to a private source with which it is hoped to undertake such investigations during the next two or three years.

Further data were accumulated during the summer of 1968 on the effectiveness on root development of treatments with 2×10^{-4} Molar TIBA (Tri-Iodo Benzoic Acid) and N_6 BZA (N_6 Benzyl Adenine), and are summarized in the following table in which brace root development is rated on a 0-5 scale. It can be concluded that, at the strengths employed, there was less influence on root development than at the higher strengths used one year ago.

	Total plant number	Treatment	No. of plants with brace root development rating* of:					
			0	1	2	3	4	5
Rootless with some roots	124	none	10	34	41	35	8	
	37	TIBA - 100 micrograms daily	6	4	22	5		
	33	TIBA - 100 micrograms each third day	2	11	14	5	1	
	40	N_6 BZA - 100 micrograms daily	3	13	19	5		
	34	N_6 BZA - 100 micrograms each third day	5	4	16	9		
	29	water (control)	2	10	13	4		

*0 = no roots; 5 = normal roots.

	Total plant number	Treatment	No. of plants with brace root development rating* of:					
			0	1	2	3	4	5
<u>Rootless</u>	94	TIBA - 100 micrograms daily	27	27	27	11	3	
<u>Rootless</u>	105	TIBA - 100 micrograms each third day	33	30	23	11	8	
	94	N ₆ BZA - 100 micrograms daily	34	34	16	10		
	107	N ₆ BZA - 100 micrograms each third day	38	27	28	13	1	
	197	none	79	56	44	11	7	
	100	water (control)	50	19	22	7	2	

Norton H. Nickerson

2. Races of maize in Panama.

Seeds from certain stocks collected in Panama, as noted in last year's Newsletter (Vol. 42), were grown in the Bahamas during the spring of 1968, and internode data on 5 - plant samples of fifteen stocks were obtained. The Bahamas experimental plot is being activated again this year in an attempt to obtain data on those stocks not yet known except from ear collections. The samples collected were analyzed for Fe, Cu, P, K, Ca, Sr, Mg, Mn and Zn by Dr. Jack Gamble of the University of Florida. In general, levels were low and did not vary significantly among the varieties or the locations collected.

Norton H. Nickerson

3. Bahamian Maize.

There are at least two "native" strains of maize in the Bahamas which apparently exhibit drought resistance. One of these appears to be an extreme Chandelle type which we have been able so far to see only in

an old government collection. Efforts are under way to obtain fresh seed specifically to study the possible genetic basis of its drought resistance.

Norton H. Nickerson

TULANE UNIVERSITY
New Orleans, Louisiana
Department of Biology

1. Field studies on hybridization and parallel variation in the wild relatives of maize in central Mexico.

Field studies of the wild relatives of maize in Mexico were begun during the fall of 1968. This field work is being undertaken in the following regions in which maize, teosinte and Tripsacum are all found growing in the same fields: Churintzio, Michoacan; Quiroga, Michoacan; Huetamo, Michoacan; Aguacate, Guerrero; Mazatlan, Guerrero; and Acachautla, Guerrero. In all these areas studied the fields were planted to maize, teosinte was wild around the margin of the fields and sometimes occurred as a weed in the fields, and Tripsacum was present within 100 yards of the field, either along stone walls or on rock outcroppings of a barranca. All of these sites are on limestone soils between 1350 and 1900 meters with the exception of Huetamo (600 m.). Although the data of the study are still in preliminary form, it is hoped that continued studies will yield field documentation on the mechanics of tripsacoid introgression via teosinte into maize and will explore the parallel variation found in Tripsacum, teosinte, and maize where the three occur together. [In the Balsas basin of Guerrero are found the most tassel-branched forms of maize, the most tassel-branched forms of teosinte (race Guerrero), and the most tassel-branched forms of Tripsacum (T. maizar). On the Central Plateau in central Mexico are encountered the maize plants with the most pronounced development of plant color, the most intensely colored teosinte race (race Central Plateau), and the Tripsacum species (T. pilosum and T. lanceolatum) with the most well-developed red plant color. There is also a parallel in the distribution of some of the most hairy forms of maize, teosinte, and Tripsacum on the Central Plateau.]

Critical field-collected clonal introductions of Tripsacum from these study areas are being maintained in the Maize Relatives - Genetics Garden of Tulane University.

H. Garrison Wilkes

UNIVERSITY OF VICTORIA
Victoria, British Columbia, Canada

1. Biochemical study of anthocyanidins produced by different R alleles.

Analyses of the anthocyanins in maize are being carried out in this laboratory for the purposes of:

- (1) Using anthocyanin formation as an indicator to study gene action at various developmental stages.
- (2) Characterizing different allelic forms of the R locus with respect to anthocyanin production.

Five different alleles of the R locus were employed for this study:

- (1) standard $\underline{R}^r \underline{R}^r$ -red seedlings, red anthers and colored aleurone.
- (2) $\underline{R}^G \underline{R}^G$ Canada (P.I. 214199) -red seedlings, green anthers and colored aleurone.
- (3) $\underline{R}^r \underline{R}^r$ Ecuador (1172) -red seedlings, red anthers and colored aleurone.
- (4) $\underline{r}^r \underline{r}^r$ -red seedlings, red anthers and colorless aleurone.
- (5) $\underline{r}^G \underline{r}^G$ -green seedlings, green anthers and colorless aleurone.

All stocks used were strains of W22 carrying $\underline{A}_1 \underline{A}_2 \underline{C}_1 \underline{C}_2 \underline{Pr} \underline{pl}$. The W22 B allele is probably \underline{B}^b (pigmented glume base and culm but otherwise weak plant-color).

Plants for this study were grown in the greenhouse. Pigmented tissues were collected and extracted with 1% HCl in MeOH, concentrated in vacuum and hydrolyzed with 4N HCl for 30 minutes. By adding a few drops of isoamyl alcohol, the hydrolysate was separated into an organic and an aqueous layer. The aglycones in the alcohol layer were spotted on a thin layer plate coated with Avicel S.F. Cellulose. The chromatograms were developed in two directions. First, formic acid: 4N HCl (2:1 v/v),

secondly with either acidified methanol water* (20:1 v/v, 0.5 ml of conc. HCl added per 100 ml of solvent), t-BuOH: 2N HCl: HAC: H₂O (6:1:1:2 v/v), or n-BuOH: HAC: H₂O (2:1:1 v/v). Essentially the same results were obtained with all these second solvents.

The number of different anthocyanidins obtained were as follows:

Genotype	Leaf Sheaths	Aleurone	Anthers
$\underline{R^r R^r}$ Standard	6	4	5
$\underline{R^r R^r}$ Ecuador	6	4	5
$\underline{R^g R^g}$ Canada	6	4	-
$\underline{r^r r^r}$	6	-	5
$\underline{r^g r^g}$	6	-	-

Anthocyanidin spots in different tissues

Tissue	Spot number						
	1	2	3	4	5	6	7
Anthers	+	-	+	+	+	-	+
Leaf Sheaths	+	+	+	+	+	+	-
Aleurone	+	-	+	+	+	-	-

Spots 1 and 3 have been identified as cyanidin and pelargonidin, respectively.

Spot 4 has been tentatively classified as peonidin.

Spots 2, 5, and 6 have the characteristics of anthocyanidins. Some were purple and some magenta. When exposed to NH₃ vapor or sprayed with Na₂CO₃ they turn bluish.

Spot 7 is orange and turns bluish purple when exposed or sprayed with NH₃ or Na₂CO₃.

*Solvents developed by Dr. D. B. Mullick
Faculty of Forestry
University of British Columbia
Canada

Kim-Tek Seah
E. D. Styles

2. The complexity of maize anthocyanins.

Anthocyanins from leaf sheaths were extracted with 1% HCl in MeOH, concentrated in vacuum at 30-40°C and freeze dried. The freeze dried material was then streaked on a cellulose plate. The chromatogram was developed in BAW solvent. Four intense purple bands and a weak orange-pink band were obtained. Each band was eluted and rechromatographed in BAW and other aqueous solvents such as aq-HCl and HAC: HCl: H₂O (3:1:8 v/v). In each case several bands were obtained from each individual eluate. This suggested that perhaps each band obtained with the BAW solvent contained more than one anthocyanin and required further purification or that the anthocyanins are labile in acidic solvents, or both. We thus extracted more anthocyanin with absolute methanol and concentrated it as before. Subsequent procedures were all carried out in neutral solvent systems except for the final step. Chromatograms were developed with BEW* solvent (4 n-BuOH: 1 Ethyl acetate: 5 H₂O). Three bands were obtained: band A purple, band B bluish-pink and band C pink, respectively. Each band was eluted and then developed with a second solvent EEW* (3 Ethanol: 1 Ethyl acetate: 1 H₂O). Band A and B each resolved into three bands, and band C into four bands. The stability of the pigments in the BEW and EEW solvents was confirmed by rechromatographing each eluate separately and redeveloping it in the same solvent system. They proved to be quite stable. Each of these bands was then eluted and developed in two directions. The solvent employed for the first direction was BAW, while for the second direction HAC: HCl: H₂O (15:3:82 v/v) was used. The chromatograms obtained showed that each individual band can be further resolved into at least 6-12 spots and characterized by different R_f values and color intensity.

As the anthocyanins were extracted and developed in such a way to minimize acidic degradation, this experiment reveals that:

- a) Maize anthocyanins are labile in acidic solvents during extraction.
- b) The four intense color bands (or anthocyanins) obtained from the BAW solvent were not simple monoglycosides, for each band

*Solvents developed by Dr. D. B. Mullick

could be further resolved into several anthocyanins.

- c) The simple monoglycosides obtained by other workers are probably products of acidic degradation of the pigment complex.

Kim-Tek Seah

E. D. Styles

UNIVERSITY OF WESTERN ONTARIO
London, Ontario
Department of Botany

1. Nuclear cycle - a correction.

In our previous contribution (MGCNL 42:175-178, 1968) we presented incorrectly the data of Clowes (1965). The correct data attributed to Clowes in our Table should read:

	"Cap. initials	Quiescent center	stele just above quiescent center	stele 200u from quiescent center
T	14	(174)	22	23
G ₁	-1	(151)	2	4
S	8	9	11	9
G ₂	5	11	7	6
M	2	(3)	2	4

Values in parentheses are derived from a value of T obtained by metaphase accumulation."

Clowes, F. A. L. 1965. The duration of the G₁ phase of mitotic cycle and its relation to radiosensitivity. The New Phytologist 64:355-359.

G. R. Douglas

2. Temperature and nuclear cycle in maize root tips.

Douglas reported (MGCNL 42:175-178, 1968) on the nuclear cycle in root tips of 'Seneca 60' at 28°C. These studies have been extended to undertake an examination of some of the factors which might influence the duration of the components of the cycle. Hereditary and environmental factors are being considered. We report at this time data from three temperatures, 20°, 30°, and 35°C, respectively.

Our materials and methods are as described by Douglas with the following modifications:

- i) Roots were grown in constant light (ca. 8 ± 2 ft. c.)
- ii) exposure following ^3H -thymidine pulsing was for 14 days.
- iii) five primary radicles (3-4 cm) were employed for each hour of each temperature.
- iv) random samples of nuclei were scored on each slide.

Table 1

Classification and frequency of nuclei scored from root tips (*Zea mays* L., hybrid 'Seneca 60') following pulse labeling (^3H thymidine, 30 minutes) at three temperatures

Class	Temperature		
	20°C	30°C	35°C
Interphase			
Labeled	173607	145893	40255
Prophase			
Labeled	3579	5417	986
Unlabeled	11903	12644	3196
Metaphase			
Labeled	1052	1408	336
Unlabeled	4215	4444	1166
Anaphase			
Labeled	359	484	97
Unlabeled	1620	1820	437
Telophase			
Labeled	1331	2346	325
Unlabeled	6236	6102	1369
TOTAL	203902	180558	48140

The number and classification of nuclei scored are listed in Table 1. The 30°C treatment experiment was conducted first followed by the 20°C and 35°C experiments. Computation of sampling error variances demonstrated that sample sizes could be reduced to the level recorded for the 35°C treatment without sacrifice of homogeneity. Our "rule of thumb" requires about 100 nuclei in the least frequent category, i.e. labeled anaphase.

Table 2
Duration of the cell cycle in the root tips of *Zea mays* L.
(hybrid 'Seneca 60') at three temperatures

Phase	Calculated Duration					
	20°		30°		35°	
	hrs.	%	hrs.	%	hrs.	%
Interphase:						
G ₁	-2.18	-17.1	-0.93	-14.1	-0.73	-19.7
S	7.00	55.1	4.00	61.1	2.00	54.1
G ₂	6.70	52.7	2.59	39.5	2.05	55.4
sub-total	11.52	90.7	5.66	86.4	3.32	89.8
Mitosis:						
Prophase	0.60	4.7	0.46	7.1	0.20	5.4
Metaphase	0.20	1.6	0.15	2.3	0.07	1.9
Anaphase	0.08	0.6	0.06	0.9	0.03	0.7
Telophase	0.29	2.3	0.22	3.3	0.08	2.2
sub-total	1.17	9.3	0.89	13.6	0.38	10.2
Total	12.69		6.55		3.70	

Wimber's (1960) proportion method for calculating the duration of M has been used to project the estimates presented in Table 2. Until we finish collecting the data at 25°C, we have refrained from applying the more extensive probit regression analysis which will yield weighted mean values and proper standard deviations.

In the absence of statistical analyses we have formed only a few impressions of the influence of temperature on nuclear cycle: Firstly, reference to Table 2 indicates that in general the cycle components retain a proportional relationship at all temperatures tested. Since independent calculations of various components have not yet been made, the percentage values reflect not only any relationship existing in the cycle but also reflect the manner in which the component-duration values were computed. Secondly, the decreasing duration intervals with increasing temperature appears consistent with the general growth habit of corn. These data would predict little or no mitotic activity at temperatures consistently below 15°C and/or above 40°C.

The greater changes in the duration of cycle components occur in the interphase nucleus; however, the percentage changes remain constant in both the interphase and mitotic nucleus. The changes in S and G₂ we report are in general agreement with the less extensive studies of Evans and Savage (1959) with Vicia faba and Wimber (1966) employing Tradescantia paludosa.

We consistently obtained negative estimates of G₁ using Wimber's (1960) proportion method. Whether or not this is an artifact of the method of computation remains to be determined. Clowes (1965) reported that G₁ is absent in maize cap initials at 18°C.

We have begun a genotype/temperature interaction study.

Literature cited

- Clowes, F. A. L. 1965. The duration of the G₁ phase of the mitotic cycle, and its relation to radiosensitivity. New Phytol. 64:355-9.
- Evans, H. J. and J. R. K. Savage. 1959. The effect of temperature on mitosis and on the action of colchicine in root meristem cells of Vicia faba. Exp. Cell Res. 18:51-61.
- Wimber, D. E. 1960. Duration of the nuclear cycle in Tradescantia paludosa root tips as measured with H³-thymidine. Amer. J. Bot. 47:828-834.

Wimber, D. E. 1966. The duration of the nuclear cycle in Tradescantia paludosa root tips at three temperatures as measured with H^3 -thymidine. Amer. J. Bot. 53:21-24.

R. S. Verma

3. Estimates of the replication patterns of individual chromosomes.

In MGCNL 42:175-178 (1968) we presented a preliminary study of the nuclear cycle in maize root tip nuclei. This investigation has been extended to determine 3H -thymidine incorporation patterns in individual chromosomes of maize root tip nuclei.

The stock used was a chromosome 3 tester in which each cell contained one B chromosome. Autoradiographs were prepared in the manner previously reported (MGCNL 42:175-178, 1968) with the following modifications:

- 1) root tips were fixed 8, 9 and 10 hours after pulse labeling.
- 2) root tips were immersed in 0.002 M 8-hydroxyquinoline for 3 hours prior to fixation.

After photographing labeled cells the silver grains were removed in the following manner:

- 1) Slides were soaked in absolute ethanol until the coverslips were removed, then were passed through an alcohol series to distilled water.
- 2) Slides were transferred to 7.5% $K_3Fe(CN)_6$ for 3 min. and then to 20% $Na_2S_2O_3 \cdot 5 H_2O$ for 3 mins.
- 3) Following 3 changes of distilled water, slides were passed through an alcohol series to absolute ethanol and mounted in Eupanol.

Cells previously photographed were rephotographed and individual chromosomes were identified on these photographs.

Mean silver grain counts over each chromosome (Table 1) were plotted against time (between fixation and pulse labeling). A linear regression coefficient was calculated for each chromosome (Table 2). We interpret a regression coefficient of a chromosome to represent its mode of replication during that portion of the S period in which the chromosome is replicating. Thus the chromosomes demonstrating initial rapid uptake of label will possess the most positive coefficients.

Table 1
Frequency data from ^3H -thymidine pulse labeling (30 min)
in maize root tip nuclei

Chromosome	Mean number of Silver Grains		
	8 hr	9 hr	10 hr
1	6.82	5.00	3.75
2	5.94	4.67	5.44
3	6.02	3.83	4.38
4	6.06	3.67	3.69
5	5.23	3.17	3.75
6	3.97	1.50	3.06
7	3.97	3.17	3.19
8	4.09	2.83	2.87
9	4.23	3.50	3.19
10	3.54	1.83	2.62
B	6.67	1.25	5.33

Table 2
Values calculated from the data recorded in Table 1

Chromosome	Regression Coefficient	Standard Error
1	-1.54	+0.11
2	-0.23	+0.33
3	-0.86	+0.44
4	-1.22	+0.38
5	-0.37	+1.02
6	-0.51	+0.61
7	-0.40	+0.13
8	-0.57	+0.21
9	-0.53	+0.07
10	-0.48	+0.41
B	-1.12	+0.53

Even though we are reporting limited data (28 cells), certain relationships are seen to emerge: Chromosomes 1, 3, 4 and the B chromosome appear to possess the most negative coefficients. Additional data from this study suggest that the B chromosome is delayed beyond mid-S in initiating uptake of label. Further, the data suggest that the terms late and early replication are ambiguous when used to describe the replication of chromosomes. The terms indicate the time within the S period at which a chromosome begins replication, but are not necessarily descriptive of the rate or mode of replication once DNA synthesis has been initiated within a chromosome.

Differences in coefficients can be tested by appropriate tests. Large standard errors, such as the one associated with chromosome 5, may indicate curvilinear as well as linear relationships. Regression analysis was possible only because of our ability to identify the somatic chromosomes in maize, and is unique in that it quantifies the patterns of replication of specific chromosomes and allows quantitative comparisons of these patterns. We are employing cytogenetic modifications of specific chromosomes in an attempt to alter the replication patterns. In addition, we can determine gross alternatives of replication patterns in chromosome segments.

G. R. Douglas

4. An abnormal chromosome 6 in maize.

One stock of maize was found having two secondary constrictions located in the distal portion of the short arm of chromosome 6 at somatic metaphase. Two tandem satellites were observed on each homologue (Fig. 1d). If in the normal stocks the secondary constriction at somatic metaphase corresponds to the heteropycnotic nucleolar organizer at pachynema, the stock described here may have two nucleolar organizers on chromosome 6. The following observations from meiosis and mitosis seem to suggest that this abnormal chromosome may have originated from a normal 6 through a paracentric inversion with one breakpoint in the nucleolar organizer and the other proximal to the organizer (Fig. 1). To conform with maize terminology, we have assigned "A6" to this abnormal no. 6 chromosome.

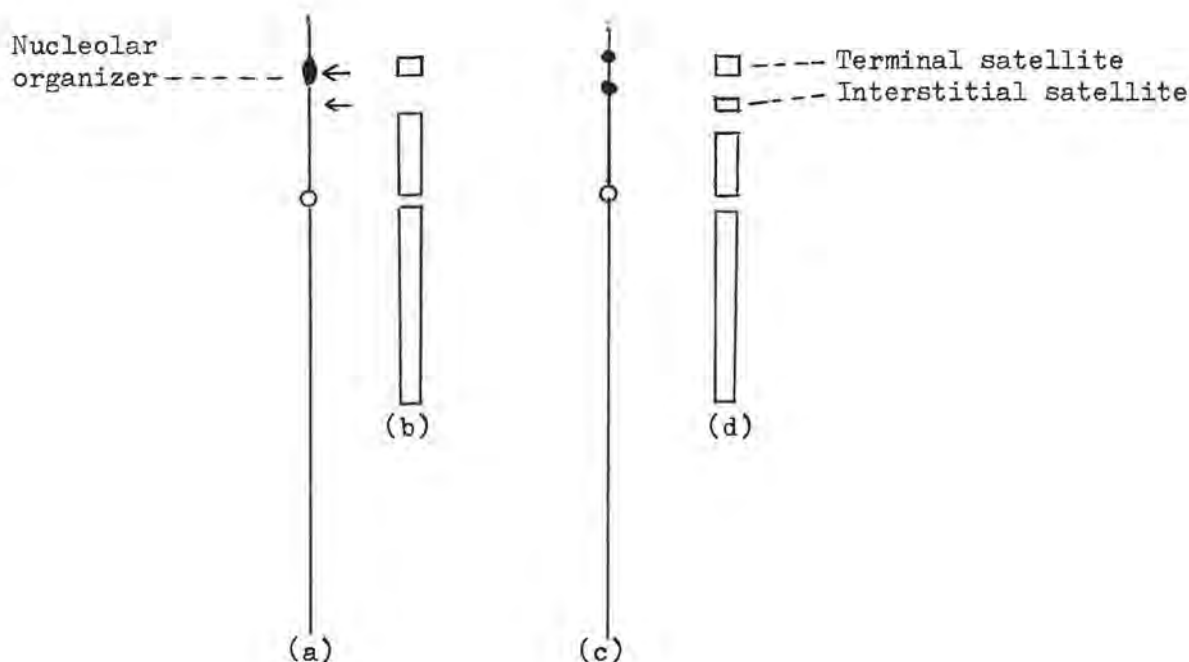


Figure 1. Diagram of the normal and abnormal chromosome 6 at pachynema and somatic metaphase.

- (a) Normal chromosome 6 at pachynema, arrows indicate break-points.
- (b) Normal chromosome 6 at somatic metaphase.
- (c) Abnormal chromosome 6 at pachynema.
- (d) Abnormal chromosome 6 at somatic metaphase.

1. Bridges and fragments were found infrequently at anaphase I in the microsporocytes of two heterozygous plants (N6/A6). Since a bridge and fragment could result from a crossover event occurring in the inverted segment, the observation of a loop at pachynema should provide direct cytological evidence for the paracentric inversion hypothesis. However, no such configuration was seen, probably because the inverted segment is too small to permit detection of a loop. We did observe some abnormalities such as two heteropycnotic regions, two nucleoli (usually one large and one small), and loose pairing of the segment distal to the nucleolar organizer region in the microsporocytes of both the homo- and heterozygous abnormal 6 plants.

2. The normal and the abnormal chromosome 6 in the somatic metaphase cells of heterozygous plants were measured and compared. The results from measurements of 44 cells are presented in Table 1. The

Table 1
Measurements of N6 and A6 chromosomes from 44 somatic metaphase cells

	Normal chromosome 6			Abnormal chromosome 6				
	Long arm	Short arm	Satellite	Long arm	Short arm	Interstitial satellite	Short arm + Int. sat.	Terminal satellite
Mean length (u)	2.68	1.39	0.54	2.60	1.03	0.40	1.43	0.49
S.E. of mean	0.051	0.024	0.019	0.053	0.024	0.021	0.032	0.018

Table 2
Transmission of the abnormal chromosome 6

Cross	Chromosome constitution in the progeny			X ² value for 1:1 or 1:2:1 ratio
	N6/N6	N6/A6	A6/A6	
N6/A6 ♀ x N6/N6 ♂	27	27		0
N6/A6 ♀ x A6/A6 ♂		28	26	0.07
N6/N6 ♀ x N6/A6 ♂	25	44		5.23*
N6/A6 (x)	26	62	49	9.39**

*Significant

**Highly significant

difference between the components of the normal 6 and the corresponding components of the abnormal 6 was tested by means of t test. There was no significant difference between the length of the satellite of the normal 6 and that of the terminal satellite of the abnormal 6 ($t = 1.7452$, d.f. = 43). The short arm of the normal 6 is apparently longer than the short arm of the abnormal 6 ($t = 11.0689$, d.f. = 43). However, when the interstitial satellite was taken as one part of the short arm of the abnormal 6, the difference between the short arm of the normal 6 and that of the abnormal 6 was not significant ($t = 1.7999$, d.f. = 43).

To study the transmission of abnormal 6, heterozygous plants were self-pollinated and backcrossed to N6/N6 and A6/A6 plants. The results from the crosses are shown in Table 2. The abnormal 6 was transmitted more frequently than the normal through the male gametes. It seems that the pollen grains with one, non-crossover, paracentric inversion on chromosome 6 may have selective advantages over the normal pollen grains (without A6) in germination and tube growth. Therefore, A6 will spread throughout the stock after several generations of self-pollination and/or sib-crossing.

C. C. Chen

5. Abnormal recovery of parental classes in the heterozygous translocation 9 - 2c.

A series of translocation stocks, all involving the short arm of chromosome 9, was obtained from the Maize Co-Op for mitotic studies. Meiotic and recombinational data were taken on a majority of the stocks. The stock in question, 9 - 2 c (9s.33 - 2s.49), T $+++$ /N $wx\ bz_1\ sh_1$, was backcrossed to the chromosome 9 tester. The data in Table 1 demonstrate (a) no recovery of crossover events between $sh_1 - bz_1$; (b) a marked reduction in crossing-over between $bz_1 - wx$; and (c) an abnormal ratio for the two parental classes ($+++$ and $wx\ bz_1\ sh_1$) from both the σ and ϕ T/N parents.

A comparison of crossing-over in the $bz_1 - wx$ region through the σ and ϕ (using X^2 and Maximum Likelihood variance estimates) showed no significant difference at the 5% level. Homogeneity tests within the σ and ϕ populations (cob to cob) showed no significance at the 5% level.

Table 1

Backcross data from T 9 - 2 c $\pm\pm\pm$ /N wx bz₁ sh₁ x wx bz₁ sh₁ tester

T/N Parent	Genotypes					Ratio : 1	% Recombination	
	+	sh ₁	+	sh ₁				
	+	bz ₁	+	bz ₁				
	+	wx	wx	+	Σ	$\frac{+}{sh_1} \frac{+}{bz_1} \frac{+}{wx}$	sh ₁ -bz ₁	bz ₁ -wx
♂	3051	1826	64	85	5026	1.69 \pm .09	0	2.96
♀	4510	2457	132	66	7165	1.84 \pm .05	0	2.76

A Wilcoxon Rank Sum test yielded a significant difference (1% level) between the ♂ (1.69 \pm .09) and ♀ (1.84 \pm .05) ratios. Thus the two populations (♂ and ♀) are each homogeneous; the recovery of the non-translocated chromosomes is, in each population, significantly higher than expected; and the two ratios are significantly different.

Abnormal segregation from a T/N has been reported previously. Nuffer (MGCNL 35:94) reported for the wx 9 translocation series a consistently higher ratio of normal to translocated chromosomes through the ♀. This effect, however, was not as consistent in the ♂. Lima-de-Faria (MGCNL 33:66) reported (T5-6y/NY) a significantly higher proportion of translocated chromosomes through the ♂. He suggested differential fertilization to account for his observed discrepancy commenting that "gametophytes carrying translocated chromosomes are apparently more viable than those with normal chromosomes."

The apparent recovery of a higher proportion of translocated chromosomes in our study supports Lima-de-Faria's suggestion of differential fertilization. However, if differential fertilization were involved, one would expect the proportion of starchy/waxy pollen grains to approximate a 1:1 ratio. From several thousand counts, we found the $\pm:w\bar{x}$ ratio to be 1.69:1, which approximates the ratio in the backcross progeny using the T/N as the ♂.

The frequencies of the configurations at diakinesis (Table 2) are similar to those suggested by Burnham (1962) for long interchange segments. However, we recorded 36.1% pollen sterility. B chromosomes and KlO are not present in this stock.

Table 2
Cytological data from T 9 - 2 c/N

	Diakinesis			Pollen	
	⊙ 4	Chain 4	Pairs	Abortion	Ratio : 1 +/wx
frequency	756	64	0	36.1%	1.69

At present, two more tests are being carried out; (a) intercross data from T/N x T/N, subsequent scoring of wx, bz₁, sh₁, and the occurrence of the three types of chromosomal combinations (TT, TN, NN); (b) screening of a large number of wx, bz₁, sh₁ genotypes in an attempt to recover crossover events between the waxy locus and the breakpoint. Recovery would permit the reciprocal test, namely the recessive alleles on the translocated chromosome and the dominant alleles on the non-translocated chromosome. Nuffer's data using the Wx allele as a marker on N, indicated a higher proportion of normal chromosomes were transmitted through the p. However, 9 - 2 c displays the reverse. By changing the allele-chromosome combination, some insight into the abnormal chromosome segregation and its relationship, if any, to the waxy or adjacent loci may be forthcoming.

W. G. Fillion

6. Biometrical analyses of somatic (root-tip) chromosomes.

The maize root-tip karyotype procedures provide an experimental system within which can be studied environmental and heritable factors affecting chromosome parameters. We have initiated a study in which we propose to examine the biometrical modifications:

1. on the entire complement, which might result from the influence of gene loci known to alter chromosome behavior;
2. on individual chromosomes as a result of cytogenetic alteration.

Below are presented representative data from one of the stocks used as a basis for several studies in this laboratory.

Table 1
Summary of maize chromosome statistics compiled from 38 metaphase
root-tip karyotypes of hybrid 'seneca 60'

	Chromosome									
	I	II	III	IV	V	VI	VII	VIII	IX	X
A. Mean Arm Ratio	1.20	1.50	1.91	1.46	1.09	2.25	2.30	2.66	1.64	1.95
standard error	0.06	0.07	0.08	0.06	0.06	0.10	0.08	0.11	0.08	0.08
B. Mean Relative Length	14.40	12.02	11.09	10.99	10.39	8.97	8.42	8.60	8.00	7.12
of <u>homologue</u>										
standard error	0.16	0.13	0.13	0.12	0.13	0.13	0.13	0.13	0.12	0.12
C. Mean Relative Length	7.81	7.11	7.25	6.51	5.39	6.16	5.85	6.21	4.94	4.69
of <u>long arm</u>										
standard error	0.09	0.10	0.08	0.08	0.06	0.09	0.08	0.08	0.07	0.61
D. Mean Relative Length	6.59	4.90	3.84	4.48	5.00	2.80	2.57	2.39	3.06	2.43
of <u>short arm</u>										
standard error	0.10	0.06	0.07	0.06	0.07	0.06	0.05	0.07	0.06	0.04
E. Variances										
Between homologues	1.96	0.96	0.51	0.90	0.60	1.05	0.47	0.71	0.55	0.43
Between long arms	0.66	0.65	0.39	0.58	0.29	0.63	0.34	0.47	0.38	0.24
Between short arms	0.96	0.27	0.37	0.21	0.23	0.27	0.12	0.33	0.30	0.10

Filion has commented (MGCN 42:175, 1968) on the use of computers in root-tip karyotype analysis. Technical difficulties with the maize system were discussed in relation to the use of the expensive and complex automated data input systems such as FIDAC, CYDAC, and the CHLOE Film Scanner.

Despite our inability to automate the mensuration of the chromosomes of maize, we feel that the opportunity to process large amounts of data in repetitious and complex calculations can still be useful for karyotype analysis. Using Fortran IV, we have written a simple but flexible program to allow rapid calculation of the various chromosome parameters for any number of chromosomes or cells. This program, now available on remote access call to our IBM 7040, may be obtained by writing the undersigned. The program accepts chromosome arm lengths and the centromere coordinate plots. The latter are obtained from a grid placed over the spread. All metaphase distributions are accepted by the program. The program permits continued evolution of the confidence limits employed to first identify and secondly, analyze the chromosomes. Presently, the chromosomes are assigned an identification (I-X) in the computer program on the basis of four criteria: relative length, arm ratio, long and short arm relative lengths. A subroutine classifies the satellited chromosome VI before identifying the rest of the complement. By integrating the normal curve for each chromosome I to X derived from previous experimentation, we can arrive at a probability expression summarizing the four criteria to yield the best possible fit for the unknown chromosome. Thus, the computer print out provides a numerical identity and an approximation of the reliability of this estimate. The program is useable whether or not all chromosomes of a metaphase spread are presented for analysis and, in addition, it is useable with aneuploid stocks.

Photographs of 38 metaphase spreads of 'Seneca 60' (x 3,000) were projected to give magnifications of approximately X 30,000. The more distinct chromatid of each chromosome was traced and measured. Following, these measurements of arm lengths were entered into the computer and processed. Table 1 contains the statistics for each chromosome. To eliminate errors due to magnification, relative lengths of arms and chromosomes per cell were indexed:

$$\frac{\text{length of segment}}{\text{total length of complement}} (x 50) = \text{relative length}$$

The arm ratio of chromosomes has been considered to be a rather stable chromosome parameter and although our data indicate variability of less than 10% for most chromosomes, chromosomes VI and VIII demonstrate unusual variability. The short arm of chromosome VI was measured in these studies to the end of the intact arm but not including the stalk and satellite. Reference to Table I indicates that excessive variation in chromosome VI is found in both arms. Likewise, chromosome VIII demonstrates considerable arm variation, particularly in the short arm. It is interesting to note that these same somatic chromosome arm ratios showed the greatest discrepancy with the published pachytene arm ratios. We are in the process of ascertaining the pachytene arm ratio of the 'Seneca 60' stock.

Several predictions can be derived from these chromosome parameters. Insofar as the corn system is "typical", this biometrical approach may be useful to investigators employing other organisms, particularly the organisms in which experimental possibilities are limited. For instance, the problem of detecting a translocation from only somatic metaphase chromosomes haunts the human cytogeneticists. The parameters presented in Table 1 can be used to provide ascertainment indices of the following magnitude:

1. The shortest minimal alteration segment capable of detection would occur in 3L. Appropriate biometrical tests will detect a difference as low as 2% of the length of 3L at a 95% confidence level based on a sample of 35 cells, if 3 is already identified.
2. The longest minimal alteration segment capable of detection would occur in 8S. A difference as low as 6% with sample size of 35 cells would be detected at the 95% confidence level if 8 is already identified.

We should point out that the two statements above do not imply identification of the chromosomes. Ascertainment of chromosome identity involves all chromosomes of the complement in our program; hence alterations in arm length may create new 'overlap' among the criteria used for

identification. In such cases, the resolving power for purposes of identification may have minimal estimates in excess of the 2% and 6% respectively, cited above.

We obtained an estimate of 35 as the number of nuclei which should be analyzed to obtain the level of significance needed to independently detect all chromosomes in the maize complement. This estimate will change as specific chromosome changes are introduced through experimental procedures.

The real value of these biometrical procedures lies not in whole nuclei analysis but in analysis of specific chromosomes. As few as 70 observations (one per 70 cells or two per 35 cells) for a single homologue will provide a discriminating power of greater than 90%.

We are extending these analyses to additional normal stocks and several aberrant stocks. Particular attention will be paid to segment specific variation as well as to the changes induced by the aberrant stocks.

J. D. Horn
D. B. Walden

UNIVERSITY OF WISCONSIN
Madison, Wisconsin
and
INDIAN AGRICULTURAL RESEARCH INSTITUTE
New Delhi, India

1. A description of R^{ch} alleles.

Even though R^{ch} was discovered more than twenty-five years ago (Anderson 1941, MGNL 15:4) no detailed description of its phenotype is available in the literature. In Dr. Brink's laboratory a good collection of R^{ch} stocks has been assembled; all of them have been repeatedly backcrossed to W22 and so possess a uniform genetic background. I worked with this collection for a considerable time and I hope the following description will be helpful to the future workers.

Three of the original collections, namely Peru Corongo 120 R^S , Peru Corongo 150 R^S , and Ecuador R^r , were not initially suspected to be cherry alleles and only during subsequent study was their ability to promote pericarp pigment discovered. Subsequently these alleles have

been found to behave differently from others in certain respects and so the original gene symbols were not changed. In general terms, however, all the \underline{R} alleles that are capable of developing cherry pericarp will be referred to as \underline{R}^{ch} alleles. The salient features of some of the cherry alleles are given in Table 1.

Table 1
Main phenotypic characteristics of some \underline{R}^{ch} alleles

Allele	Identification	Aleurone phenotype ($\underline{R} \underline{r} \underline{r}$)	Pigmentation		
			Roots and coleoptile	Leaves	Anthers
Stadler \underline{R}^{ch}	-	Pale mottling	+	-	+
New Mexican 1 \underline{R}^{ch}	PI 218151	Pale mottling	+	-	+
New Mexican 2 \underline{R}^{ch}	PI 218159	Pale mottling	+	-	+
Pueblo \underline{R}^{ch}	-	Pale mottling	+	-	+
Ecuador \underline{R}^r	1172	Self color	+	+	+
Peru Corongo 120 \underline{R}^g	-	Self color	+	+	-
Peru Corongo 150 \underline{R}^g	-	Self color	+	+	-

The \underline{R}^{ch} seedlings in germinating pans under constant light resemble those of \underline{R}^r except the coleoptiles and roots show relatively less anthocyanin pigment. The presence of \underline{Pl} has no effect at this stage of plant growth. The seedlings can develop some pigment in darkness, but this is neither a constant nor a distinct feature. The mature \underline{R}^{ch} plants can be distinguished from other genotypes by examining the nodes which are characterized by deep red colour. The presence of \underline{Pl} usually accentuates this character but if both \underline{B} and \underline{Pl} are present then identification by this method will not be possible. Anther colour when present is in small streaks on the anther walls; when \underline{Pl} is absent and \underline{R}^{ch} is heterozygous with a green anthered allele, then the anthers are practically green. The anther colour of Ecuador \underline{R}^r is as red as standard \underline{R}^r , and hence is an exception. Pink silk colour is a common property of all cherry alleles in the Wisconsin collection. This is also true for \underline{r}^{ch} .

It should be noted, however, that while all cherry alleles are characterized by pink silks the converse is not true. Argentina \underline{R}^r , \underline{R}^{nj} and many varieties of sweet corn, none of which react with \underline{Pl} to produce pericarp colour, were found to possess pink silks.

The appearance of anthocyanin pigment in the aleurone of \underline{R}^{ch} is not different from that of \underline{R}^r , but the kernels with one dose do not exhibit the mottling characteristic of standard \underline{R}^r , but show a phenotype with dilute coloured patches on dark background. The nonparamutable \underline{R}^{ch} alleles, like Ecuador \underline{R}^r , do not show any type of mottling. Stadler \underline{R}^{ch} , Pueblo \underline{R}^{ch} , and both alleles from New Mexico have been found to possess one or two dominant modifiers which partially inhibit the aleurone pigment. The modifiers are not known to affect any other property of \underline{R}^{ch} . Standard \underline{R}^r and Ecuador \underline{R}^r are not sensitive to their action.

The basic feature of all cherry alleles is their ability to promote formation of a deep red, water soluble pigment in the pericarp when another factor, \underline{Pl} , is also present. It has been found that even $\underline{b} \underline{pl} \underline{R}^{ch}$ plants develop pericarp pigment if the ears are exposed to light by removing the husks. The details of this technique have been already reported (MGNL 39:178).

Plants of Ecuador \underline{R}^r and both Peru Corongo alleles, in addition to the above mentioned features, show some red pigment in the leaf blades, and more prominently in the mid-rib region. The pigment develops when the plants are 8-10 weeks old and is best scored after two or three cool nights. When these stocks were grown in India, plants never developed leaf colour in the summer season. Presence of \underline{Pl} accentuates this character.

From the preferential segregation pattern, both New Mexican \underline{R}^{ch} stocks are inferred to carry the heterochromatic knob on chromosome-10. On W22 background at least, when the knob is present in homozygous condition, the plants bend and tend to be twisted.

The aleurone-pigmenting ability of Stadler, Pueblo and New Mexican \underline{R}^{ch} alleles is reduced when they pass through the heterozygous condition of \underline{R}^{st} . In this sense, they are paramutable. Their pigmenting ability in the pericarp is not affected. Ecuador and Peru Corongo alleles are not paramutable.

Two less-studied \underline{R}^{ch} alleles are also present in the Wisconsin collection; of these two, Costa Rica \underline{R}^{ch} (obtained from Dr. Mangelsdorf) more closely resembles Stadler \underline{R}^{ch} . The second one (maize morado, courtesy of Dr. Greenblatt) is still not well backcrossed. The paramutability of these two stocks has not yet been tested.

To complete this account, a few words about \underline{r}^{ch} may be added. This allele has long been known to possess colourless aleurone, red anthers, pink silks, and cherry pericarp (in the presence of \underline{Pl}). This is known to occur in nature and was also obtained as a mutant from \underline{R}^{ch} .

The interaction of \underline{R}^{ch} and \underline{r}^{ch} with other genes like \underline{C}_1 is very interesting. They will be described in another publication.

G. R. K. Sastry*

*Present address:

Beit Memorial Research Fellow
Department of Genetics
University of Leeds
Leeds, LS2 9JT, U.K.

2. Recombinational analyses of Ecuador \underline{R}^r (1172) and Peru Corongo \underline{R}^g alleles.

In 1964, Bray presented evidence (MGNL 38:134) for the presence of a plant colour factor closely and distally linked to the \underline{R} locus (1 to 2 units) in Ecuador \underline{R}^r . The production of pericarp colour in the presence of \underline{Pl} and the production of pink silks and red colour in the leaves were attributed to this factor. Since the appearance of Bray's work two more alleles from Peru (Peru Corongo 120 and 150) were also found to possess all the features of Ecuador \underline{R}^r except that their anther colour is green (hence \underline{R}^g). A recombinational analysis of these alleles was conducted to study the problem further.

$\underline{R} \underline{R}^{st}$ and $\underline{R} \underline{r}^g$ plants of all three alleles were pollinated by W22 $\underline{r}^g \underline{r}^g$. From the resulting ears $\underline{R} \underline{r}^g$ and $\underline{R}^{st} \underline{r}^g$ or $\underline{r}^g \underline{r}^g$ kernels were planted separately in detasseling plots. (These kernels were not in equal number and hence the discrepancy in parental classes in Tables 2 and 3). The staminate parent in the detasseling plots was W22 $\underline{r}^g \underline{r}^g$.

When the plants were 8-10 weeks old the leaf colour was checked and all exceptional plants were tagged. Notes on silk colour were taken at pollination time and all exceptional plants were labelled. Two to

three weeks following pollination the ears of exceptional plants were exposed to sunlight to determine their ability to produce pericarp pigment.

When the data thus obtained were summarized, three facts emerged: (1) In both Ecuador \underline{R}^r and Peru Corongo alleles, pink silks and cherry pericarp are conditioned by different and separable elements. These will be referred to as (Si) for silk colour and (Ch) for pericarp colour. The leaf colour is conditioned by a different element which is designated as (Lc). (2) All three components are situated distally to (P) (S). ((P) = coloured anthers and (S) = coloured aleurone as in Stadler's terminology). (3) The different plant colour components of both Ecuador \underline{R}^r and Peru Corongo \underline{R}^g complexes are easily separable.

Table 2

Pooled linkage data for different components of Ecuador \underline{R}^r complex:
frequencies of different genotypes obtained from the crosses
Ecuador $\underline{R}^r/\underline{r}^g \times \underline{r}^g/\underline{r}^g$ and Ecuador $\underline{R}^r/\underline{R}^{st} \times \underline{r}^g/\underline{r}^g$.
(In the following table \underline{r} means either \underline{r}^g or \underline{R}^{st} .)

Combination	Genotype	No. plants	Percentage
Parental	$\underline{R}-\underline{Si}-\underline{Lc}$	482	
Parental	$\underline{r}-\underline{si}-\underline{lc}$	648	
Recomb.-I	$\underline{R}-\underline{si}-\underline{lc}$	15	
Recomb.-I	$\underline{r}-\underline{Si}-\underline{Lc}$	9	2.06
Recomb.-II	$\underline{R}-\underline{Si}-\underline{lc}$	3	
Recomb.-II	$\underline{r}-\underline{si}-\underline{Lc}$	3	0.51
Doubles	$\underline{R}-\underline{si}-\underline{Lc}$	5	
Doubles	$\underline{r}-\underline{Si}-\underline{lc}$	0	0.43
Total plants		1165	

Expected double crossovers = 0.0234%
Observed double crossovers = 0.4300%
Coefficient of coincidence = 18.70%

Tentative map of Ecuador \underline{R}^r complex

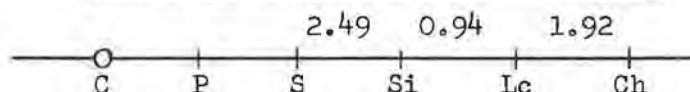


Table 3

Linkage data for different components of Peru Corongo R^G complex: frequencies of different genotypes obtained from the crosses Peru Corongo $R^G/r^G \times r^G/r^G$ and Peru Corongo $R^G/R^{st} \times r^G/r^G$. (Data of both Peru Corongo alleles were pooled. In the following table r means either r^G or R^{st} .)

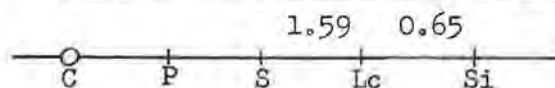
Combination	Genotype	No. plants	Percentage
Parental	<u>R-Lc-Si</u>	395	
Parental	<u>r-lc-si</u>	652	
Recomb.-I	<u>R-lc-si</u>	7	
Recomb.-I	<u>r-Lc-Si</u>	9	1.50
Recomb.-II	<u>R-Lc-si</u>	4	
Recomb.-II	<u>r-lc-Si</u>	2	0.56
Doubles	<u>R-lc-Si</u>	1	
Doubles	<u>r-Lc-si</u>	0	0.09
Total plants		1070	

Expected double crossovers = 0.01%

Observed double crossovers = 0.09%

Coefficient of coincidence = 9.00%

Tentative map of Peru Corongo R^G complex



The linkage data are presented in Tables 2 and 3. Double recombinants in Ecuador R^r were found to be almost equal to recombinants between (Si) and (Lc) (region II). If the double recombinants are considered as recombinants in region II, then the position of (Si) and (Lc) will be switched as in Peru Corongo alleles. Tentative genetic maps are also given for Ecuador and Peru Corongo alleles under Tables 2 and 3, respectively. The position of (Ch) in the Ecuador R^r map has been fixed by studying its distribution in plants which are recombinants for (Si) and (Lc). The presence of more double recombinants than expected resulted in high negative interference (coefficient of coincidence in

Ecuador $\underline{R}^r = 18.70$ and in Peru Corongo $\underline{R}^g = 9.00$). No simple explanation can be given for this high negative interference, although some analogies can be drawn from similar phenomena occurring in organisms like *Neurospora*, *Aspergillus* and yeast. However, these analogies necessarily require elaborate and complex models for which no evidence is available in the present experiments. On the other hand, if some or all plant colour components are transposable, then also the results simulate "loose linkage" and "negative interference".

Mutation studies of Stadler \underline{R}^{ch} and New Mexico \underline{R}^{ch} alleles (described above) indicated that the two components (Si) and (Ch) are between (P) and (S).

G. R. K. Sastry*

*Present address: University of Leeds

3. Further studies on mutable \underline{R} from Bolivia.

In the 1965 Maize News Letter a short note on a new mutable allele from Bolivia was included. This material was grown in India during the winters of 1966 and 1967. The material obtained from the two crops showed a very low mutable pattern in aleurone tissue compared to the material grown in Wisconsin. Fully coloured plants (from fully coloured aleurone mutants) also showed less pigmentation. This may be due to higher temperatures in India. Such changes were not seen in any other \underline{R} alleles.

An attempt to reconstitute mutable \underline{R} from fully coloured mutants has failed.

G. R. K. Sastry*

*Present address: University of Leeds

ADDENDUM:

UNIVERSITY OF OSAKA PREFECTURE
Sakai, Osaka, Japan
Laboratory of Genetics and Plant Breeding

1. Electrophoresis of embryo proteins of maize.

Water soluble components of the proteins of the maize embryo were separated by acrylamide gel electrophoresis as described by Steward et al. (1965) using a 7.5% polyacrylamide gel column 45 mm deep. For the preparation of extracts, 1 to 1.5 embryos per column were homogenized in tris-glycine buffer and the resulting homogenates were centrifuged at 4000 rpm for 10 minutes. The supernatant was directly added to the tube above an upper gel layer (3.75%, 14 mm deep). The electric current was regulated to 3 mA/column between upper and lower electrode vessels containing tris-glycine buffer of 0.1 M and pH 8.6, and lasted about 80 min until the indicator dye (BPB) attained the lower end of the column. The gels were stained in amido-black solution for one hour, then destained electrolytically using 7% acetic acid and in most cases the same apparatus as used for electrophoresis. The materials used are shown in Table 1. Since the stocks were unfortunately not isogenic in background, electrophoretic differences among lines cannot be directly related to the genotypes.

Table 1
The list of genotypes used

Line	Genotype	Chromosome having recessive genes
1	<u>ws</u> ₃ , <u>lg</u> ₁ , <u>gl</u> ₂ , <u>b</u>	#2
2	<u>ra</u> ₂ , <u>lg</u> ₂	#3
3	<u>la</u> ₁ , <u>su</u> ₁ , <u>gl</u> ₃	#4
4	<u>a</u> ₂ , <u>bm</u> ₁ , <u>bt</u> ₁ , <u>bv</u> ₁ , <u>pr</u>	#5
5	<u>o</u> ₂ , <u>v</u> ₅ , <u>ra</u> ₁ , <u>gl</u> ₁	#7
6	<u>c</u> , <u>sh</u> ₁ , <u>wx</u> ₁ , <u>gl</u> ₁₅	#9
Tama, Flint stock carrying the dominant loci for all recessive genes listed above.		

A total of 18 discernible bands was obtained in Tama, and the other lines lacked one or two of them. The bands were labelled 1a to 6, with 6 nearest the origin, as shown in Fig. 1. The genotypes (or lines) differed from one another quantitatively rather than qualitatively. Line 1 was characterized by the absence of 1b and 1d. Lines 2 and 3 resembled each other, but line 3 was differentiated by the absence of the 2d band. Line 4 has the 3d band characteristically faint. Both lines 5 and 6 had faint bands at 1b and 1d, but line 6 differed from line 5 in having a strong 2b and faint 2d bands. It was of interest that all the protein bands in line 5 tended to distribute in slower side of column, while in the hybrid of line 5 x Tama the proteins were normally distributed, losing this tendency.

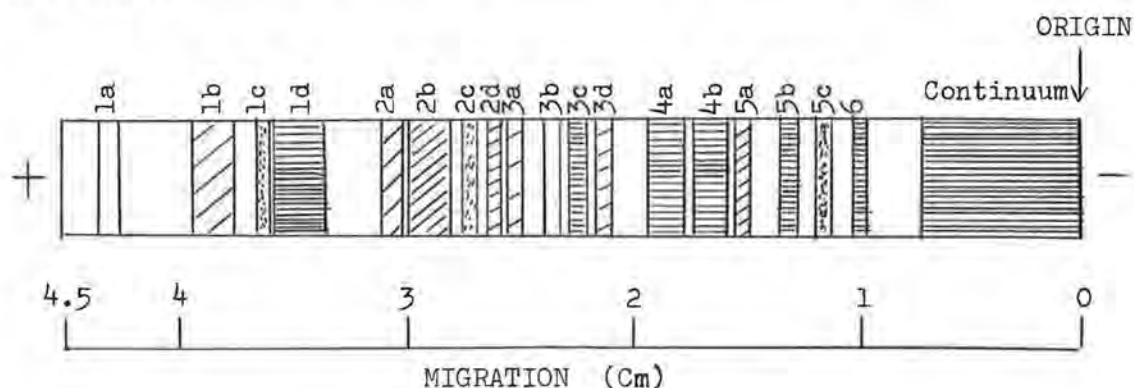


Fig. 1. Idiogrammatic pattern of the protein bands of the Tama embryo.

The minute inspection of protein bands including their qualitative and quantitative nature may reveal molecular relationships between genotypes within species.

Kenjiro Kinugawa
Yoshihiro Tsujimoto
Kazuko Hiramatsu

2. Receptivity to gametes in the female inflorescence and the time elapsed from pollination to fertilization in maize.

The female inflorescence in maize loses the ability to receive male gametes if it is maintained intact for a long time without pollination. In the present experiment, H-73, a homozygous diploid line, was

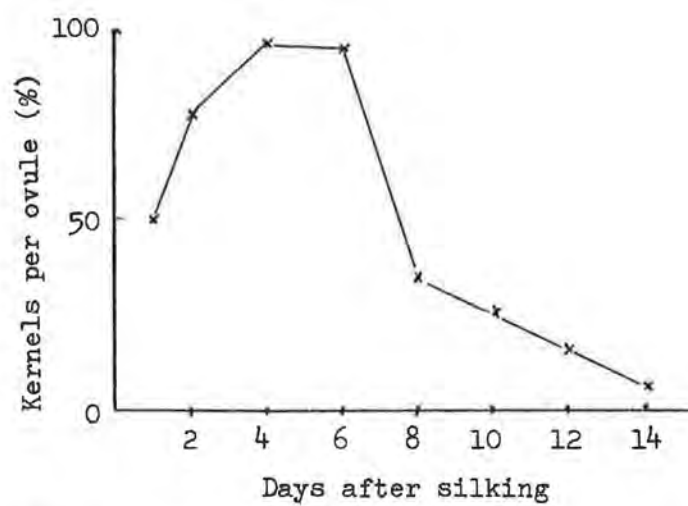


Fig. 1. The time of pollination and the receptivity of female inflorescence.

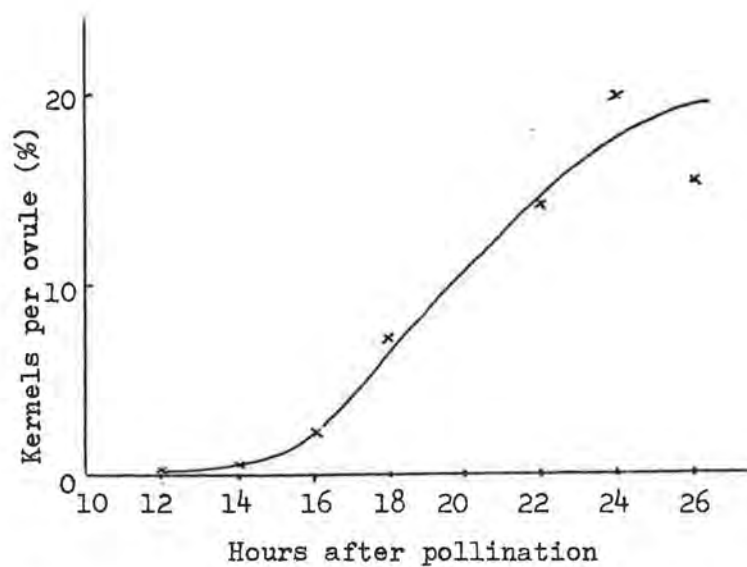


Fig. 2. The time required for fertilization.

used. The silks were cut off together with the uppermost part of the husks at various times after silking and pollinated on the following day. In Fig. 1, the kernels per ovule in percent, which were counted one month after pollination, were plotted against the time of pollination. The figure shows that the kernels per ovule rose until 4 to 6 days after the start of silking, then diminished gradually. The early rise in receptivity coincided with the increase in the number of silks emerged; the kernels usually crowded around the middle part of the cob following early pollination, while the filling of the tip with kernels was achieved by pollination at a rather advanced time. The silks were cut off at 1 cm above the husks and then pollinated. At various times after pollination, the silks were carefully removed from all ovules on the stripped inflorescences; then the inflorescences were wrapped again in husks and paper envelopes. The results were examined one month later. No kernels set on the ears where silks had been removed up to 12 to 16 hours after pollination, while the numbers of kernels increased as the time of removal of silks was prolonged. Since the curve in Fig. 2 roughly coincided with the cumulative frequency curve of silk length, the time necessary from pollination to fertilization may be proportional to the length of the silks.

Kenjiro Kinugawa
Kazuko Hiramatsu
Yoshihiro Tsujimoto

3. Mutation frequencies of five endosperm loci induced by u.v. irradiation of maize pollen.

Pollen grains of Tama (inbred flint stock), homozygous for Sh₁, Bt₁, O₂, Su₁, and Wx₁, were irradiated with ultraviolet light from germicidal lamps in a dark room under the following conditions, and stored within black vials placed in a cool environment:

Year	intensity (ergs/mm ² /sec)	dosage (ergs/mm ²)
1968	1080	1.8 - 4.5 x 10 ³

Four recessive tester stocks (la su₁ Tu gl₃, a₂ bm₁ bt₁ bv pr, o₂ v₅ ra₁, and c sh₁ wx₁ gl₁₅ in genotype, respectively) were pollinated by the irradiated dominant pollen. Immediately after pollination the treated ear was wrapped in aluminum foil for 24 hours, and the pollen was

prevented from photoreactivation except at the time of pollination when the pollen was exposed to daylight for only 15 to 30 seconds. The results for whole and chimeral mutations are separately shown in Table 1. The mutation frequency was the highest for o_2 and progressively lower for su_1 , wx_1 , bt_1 and sh_1 . The higher frequency in the mutation of $Sh_1 Wx_1$ to $sh_1 wx_1$ was presumably caused by aberrations in the short arm of chromosome 9, because if mutations occurred independently in both loci, double mutations would have appeared in a much smaller frequency than the present value. The high o_2 mutation may be due to the terminal position of the locus in the short arm of chromosome 7. However, the frequent mutations of Su_1 would not support this hypothesis, because Su_1 is near the centromere in chromosome 4. This locus may include some molecular elements sensitive to u.v.

Table 1
Mutation rates at five endosperm loci of maize pollinated
with UV-irradiated pollen grains in 1968

Mutation	Number of seeds set	Whole mutation		Chimeral mutation		Total ($\times 10^{-4}$)
		obs.	rates ($\times 10^{-4}$)	obs.	rates ($\times 10^{-4}$)	
$Su_1 \rightarrow su_1$	15283	59	41.30	113	79.18	120.40
$Bt_1 \rightarrow bt_1$	1252	2	15.96	7	55.97	71.87
$o_2 \rightarrow o_2$	15020	86	57.21	201	133.79	190.95
$Sh_1 \rightarrow sh_1$	12502	17	13.59	24	19.19	32.75
$Wx_1 \rightarrow wx_1$		19	15.19	74	59.18	74.34
$Sh_1 Wx_1 \rightarrow sh_1 wx_1$		24	19.99	29	23.19	43.18

Kenjiro Kinugawa
Kazuko Hiramatsu
Yoshihiko Tsujimoto

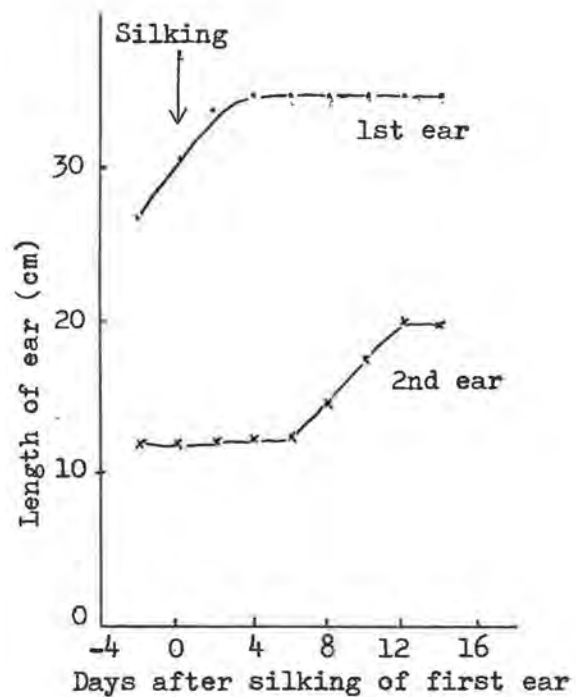


Fig. 1. The growth of ears when first and second ear present.

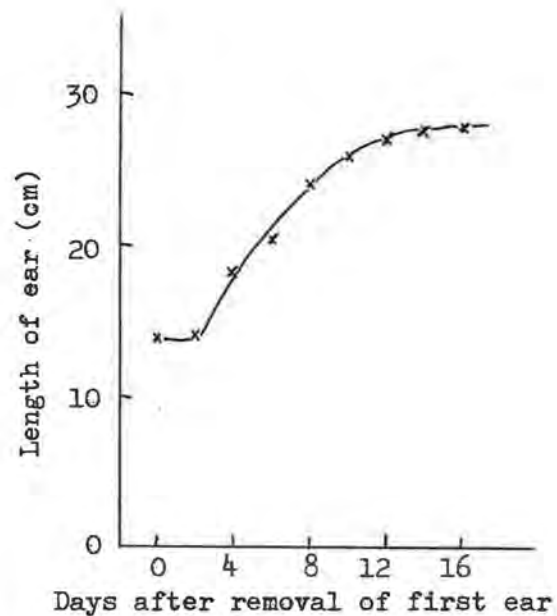


Fig. 2. The growth of second ear. The first ear was removed at time 0.

4. Regulation of ear growth by the organs of upper position in maize.

The experiment was conducted in 1968 using H-73, a homozygous diploid line. In the maturation period of the plant, the second ear from the top usually appeared from the leaf sheath earlier than the first ear, then entered into a temporary suspension for several days. The second ear resumed its active growth after the growth of the first ear attained nearly saturation. This is shown in Fig. 1, where the length (cm) of first and second ears was plotted against days scored from the date of silking of the first ear. Although the lengths of the youngermost ears lying concealed within the leaf sheath could not be measured, the two step growth of the second ear was easily estimated from the curve. The interposed suspension of second ear growth possibly resulted from the developing first ear, because the period of suspension coincided well with the logarithmic growth of the first ear. Then, first ears were removed as early as possible and the growth of the second ear was measured with time. Uniformly growing plants were selected for the experiment, and at the time of examination, all second ears were in the state of interposed suspension. As shown in Fig. 2, the second ears in seven out of 10 plants began to grow shortly after removal of the first ears. The reasons why the development of the first ear is retarded as compared with second ear in an earlier period have not been examined. However, it seems possible that the developing young tassel suppressed the first ear development. In many cases, it was observed that the first ears entered into prominent growth after the main emergence of tassels was finished.

Kenjiro Kinugawa
Yoshihiro Tsujimoto
Kazuko Hiramatsu

IV. REPORT ON MAIZE COOPERATIVE

A total of 168 seed requests were supplied during 1968. This was the largest number of requests for a one year period. A total of 122 (72 percent) of the requests were from 26 states in the U.S. and 46 (28 percent) requests were from 22 foreign countries. In addition, 73 percent of the requests were from Geneticists, 17 percent from Plant Breeders and 10 percent for educational purposes. A total of 88 requests for opaque-2 and floury-2 have been received during the three year period 1965-1968; 65 of the requests were from the U.S. and 23 from foreign countries.

During the summer of 1968 seed increases were made on certain maize genetic testers for chromosomes 1, 2, 3, 4, 6, and 9. In addition, about 128 translocation stocks were grown to increase seed and obtain desirable genotypes. Also 22 inversion stocks were grown for seed increase and confirmation of pedigrees. Seed increases were also obtained on tetraploid marker stocks, T and S sterile cytoplasms along with certain Rf₁ and Rf₂ restorer combinations.

A total of about 800 allele tests were made on endosperm mutants and seedling traits in order to test certain cultures that have accumulated over the years. The endosperm and seedling traits found not allelic to the standard series will be crossed to A-B and waxy translocation series for gene location studies.

Work has been finished on assembling the translocation stocks from Dr. E. G. Anderson's collection. A total of 865 reciprocal translocations are now catalogued and in the collection, 734 of these have been sent to Fort Collins, Colorado for safe keeping. It is planned to send the remainder to Fort Collins in the near future. The attached catalogue of reciprocal translocation stocks summarizes this collection and includes the stocks that are available.

A complete list of chromosome marker stocks currently available can be found in the 1968 News Letter Vol. 42 (p. 186-194).

Requests for seed and correspondence relative to the stock program should be addressed to Dr. R. J. Lambert, S-116 Turner Hall, Department of Agronomy, University of Illinois, Urbana, Illinois 61801.

R. J. Lambert

List of Reciprocal Translocation Stocks Maintained
by the Maize Genetics Cooperative

Translocation		Break Points*		Translocation		Break Points	
1-2	7039	1S.90	2S.89	1-3	5982	1S.77	3L.66
1-2	d	1S.78	2L.56	1-3	8995	1S.49	3L.06
1-2	c	1S.77	2L.33	1-3	k	1S.17	3L.34
1-2	e	1S.61	2L.47	1-3	a	1S.15	3L.17
1-2	4464	1S.53	2L.28	1-3	c	1S.14	3L.14
1-2	b	1S.43	2S.36	1-3	h	1S.06	3L.04
1-2	036-7	1S.37	2L.33	1-3	013-9	1ctr.	3ctr.
1-2	5255	1S.25	2S.31	1-3	6861	1L.04	3L.65
1-2	5896	1S.22	2S.30	1-3	8048	1L.11	3S.18
1-2	004-11	1S.13	2S.36	1-3	j	1L.11	3L.13
1-2	8628	1ctr.	2L.49	1-3	6884	1L.17	3L.19
1-2	5946	1ctr.	2ctr.	1-3	024-14	1L.27	3S.49
1-2	028-17	1ctr.	2ctr.	1-3	8637	1L.37	3S.50
1-2	4937	1L.10	2S.15	1-3	4759	1L.39	3L.20
1-2	5453	1L.11	2S.58	1-3	e	1L.58	3L.45
1-2	051-1	1L.16	2S.30	1-3	8405	1L.60	3L.31
1-2	018-18	1L.16	2L.44	1-3	d	1L.61	3S.75
1-2	5539	1L.21	2L.61	1-3	5476	1L.66	3L.87
1-2	5523	1L.27	2S.62	1-3	i	1L.68	3S.30
1-2	041-9	1L.27	2S.57	1-3	5267	1L.72	3L.73
1-2	6892	1L.30	2L.35	1-3	4314	1L.81	3L.89
1-2	6427	1L.43	2L.50	1-3	5242	1L.90	3L.65
1-2	7211	1L.57	2L.79				
1-2	6883	1L.63	2L.52	1-4	h	1S.94	4L.52
1-2	017-3	1L.67	2S.58	1-4	002-19	1S.87	4L.42
1-2	5376	1L.77	2L.08	1-4	5680	1S.87	4L.45
				1-4	4308	1S.65	4L.58
1-3	5883	1S.88	3S.60	1-4	b	1S.55	4L.83
1-3	5597	1S.77	3L.48	1-4	8602	1S.41	4L.81

*Break points taken from Longley, A. E.; Crops Research Bulletin, ARS 34-16, January, 1961.

Translocation		Break Points		Translocation		Break Points	
1-4	064-20	1S.23	4L.19	1-5	e	1L.03	5L.09
1-4	5566	1S.21	4L.26			1S.13	5S.24**
1-4	8368	1S.14	4S.30	1-5	f	1L.07	5L.09
1-4	K-40	1S.13	4S.42	1-5	6401	1L.14	5S.20
1-4	8663	1S.09	4S.36	1-5	7219	1L.15	5S.19
1-4	5629	1L.10	4L.10			1S.18	5L.39**
1-4	039-15	1L.14	4S.26	1-5	h	1L.18	5L.53
1-4	6422	1L.16	4S.11	1-5	c	1L.34	5L.29
1-4	5373	1L.17	4S.29	1-5	070-12	1L.39	5S.71
1-4	f	1L.25	4L.16	1-5	7212	1L.44	5S.28
1-4	8249	1L.26	4L.63	1-5	4597	1L.52	5S.43
1-4	d	1L.27	4L.30	1-5	a	1L.52	5S.42
1-4	c	1L.33	4S.23			1L.62	5L.44**
1-4	8563	1L.39	4S.21	1-5	g	1L.58	5S.85
1-4	4692	1L.46	4L.15	1-5	8041	1L.80	5L.15
1-4	a	1L.51	4S.69			1L.80	5S.10**
1-4	5438	1L.93	4L.81	1-5	7267	1L.92	5L.82
1-4	g	1L.95	4L.35				
1-5	5045	1S.94	5L.50	1-6	8452	1S.80	6L.52
1-5	058-2	1S.88	5S.62	1-6	8609	1S.79	6L.59
1-5	4613	1S.78	5L.22	1-6	028-13	1S.56	6L.54
1-5	5525	1S.75	5S.53	1-6	7097	1S.46	6L.62
1-5	i	1S.71	5S.74	1-6	7352	1S.40	6L.60
1-5	6899	1S.32	5S.20	1-6	e	1S.37	6L.21
		1S.40	5L.10**				6S **
1-5	b	1S.17	5L.10	1-6	055-10	1S.29	6L.48
1-5	043-15	1S.10	5L.63	1-6	5013	1S.26	6L.28
1-5	5512	1S.08	5L.70	1-6	5495	1S.25	6S.80
1-5	6197	1S.02	5L.02	1-6	c	1S.25	6L.27
1-5	8782	1ctr.	5ctr.	1-6	6189	1S.23	6L.17
		L-S or	S-L **				6S **
				1-6	4986	1S.21	6S.78
				1-6	5077	1S.20	6L.60
				1-6	h	1L.03	6L.17
				1-6	d	1L.13	6S.74

**Break points determined by Dr. C. R. Burnham and students, University of Minnesota.

Translocation		Break Points		Translocation		Break Points	
1-6	g	1L.16	6L.84	1-8	8919	1S.53	8L.44
1-6	a	1L.20	6L.54	1-8	4307-4	1S.42	8L.61
1-6	8415	1L.29	6S.82	1-8	001-13	1S.39	8L.67
1-6	f	1L.32	6L.42	1-8	4685	1S.20	8L.21
1-6	070-1	1L.40	6L.58	1-8	6591	1S.18	8S.43
1-6	5225	1L.61	6L.72	1-8	008-17	1S.16	8L.20
1-6	4456	1L.71	6L.30	1-8	5588	1S.10	8S.32
1-6	8658	1L.79	6L.91	1-8	055-23	1ctr.	8ctr.
				1-8	064-13	1ctr.	8ctr.
1-7	4742	1S.95	7L.03	1-8	4676	1L.04	8S.06
1-7	g	1S.79	7S.22	1-8	5619	1L.07	8L.16
1-7	4837	1S.73	7L.55	1-8	5634	1L.08	8S.28
1-7	f	1S.72	7L.80	1-8	5384	1L.10	8L.59
1-7	4444	1S.65	7S.50	1-8	8683	1L.11	8ctr.
1-7	4405	1S.43	7S.46	1-8	8640	1L.11	8L.16
1-7	6796	1S.40	7S.39	1-8	020-19	1L.11	8L.38
1-7	010-12	1S.35	7L.57	1-8	4748	1L.12	8L.15
1-7	i	1S.31	7L.26	1-8	036-4	1L.18	8L.59
1-7	4302-31	1S.15	7L.12	1-8	005-7	1L.22	8L.78
1-7	A-37	1L.10	7L.56	1-8	7509	1L.28	8L.21
1-7	5871	1L.12	7L.24	1-8	5752	1L.36	8L.25
1-7	4891	1L.12	7L.69	1-8	a	1L.41	8S.52
1-7	j	1L.20	7L.61	1-8	026-2	1L.49	8L.80
1-7	5339	1L.24	7L.14	1-8	6766	1L.54	8L.77
1-7	a	1L.28	7L.13	1-8	b	1L.59	8L.82
1-7	e	1L.39	7L.11	1-8	5821	1L.65	8L.31
1-7	c	1L.39	7L.14	1-8	6697	1L.89	8L.52
1-7	h	1L.46	7L.19	1-8	5910	1L.93	8L.67
1-7	4420	1L.47	7L.90	1-8	5704	1L.96	8S.67
1-7	b	1L.53	7S.12**				
1-7	d	1L.81	7S.44	1-9	024-7	1S.71	9L.13
1-7	5693	1L.92	7L.18	1-9	8302	1S.55	9L.29

Translocation		Break Points		Translocation		Break Points	
1-9	8001	1S.51	9L.24	2-3	023-5	2S.80	3L.70
1-9	c	1S.48	9L.22	2-3	8662	2S.78	3L.83
1-9	7535	1S.33	9S.27	2-3	e	2S.76	3L.48
1-9	8918	1S.21	9L.20	2-3	5800	2S.73	3S.81
1-9	6762	1S.16	9L.53	2-3	5304	2S.62	3L.29
1-9	a	1S.13	9L.15	2-3	c	2S.46	3S.52
1-9	8460	1S.13	9L.24	2-3	6270	2S.46	3L.60
1-9	5622	1L.10	9L.12	2-3	014-12	2S.43	3L.51
1-9	4995	1L.19	9S.20	2-3	6862	2S.39	3L.20
1-9	8886	1L.33	9L.23	2-3	4369	2S.19	3S.26
1-9	4997	1L.37	9S.28	2-3	010-10	2S.17	3L.13
1-9	d	1L.42	9L.25	2-3	4301-111	2ctr.	3ctr.
1-9	b	1L.50	9L.60	2-3	023-2	2ctr.	3ctr.
1-9	4398	1L.51	9S.19	2-3	055-7	2L.10	3S.31
1-9	8389	1L.74	9L.13	2-3	005-14	2L.12	3S.29
1-9	035-10	1L.89	9S.67	2-3	h	2L.14	3L.07
				2-3	8483	2L.14	3L.12
1-10	g	1S.80	10L.21	2-3	I-10	2L.19	3S.51
1-10	007-19	1S.07	10L.08	2-3	g	2L.21	3S.21
1-10	f	1S.04	10L.30	2-3	7285	2L.26	3L.39
1-10	4885	1ctr.	10ctr.	2-3	033-4	2L.27	3L.23
1-10	8770	1L.09	10L.38	2-3	f	2L.35	3S.60
1-10	e	1L.16	10L.31	2-3	b	2L.45	3L.08
1-10	068-14	1L.16	10L.79	2-3	d	2L.67	3L.48
1-10	5273	1L.17	10L.69	2-3	4303-74	2L.73	3L.68
1-10	b	1L.19	10S.39	2-3	6750	2L.76	3S.53
1-10	a	1L.29	10L.33	2-3	6284	2L.81	3L.75
1-10	c	1L.43	10L.74				
1-10	8491	1L.45	10L.76	2-4	5157	2S.86	4L.07
1-10	d	1L.50	10L.68	2-4	8865	2S.52	4L.27
1-10	015-9	1L.67	10S.46	2-4	060-8	2S.50	4L.37
1-10	8375	1L.69	10L.64	2-4	018-3	2S.38	4L.47
1-10	001-3	1L.86	10L.48	2-4	5495	2S.27	4L.10

Translocation		Break Points		Translocation		Break Points	
2-4	j	2ctr.	4ctr.	2-5	5098	2L.13	5S.23
2-4	8407	2ctr.	4ctr.	2-5	a	2L.14	5L.15
2-4	g	2L.13	4S.31	2-5	c	2L.16	5S.48
2-4	010-4	2L.13	4S.31	2-5	015-3	2L.16	5S.69
2-4	k	2L.13	4L.04	2-5	002-16	2L.25	5L.35
2-4	004-13	2L.14	4S.51	2-5	023-15	2L.28	5S.30
2-4	4374	2L.15	4L.23	2-5	032-9	2L.40	5S.31
2-4	8027	2L.15	4L.43	2-5	062-3	2L.45	5S.34
2-4	d	2L.17	4L.45	2-5	5876	2L.47	5L.46
2-4	5951	2L.18	4S.26	2-5	5645	2L.60	5S.85
2-4	a	2L.30	4L.21	2-5	6885	2L.63	5S.79
2-4	e	2L.31	4S.47	2-5	5602	2L.73	5L.77
2-4	m	2L.34	4S.47	2-5	8321	2L.86	5L.11
2-4	057-19	2L.35	4S.51	2-5	f	2L.91	5L.10
2-4	017-18	2L.39	4L.19	2-5	d	2L.91	5L.86
2-4	6266	2L.40	4L.27	2-5	4578	2L.92	5S.71
2-4	011-7	2L.53	4L.76				
2-4	l	2L.59	4S.40	2-6	8786	2S.90	6S.77
2-4	f	2L.75	4L.12				6S.org.**
2-4	052-15	2L.75	4L.66	2-6	001-5	2S.72	6S.87
2-4	c	2L.81	4S.09				org.**
2-4	b	2L.81	4L.53	2-6	b	2S.69	6L.49
				2-6	060-5	2S.36	6L.18
				2-6	027-4	2S.34	6L.11
						2L.10	6S.org.**
2-5	g	2S.79	5S.24	2-6	5472	2S.25	6L.15
2-5	059-17	2S.73	5S.61	2-6	e	2L.18	6L.20
2-5	019-1	2S.67	5S.51				6S.**
2-5	4741	2S.47	5L.47	2-6	6931	2L.24	6L.23
2-5	009-19	2S.29	5L.11	2-6	"78"	2L.24	6L.29
2-5	025-4	2S.26	5L.78	2-6	a	2L.28	6L.20
2-5	e	2S.19	5S.28			2S.40	6S.50**
2-5	059-1	2ctr.	5S.84	2-6	c	2L.37	6L.25
2-5	b	2L.06	5S.09			2S	**
2-5	6580	2L.09	5S.09	2-6	d	2L.41	6L.45
				2-6	4717	2L.77	6L.27
				2-6	5419	2L.82	6S.79
							6S.org.**

Translocation		Break Points		Translocation		Break Points	
2-6	8441	2L.94	6S.79 6S.org.**	2-8	031-7	2L.30	8S.44
				2-8	"84"	2L.32	8L.30
2-7	5279	2S.93	7L.25	2-8	051-15	2L.62	8L.48
2-7	5144	2S.35	7L.08	2-8	062-15	2L.70	8L.26
2-7	022-4	2S.30	7L.24	2-8	g	2L.71	8S.71
2-7	8045	2S.12	7L.06	2-8	051-7	2L.83	8L.74
2-7	d	2L.16	7L.18	2-8	48-45-6	2L.84	8L.68
2-7	4400	2L.24	7L.32	2-8	8376	2L.95	8L.03
2-7	f	2L.30	7L.68	2-8	037-5	2L.95	8L.54
2-7	b	2L.37	7L.12				
2-7	c	2L.47	7S.34	2-9	7096	2S.57	9L.66
2-7	4519	2L.65	7L.66	2-9	c	2S.49	9S.33
2-7	5783	2L.66	7L.10	2-9	a	2S.36	9L.58
2-7	038-12	2L.75	7S.68	2-9	055-14	2S.28	9L.27
2-7	8322	2L.76	7L.74	2-9	5711	2S.24	9L.23
2-7	e	2L.82	7L.63	2-9	b	2S.18	9L.22
				2-9	062-11	2L.21	9S.53
2-8	013-17	2S.89	8L.61	2-9	5257	2L.28	9L.20
2-8	4711	2S.86	8L.67	2-9	6656	2L.32	9S.31
2-8	011-20	2S.58	8L.28	2-9	5208	2L.76	9L.68
2-8	c	2S.15	8S.11	2-9	d	2L.83	9L.27
2-8	f	2ctr.	8ctr.				
2-8	006-10	2ctr.	8ctr.	2-10	043-10	2S.89	10L.40
2-8	d	2L.05	8L.10	2-10	5651	2S.71	10L.62
2-8	e	2L.07	8L.10	2-10	b	2S.50	10L.75
2-8	4414	2L.12	8L.14	2-10	8864	2S.10	10L.76
2-8	7069	2L.13	8L.14	2-10	4484	2S.09	10L.14
2-8	003-5	2L.19	8S.72	2-10	5830	2L.12	10L.12
2-8	b	2L.20	8L.18	2-10	a	2L.16	10L.55
2-8	5454	2L.21	8S.39	2-10	I-3	2L.30	10S.40
2-8	h	2L.23	8L.22	2-10	5561	2L.35	10S.16
2-8	5484	2L.24	8S.58	2-10	8219	2L.50	10L.35

Translocation		Break Points		Translocation		Break Points	
2-10	6853	2L.79	10L.86	3-5	h	3L.55	5L.22
2-10	035-2	2L.85	10L.49	3-5	b	3L.61	5L.57
				3-5	c	3L.62	5L.27
3-4	8969	3S.75	4L.75	3-5	7043	3L.63	5L.61
3-4	8397	3S.74	4S.55	3-5	8351	3L.75	5L.68
3-4	8634	3S.71	4L.75	3-5	6346	3L.94	5L.83
3-4	5156	3S.47	4L.67				
3-4	5920	3S.28	4L.73	3-6	b	3S.73	6S.82
3-4	012-16	3S.27	4S.30				6S.org.**
3-4	4662	3S.24	4S.67	3-6	060-4	3S.62	6L.08
3-4	4726	3S.16	4L.15	3-6	4349	3S.58	6L.70
3-4	5891	3ctr.	4ctr.	3-6	c	3S.56	6L.54
3-4	5074-6	3ctr.	4ctr.	3-6	016-17	3S.48	6L.30
3-4	A-21	3L.07	4L.85	3-6	032-3	3S.41	6S.78
3-4	006-17	3L.10	4S.45	3-6	030-8	3S.27	6S.81
3-4	037-9	3L.10	4L.14	3-6	8963	3S.23	6L.14
3-4	8443	3L.12	4L.13	3-6	a	3L.06	6L.30
3-4	6534	3L.48	4L.89	3-6	7067	3L.07	6L.75
				3-6	6349	3L.10	6L.15
3-5	4635	3S.44	5S.48	3-6	055-5	3L.16	6L.32
3-5	e	3S.34	5S.16	3-6	8145	3L.17	6L.26
3-5	6473	3S.32	5S.26	3-6	5368	3L.22	6L.20
3-5	6462	3S.31	5L.47	3-6	d	3L.23	6L.82
3-5	4880	3ctr.	5ctr.	3-6	5201	3L.26	6L.21
3-5	4898	3ctr.	5ctr.	3-6	6566	3L.41	6L.35
3-5	6695	3ctr.	5ctr.				6S.**
3-5	g	3L.01	5S.73	3-6	8672	3L.47	6L.87
3-5	8104	3L.05	5L.08	3-6	7162	3L.52	6L.53
3-5	8528	3L.06	5L.72	3-6	054-12	3L.72	6L.75
3-5	039-13	3L.13	5L.14	3-7	b	3S.92	7L.03
3-5	5874	3L.16	5L.21	3-7	001-15	3S.38	7L.30
3-5	5521	3L.17	5L.48	3-7	004-7	3S.38	7L.26
3-5	a	3L.28	5L.60	3-7	a	3S.25	7L.18

Translocation		Break Points		Translocation		Break Points	
3-7	4670	3S.20	7L.76	3-8	a	3L.41	8L.61
3-7	4773	3S.11	7L.07	3-8	6261	3L.49	8L.40
3-7	5724	3ctr.	7ctr.	3-8	h	3L.53	8S.46
3-7	5955	3L.10	7L.58	3-8	8350	3L.75	8S.60
3-7	029-3	3L.11	7L.13	3-8	4340	3L.88	8L.72
3-7	5378	3L.13	7L.73	3-8	4301-39	3L.92	8L.82
3-7	e	3L.25	7S.56				
3-7	6466	3L.36	7L.14	3-9	054-18	3S.88	9L.82
3-7	c	3L.46	7L.45	3-9	6722	3S.66	9S.66
3-7	5471	3L.64	7L.58	3-9	7041	3S.59	9L.70
3-7	d	3L.64	7L.81	3-9	5643	3S.55	9L.64
3-7	8006	3L.88	7L.90	3-9	8447	3S.44	9L.14
				3-9	030-2	3S.39	9L.30
3-8	024-11	3S.65	8L.49	3-9	8465	3S.27	9L.41
3-8	6373	3S.53	8L.68	3-9	8032	3S.26	9L.96
3-8	e	3S.36	8L.21	3-9	020-5	3ctr.	9ctr.
3-8	4626	3S.30	8L.31	3-9	e	3L.02	9L.29
3-8	6439	3S.30	8L.15	3-9	5775	3L.09	9S.24
3-8	8666	3S.30	8L.14	3-9	c	3L.09	9L.12
3-8	8667	3S.30	8L.14			3S.15	9S.20**
3-8	8670	3S.30	8L.14	3-9	h	3L.09	9L.33
3-8	8367	3S.28	8S.52	3-9	a	3L.11	9L.16
3-8	5558	3S.26	8S.74	3-9	d	3L.13	9L.26
3-8	c	3S.23	8L.85	3-9	g	3L.40	9L.14
3-8	4303-12	3ctr.	8ctr.	3-9	b	3L.48	9L.53
3-8	4872	3ctr.	8ctr.	3-9	5285	3L.51	9L.49
3-8	043-14	3L.02	8S.40	3-9	4727	3L.54	9L.42
3-8	7362	3L.07	8L.69	3-9	f	3L.63	9S.69
3-8	f	3L.08	8L.10	3-9	8562	3L.65	9L.22
3-8	g	3L.12	8L.19	3-9	4963	3L.76	9L.57
3-8	b	3L.16	8L.23				
3-8	8023	3L.18	8L.16	3-10	7464	3S.49	10L.60
3-8	4874	3L.28	8L.32	3-10	8412	3S.39	10S.36

Translocation		Break Points		Translocation		Break Points	
3-10	8349	3S.38	10ctr.	4-5	018-4	4L.61	5L.67
3-10	4382	3S.38	10L.29	4-5	027-10	4L.61	5L.79
3-10	5892	3S.17	10L.25	4-5	021-3	4L.62	5S.71
3-10	a	3L.16	10L.22	4-5	8395	4L.63	5L.82
3-10	b	3L.19	10L.27	4-5	b	4L.76	5L.68
3-10	c	3L.22	10L.30				
3-10	6691	3L.30	10L.87	4-6	b	4S.80	6L.16
3-10	036-15	3L.48	10L.64	4-6	e	4S.62	6L.56
3-10	044-10	3L.77	10L.72	4-6	7328	4S.53	6S.89
				4-6	8380	4S.47	6L.18
4-5	e	4S.41	5S.32	4-6	5227	4S.46	6S.84
4-5	g	4S.38	5L.30	4-6	025-12	4S.44	6L.34
4-5	8108	4S.37	5S.72	4-6	4341	4S.37	6S.81
4-5	5529	4S.37	5L.46	4-6	c	4S.33	6S.83
4-5	8069	4S.34	5S.71	4-6	011-16	4S.31	6L.33
4-5	c	4S.34	5L.27	4-6	4447	4S.28	6L.14
4-5	6831	4S.32	5S.59	4-6	6623	4L.18	6L.31
4-5	6560	4S.32	5S.21	4-6	8591	4L.17	6L.24
4-5	002-12	4S.29	5S.36	4-6		6S.	**
4-5	4305-8	4S.27	5L.28	4-6	055-8	4L.29	6L.25
4-5	4472	4S.25	5S.19	4-6	8428	4L.32	6L.28
4-5	d	4S.21	5L.22	4-6	8764	4L.32	6L.90
4-5	k	4S.06	5L.13	4-6	a	4L.37	6L.43
4-5	7078	4L.05	5L.10	4-6	d	4L.49	6L.53
4-5	i	4L.10	5S.15	4-6	003-16	4L.50	6S.90
4-5	h	4L.13	5L.08	4-6	7037	4L.61	6S.77
4-5	a	4L.19	5S.29	4-6	8927	4L.70	6L.18
4-5	j	4L.21	5L.36	4-6	038-11	4L.78	6L.29
4-5	8622	4L.30	5L.52	4-6	8339	4L.87	6L.79
4-5	006-7	4L.43	5S.25				
4-5	7136	4L.45	5L.33	4-7	8103	4S.81	7L.76
4-5	f	4L.50	5L.80	4-7	6575	4S.38	7S.32
4-5	6743	4L.56	5S.59	4-7	a	4S.32	7L.06

Translocation		Break Points		Translocation		Break Points	
4-7	48-40-8	4S.32	7L.64	4-9	5884	4L.40	9L.49
4-7	7347	4S.31	7L.66	4-9	f	4L.55	9L.18
4-7	7108	4S.17	7S.45	4-9	5574	4L.80	9L.87
4-7	4698	4L.08	7L.74	4-9	c	4L.82	9L.29
4-7	7067	4L.17	7S.60	4-9	b	4L.90	9L.29
4-7	027-17	4L.17	7L.31	4-9	8636	4L.94	9S.09
4-7	8374	4L.24	7L.55	4-9	8649	4L.94	9S.09
4-7	4483	4L.39	7L.61				
				4-10	c	4S.64	10L.18
4-8	036-16	4S.66	8L.69	4-10	9028	4S.57	10L.89
4-8	a	4S.59	8L.19	4-10	8541	4S.45	10ctr.
4-8	8987	4S.58	8L.76	4-10	d	4S.36	10L.36
4-8	8607	4S.42	8L.35	4-10	6662	4L.04	10L.03
4-8	8004	4S.27	8L.84	4-10	e	4L.14	10L.14
4-8	5339	4S.22	8L.71	4-10	b	4L.15	10L.60
4-8	8456	4S.22	8L.75	4-10	021-5	4L.34	10L.33
4-8	b	4S.18	8L.16	4-10	073-8	4L.41	10S.74
4-8	6063	4S.02	8L.05	4-10	6587	4L.55	10L.51
4-8	6926	4L.60	8L.71	4-10	057-14	4L.56	10S.48
4-8	6363	4L.76	8L.30	4-10	024-16	4L.75	10L.18
				4-10	f	4L.94	10L.14
4-9	e	4S.53	9L.26				
4-9	4307-12	4S.48	9L.55	5-6	5622	5S.94	6L.92
4-9	g	4S.27	9L.27			5S.87	6L.47**
4-9	5918	4S.24	9L.18	5-6	8818	5S.91	6L.93
						5L.91	6L.93**
4-9	4304-82	4S.22	9L.37	5-6	6522	5S.87	6L.70
4-9	6222	4L.03	9S.68	5-6	6559	5S.72	6L.09
4-9	6504	4L.09	9S.83	5-6	d	5S.64	6S.89
4-9	d	4L.12	9L.17			5S.58	6S.Sat.**
4-9	a	4L.16	9L.58	5-6	040-1	5S.48	6S.82
4-9	004-7	4L.28	9L.26	5-6	f	5S.37	6S.76
4-9	4373	4L.29	9L.39	5-6	8590	5S.29	6L.25
4-9	5657	4L.33	9S.25			5S.25	6L.61**
				5-6	4933	5S.23	6L.89

Translocation		Break Points		Translocation		Break Points	
5-6	5765	5S.19	6L.32	5-7	8671	5L.96	7L.67
5-6	5906	5S.15	6L.13				
5-6	e	5L.11	6L.60	5-8	8420	5S.90	8L.33
5-6	4669	5L.13	6L.40	5-8	8746	5S.84	8L.25
5-6	6062	5L.20	6L.78	5-8	5013	5S.67	8L.59
5-6	5685	5L.27	6L.20	5-8	6612	5S.59	8L.66
		5S.24	6L.23**				
5-6	4934	5L.34	6L.89	5-8	013-11	5S.59	8S.63
5-6	a	5L.35	6L.43	5-8	d	5S.55	8L.12
5-6	4666	5L.35	6L.86	5-8	5570	5S.47	8S.35
5-6	004-17	5L.60	6L.24	5-8	c	5S.24	8L.20
5-6	b	5L.72	6L.21	5-8	b	5S.23	8L.23
5-6	8219	5L.76	6S.84	5-8	5575	5S.21	8S.22
		5L.69	6S.Sat.**				
5-6	c	5L.81	6L.08	5-8	7068	5S.18	8L.18
		5L.89	6S.00**				
5-6	8696	5L.89	6S.80	5-8	5777	5S.13	8L.19
				5-8	6402	5S.07	8L.07
				5-8	A-50	5S.07	8L.11
5-7	d	5S.63	7S.33	5-8	6406	5ctr.	8ctr.
5-7	064-18	5S.61	7S.49	5-8	f	5L.02	8S.08
5-7	061-4	5S.54	7L.30	5-8	6289	5L.06	8L.54
5-7	5143	5S.51	7L.10	5-8	045-6	5L.08	8L.13
5-7	e	5S.40	7S.18	5-8	002-17	5L.11	8L.28
5-7	013-3	5S.36	7S.35	5-8	8997	5L.16	8L.08
5-7	4306-4	5S.32	7L.35	5-8	014-5	5L.19	8L.18
5-7	8679	5S.09	7S.26	5-8	053-4	5L.21	8S.48
5-7	062-18	5ctr.	7ctr.	5-8	4636	5L.23	8L.79
5-7	023-13	5L.12	7L.15	5-8	g	5L.28	8S.44
5-7	b	5L.18	7S.36	5-8	007-17	5L.32	8S.47
5-7	6293	5L.26	7L.63	5-8	5866	5L.32	8L.77
5-7	8630	5L.38	7L.24	5-8	7102	5L.48	8S.10
5-7	c	5L.42	7L.72	5-8	030-1	5L.48	8L.78
5-7	5179	5L.55	7L.73	5-8	a	5L.49	8S.58
5-7	a	5L.78	7L.72	5-8	8806	5L.72	8S.59
5-7	f	5L.80	7L.85	5-8	8796	5L.76	8L.11

Translocation		Break Points		Translocation		Break Points	
6-7	4337	6L.37	7L.13	6-9	8906	6L.27	9L.59
6-7	6598	6L.43	7L.61	6-9	043-1	6L.36	9L.36
6-7	4594	6L.52	7S.67	6-9	5964	6L.47	9L.83
6-7	027-6	6L.66	7L.97	6-9	8768	6L.89	9S.61
6-7	a	6L.73	7L.68				
6-7	7402	6L.97	7L.14	6-10	f	6S.92	10S.28
				6-10	5253	6S.80	10L.41
6-8	058-1	6ctr.	8L.46	6-10	5519	6S.75	10L.17
6-8	6187	6L.19	8L.51	6-10	b	6L.12	10L.29
6-8	6873	6L.21	8L.29	6-10	e	6L.14	10S.43
6-8	5028	6L.21	8L.31	6-10	d	6L.16	10L.29
6-8	c	6L.27	8L.50	6-10	8645	6L.21	10L.28
6-8	5605	6L.36	8L.22	6-10	8651	6L.27	10L.48
6-8	a	6L.41	8L.80	6-10	h	6L.47	10L.87
6-8	024-1	6L.42	8L.74	6-10	044-8	6L.48	10L.51
6-8	d	6L.51	8L.77	6-10	c	6L.51	10S.36
6-8	b	6L.79	8S.76	6-10	8904	6L.51	10L.83
				6-10	4307-12	6L.74	10S.71
6-9	017-14	6S.80	9L.50	6-10	a	6L.75	10L.15
6-9	4778	6S.80	9L.30	6-10	4833	6L.83	10S.78
6-9	a	6S.79	9L.40	6-10	g	6L.85	10L.20
		6S.org.	**	6-10	5780	6L.93	10L.13
6-9	d	6S.73	9L.82				
6-9	067-6	6S.39	9L.47	7-8	5828	7S.31	8L.10
6-9	5454	6ctr.	9S.75	7-8	6531	7ctr.	8ctr.
6-9	84-39	6L.06	9S.73	7-8	6981	7ctr.	8ctr.
6-9	b	6L.10	9S.37	7-8	8580	7ctr.	8ctr.
6-9	4505	6L.13	9ctr.	7-8	004-3	7ctr.	8ctr.
6-9	c	6L.15	9L.29	7-8	016-15	7ctr.	8ctr.
6-9	8536	6L.18	9S.81	7-8	034-17	7L.05	8S.59
6-9	e	6L.18	9L.24	7-8	5499	7L.05	8L.08
6-9	6270	6L.19	9L.28	7-8	062-16	7L.15	8L.17
6-9	6019	6L.27	9L.26				

Translocation		Break Points		Translocation		Break Points	
7-8	014-17	7L.18	8L.30	8-9	b	8S.67	9L.75
7-8	4536	7L.34	8L.47	8-9	4643	8S.37	9L.11
7-8	038-8	7L.52	8L.46	8-9	c	8ctr.	9ctr.
7-8	7149	7L.56	8L.65	8-9	8525	8L.06	9S.63
7-8	5479	7L.70	8S.21	8-9	5391	8L.07	9S.33
7-8	021-1	7L.72	8L.49	8-9	d	8L.09	9S.16
7-8	4824	7L.83	8L.25	8-9	a	8L.13	9L.38
				8-9	8951	8L.13	9L.77
7-9	b	7S.76	9S.19	8-9	043-6	8L.17	9S.34
7-9	071-1	7S.70	9L.07	8-9	e	8L.32	9L.25
7-9	8659	7S.55	9S.35	8-9	6673	8L.35	9S.31
7-9	053-8	7S.51	9L.77	8-9	4775	8L.42	9L.68
7-9	5074	7S.48	9L.53	8-9	4593	8L.69	9L.65
7-9	8558	7S.22	9L.16	8-9	6921	8L.85	9L.15
7-9	4363	7ctr.	9ctr.	8-9	5300	8L.85	9S.43
7-9	6225	7ctr.	9ctr.	8-9	4453	8L.86	9S.68
7-9	8383	7ctr.	9ctr.				
7-9	6482	7L.01	9S.97	8-10	b	8ctr.	10ctr.
7-9	7074	7L.03	9S.80	8-10	5585	8ctr.	10ctr.
7-9	c	7L.14	9L.22	8-10	6653	8L.04	10L.06
7-9	4713	7L.60	9ctr.	8-10	9020	8L.13	10S.50
7-9	027-9	7L.61	9S.18	8-10	6488	8L.14	10S.34
7-9	6978	7L.62	9S.83	8-10	5287	8L.17	10S.33
7-9	a	7L.63	9S.07	8-10	034-19	8L.24	10L.28
7-9	032-13	7L.82	9L.88	8-10	001-5	8L.30	10S.57
7-9	5381	7L.85	9L.78	8-10	d	8L.39	10L.16
				8-10	c	8L.41	10S.56
7-10	7356	7S.75	10L.88	8-10	6128	8L.43	10S.49
7-10	022-15	7ctr.	10ctr.	8-10	a	8L.48	10S.48
7-10	015-12	7ctr.	10ctr.	8-10	5944	8L.75	10L.40
7-10	019-3	7L.17	10L.47	8-10	e	8L.84	10S.37
7-10	4422	7L.79	10ctr.				

Translocation		Break Points		Translocation		Break Points	
9-10	059-10	9S.31	10L.53	9-10	5488	9L.57	10L.89
9-10	8630	9S.28	10L.37	9-10	041-4	9L.67	10L.92
9-10	b	9S.13	10S.40	9-10	041-6	9L.70	10L.90
9-10	4303-9	9L.26	10S.44	9-10	7103	9L.73	10L.88

The following reciprocal translocation stocks do not involve the same chromosomes and/or breakpoints as originally reported. The new information was kindly supplied by Dr. C. R. Burnham and Associates of the University of Minnesota.

Translocation	Symbol	Breakpoint from Longley ARS-34-16		New information Burnham and Associates	
1-5	8347	1S.84	5L.51	1	2
1-5	8972	1S.56	5S.29		1 not correct
1-5	018-5	1S.53	5L.52	1	2
1-5	055-4	1S.32	5L.31	1	or 5 not correct
1-5	040-3	1S.17	5L.61	1	or 5 not correct
1-5	024-5	1S.09	5L.98	1	2
1-5	4331	1L.03	5S.02	7	10
1-5	6178	1L.04	5L.05	1	2
1-5	8388	1L.30	5S.25	1	2
2-6	4394	2S.91	6L.12	4	6
2-6	6671	2S.22	6L.22	5S.49	6L.35
2-6	5648	2L.25	6L.19	1	6
2-6	9002	2L.57	6L.56	1	6
2-6	f	2L.79	6L.87	1	6
5-6	8665	5L.58	6L.25	Independent of chrom. 5 genes	
5-10	6061	5S.60	10L.57	2	10

Also in the Maize Genetics Co-op collection are 48 cultures with no chromosome information and 27 cultures with chromosomes involved determined but no breakpoints. This seed is available to anyone who wishes to determine the chromosomes and breakpoints or would like to use the material as unknowns in cytogenetic courses.

V. RECENT MAIZE PUBLICATIONS

- Ahmad, M. The effects of organo-phosphorous insecticides on the meristematic tissue of the corn plant. Diss. Abstr. 27:3355. 1967. (Abstr.)
- Alexander, D. E. and R. J. Lambert. Relationship of kernel oil content to yield in maize. Crop Sci. 8:273-275. 1968.
- Amano, E. Comparison of ethyl methanesulfonate- and radiation-induced waxy mutants in maize. Mutation Res. 5:41-46. 1968.
- Andrew, R. H. Ear to row selection for early maturity in sweet corn. Crop Sci. 9:51-55. 1969.
- Angeles A., H. H. Ontogenetic study of the vegetative shoot apex of corn grass, a mutant in maize. Diss. Abstr. 27:2591. 1967. (Abstr.)
- Balint, A. Heterosis and chromosome alterations. Magyar Tud. Akad. Agrartud. Oszt. Kozl 26:445-449. 1967.
- Bianchi, A. Quantitative relationship between genetic effects and x-ray doses in maize. Symp. Genet. Biol. Ital. 13:491-498. 1966.
- Bianchi, A. A fine analysis of crossing over in maize. Genetica Agraria 22:136-149. 1968.
- Bianchi, A. and F. Salamini. Heredity and genetic improvement in maize. Sementi Elette 13:256-313. 1967.
- Bingham, E. T., C. R. Burnham, and C. E. Gates. Double and single backcross linkage estimates in autotetraploid maize. Genetics 59:399-410. 1968.
- Bogyo, T. P. Inheritance of resistance to Helminthosporium turcicum in maize. 1951-1965. Agric. Res. Pretoria 1966:103-104. 1966. (Abstr.)
- Briggs, R. W. Effects of post-treatment hydration and dehydration of ethyl methanesulfonate-treated maize seeds. In Mutations in Plant Breeding II, pp. 47-55, Intern. Atomic Energy Agency, Vienna. 1968.
- Brown, A. H. D. and R. W. Allard. Inheritance of isozyme differences among the inbred parents of a reciprocal recurrent selection population of maize. Crop Sci. 9:72-75. 1969.
- Brown, W. L. Results of non-selective inbreeding in maize. Der Zuchter 37:155-159. 1967.

- Burnham, C. R. A new method of using interchanges as chromosome markers. *Crop Sci.* 8:357-361. 1968.
- Casas, E., W. D. Hanson, and E. J. Wellhausen. Genetic relationships among collections representing three Mexican race composites of Zea mays L. *Genetics* 59:299-310. 1968.
- Castro G., M., C. O. Gardner, and J. H. Lonnquist. Cumulative gene effects and the nature of heterosis in maize crosses involving genetically diverse races. *Crop Sci.* 8:97-101. 1968.
- Chao, S. E. and J. G. Scandalios. Genetic and developmental studies of amylase isozymes in maize. *Genetics* 60:168-169. 1968. (Abstr.)
- Chase, S. S. Complementary pollen fertility restoration in maize. *J. Hered.* 59:240-242. 1968.
- Chase, S. S. and D. K. Nanda. Number of leaves and maturity classification in Zea mays L. *Crop Sci.* 7:431-432. 1967.
- Okanikov, D. I. et al. On the possibility of inducing male sterility in maize by means of certain chemical compounds. *Agrohimija* 1964 (7):123-129. 1964.
- Coe, E. H., Jr. Heritable repression due to paramutation in maize. *Science* 162:925. 1968.
- Creech, R. G. Carbohydrate synthesis in maize. *Adv. Agron.* 8:275-322. 1968.
- Creech, R. G., R. J. Snyder, E. V. Wann and W. H. Lachman. Genotypic-environmental interactions for sweet corn hybrids produced by the conventional and cytoplasmic-genetic male sterile methods. *Crop Sci.* 8:380-383. 1968.
- Crum, C. W. Epistatic effects and inbreeding depression of three performance levels in two populations of Zea mays L. *Diss. Abstr.* 27:2988. 1967. (Abstr.)
- Curtis, P. E., E. R. Leng, and R. H. Hageman. Development changes in oil and fatty acid content of maize strains varying in oil content. *Crop Sci.* 8:689-693. 1968.
- Da Silva, W. J. and J. H. Lonnquist. Genetic variances in populations developed from full-sib and S_1 testcross progeny selection in an open-pollinated variety of maize. *Crop Sci.* 8:201-204. 1968.
- Diaconu, P. Cytological studies of cytoplasmic male sterility in maize. *An. Inst. Cerc. Cereal Pl. Teh. Fundulea* 33:227-238. 1967.
- Duclos, L. A. and P. L. Crane. Comparative performance of top crosses and S_1 progeny for improving populations of corn (Zea mays L.). *Crop Sci.* 8:191-194. 1968.

- Duclos, Z. M., F. L. Patterson and H. H. Kramer. Performance of inbred lines in hybrid combination during transfer of a single gene (ae) locus in maize (Zea mays L.). Crop Sci. 7:433-437. 1967.
- Eberhart, S. A. and A. R. Hallauer. Genetic effects for yield in single, three-way, and double-cross maize hybrids. Crop Sci. 8:377-379. 1968.
- Efron, Y. and H. L. Everett. Evaluation of exotic germ plasm for improving corn hybrids in Northern United States. Crop Sci. 9:44-47. 1969.
- Efron, Y. and D. Schwartz. In vivo inactivation of maize alcohol dehydrogenase by a two-factor system. P. N. A. S. 61:586-591. 1968.
- El-Eryani, A. A. and A. A. Fleming. Differences in free amino acids between stocks of the same long-time inbred line of maize (Zea mays L.). Crop Sci. 8:529-532. 1968.
- Faludi-Daniel, A., L. Fridvalszky and I. Gyurjan. Pigment composition and plastid structure in leaves of carotenoid mutants of maize. Planta 78:184-195. 1968.
- Ficsor, G. and W. C. Beckman. Mutagenic inactivation of EMS in water and in germinating maize kernels. Genetics 60:176-177. 1968. (Abstr.)
- Fleming, A. A. and E. H. Wood. Effects of soil moisture and plant population on corn hybrids at a high fertility level. Abst. Amer. Soc. Agron. 1967. (Abstr.)
- Focke, R. Physiological and genetic studies of dry-matter production in maize leaves in relation to transport and yield. Der Zuchter 37: 371-376. 1967.
- Freeman, J. E., N. W. Kramer, and S. A. Watson. Gelatinization of starches from corn (Zea mays L.) and sorghum (Sorghum bicolor L. Moench): Effects of genetic and environmental factors. Crop Sci. 8:409-413. 1968.
- Freeman, J. E., K. R. Yahl and S. A. Watson. A chemical and structural analysis of the caryopsis of an autotetraploid corn (Zea mays L.). Crop Sci. 7:655-658. 1967.
- Fuchs, A. Relationships between organogenesis and yield structure in Zea mays. Z. für Pflanzenzucht 60:260-283. 1968.
- Galinat, W. C. and R. G. Campbell. The diffusion of eight-rowed maize from the Southwest to the Central Plains. Mass. Agric. Exper. Sta. Monograph No. 1. 16 pp. 1967.

- Galinat, W. C. and S. S. Chase. Review of Manifestation of teosinte and "Tripsacum" introgression in Corn Belt maize, by G. S. Johnston. 73 pp. The Bussey Institution of Harvard Univ. Econ. Botany 22: 305-306. 1968.
- Gee, M. S., O. E. Nelson, and J. Kuc'. Abnormal lignins produced by the brown-midrib mutants of maize II. Comparative studies on normal and brown-midrib-1 dimethylformamide lignins. Arch. Biochem. Biophys. 123:403-408. 1968.
- Genter, C. F. Inbreeding without inbreeding depression. Proc. Ann. Hybrid Corn Industry Res. Conf. 22:82-90. 1968.
- Ghidoni, A. Transmission and phenotypic effect of a duplicate chromosome segment in maize. Molec. Gen. Genetics 101:317-332. 1968.
- Goodman, M. M. The races of maize: I. The use of Mahalanobis' generalized distances to measure morphological similarity. Fitotecnica Latinoamericana 4:1-22. 1967.
- Goodman, M. M. A measure of "overall variability" in populations. Biometrics 24:189-192. 1968.
- Goodman, M. M. The races of maize: II. Use of multivariate analysis of variance to measure morphological similarity. Crop Sci. 8:693-698. 1968.
- Goodsell, S. F. Potassium in mature kernels of normal and opaque-2 maize. Crop Sci. 8:281-283. 1968.
- Greenblatt, I. M. The mechanism of modulator transposition in maize. Genetics 58:585-597. 1968.
- Grogan, C. O. and C. W. Blessin. Characterization of major carotenoids in yellow maize lines of differing pigment concentration. Crop Sci. 8:730-732. 1968.
- Grogan, C. O. and E. E. Rosenkranz. Genetics of host reaction to corn stunt virus. Crop Sci. 8:251-254. 1968.
- Gross, J. A. Development, physiology and biochemistry of corn and wheat pollen. Botanical Review 34(3):333-358. 1968.
- Hageman, R. H., E. R. Leng, and J. W. Dudley. A biochemical approach to corn breeding. Advanc. Agron. 19:45-86. 1967.
- Hallauer, A. R. Performance of single cross hybrids developed from two-ear varieties. Proc. Ann. Hybrid Corn Industry Res. Conf. 22:74-81. 1968.
- Hallauer, A. R. and J. H. Sears. Second phase in the evaluation of synthetic varieties of maize for yield. Crop Sci. 8:448-451. 1968.

- Hallauer, A. R. and J. H. Sears. Mass selection for yield in two varieties of maize. *Crop Sci.* 9:47-51. 1969.
- Harris, J. W. Isozymes of the E₄ esterase in maize. *Genetics* 60:186-187. 1968. (Abstr.)
- Helm, J. L., V. L. Fergason, and M. S. Zuber. Effect of planting date on high-amylose corn (Zea mays L.). *Agron. Jour.* 60:530-531. 1968.
- Higgs, R. L. and W. A. Russell. Genetic variation in quantitative characters in maize inbreds. I. Variation among and within Corn Belt seed sources of six inbreds. *Crop Sci.* 8:345-348. 1968.
- Higgs, R. L. and W. A. Russell. Genetic variation in quantitative characters in maize inbreds. II. Effects on performance in single-cross and double-cross hybrids. *Crop Sci.* 8:349-351. 1968.
- Hooker, A. L. Diseases and yield barriers. *Proc. Ann. Hybrid Corn Industry Res. Conf.* 22:30-36. 1967.
- Hocker, A. L. Inheritance of resistance in maize to rust caused by Puccinia sorghi. First Intl. Cong. Plant Path., London. Abstracts of papers, p. 91. 1968.
- Horner, E. S. Effect of a generation of inbreeding on genetic variation in corn (Zea mays L.) as related to recurrent selection procedures. *Crop Sci.* 8:32-35. 1968.
- Hunter, R. B. and E. E. Gamble. Effect of cytoplasmic source on the performance of double-cross hybrids in maize, Zea mays L. *Crop Sci.* 8:278-281. 1968.
- Ikenberry, R. W. and D. C. Foley. Cellulase activity in corn stalks infected with Fusarium moniliforme Sheld. and its relation to stalk rot. *Iowa St. J. Sci.* 42:47-61. 1967.
- Ivanov, V. B. Growth of root cells of maize seedlings after irradiation with high doses of x-rays. I. Inhibition of cell division, duration and rate of root growth after irradiation. *Citologia* 9:958-966. 1967.
- Jinahyon, S. Evaluation of recurrent selection for stalk-rot resistance in maize and the associated changes in other agronomic characters. *Diss. Abstr.* 27:3758. 1967. (Abstr.)
- Kendelic, V. Mutational changes induced by ionizing radiation in inbred lines of maize with a view to producing new genotypes having useful properties. *Bull. Sci. Cons. Acad. Yougosl.* 12:292. 1967. (Abstr.)

- Kuc', J., O. E. Nelson, and P. Flanagan. Degradation of abnormal lignins in the brown-midrib mutants and double mutants of maize. *Phytochem.* 7:1435-1436. 1968.
- La Fontaine, G. S. and V. Ulrich. A biochemical basis for heterosis in the RNA synthesizing mechanism of maize. *Genetics* 60:194. 1968. (Abstr.)
- Laible, C. A. and V. A. Dirks. Genetic variance and selective value of ear number in corn (Zea mays L.). *Crop Sci.* 8:540-543. 1968.
- Lambert, R. J., V. Reich, and L. Silvela Sangro. Quantitative and qualitative changes in oil content of the grain of Z. mays (L.) during ripening and germination. *Bol. Inst. Invest. Agron. Madr.* 27:63-70. 1967.
- Larson, R. L. and E. H. Coe, Jr. Enzymatic action of the Bz1 anthocyanin factor in maize. *Proc. XII Int. Cong. Genetics* 1:131. 1968. (Abstr.)
- Lim, S. M., J. D. Paxton, and A. L. Hooker. Phytoalexin production in corn resistant to Helminthosporium turcicum. *Phytopath.* 58:720-721. 1968.
- Lonnquist, J. H. Further evidence on testcross versus line performance in maize. *Crop Sci.* 8:50-53. 1968.
- Lonnquist, J. H. and N. E. Williams. Development of maize hybrids through selection among full-sib families. *Crop Sci.* 7:369-370. 1967.
- Maguire, M. P. Effect of heat treatment on crossover frequency in maize. *J. Cell. Biol.* 39:171a-172a. 1968. (Abstr.)
- Maguire, M. P. Nomarski interference contrast resolution of subchromatid structure. *Proc. Nat. Acad. Sci.* 60:533-536. 1968.
- Maguire, M. P. The effect of synaptic partner change on crossover frequency in adjacent regions of a trivalent. *Genetics* 59:381-390. 1968.
- Maguire, M. P. Chromosome pairing in altered constitutions and models of synapsis and crossing over. *Genet. Res.* 12:21-27. 1968.
- Maguire, M. P. Apparent increase in recombination frequency in regions adjacent to segmental crossover inhibition. *Genetics* 60:200-201. 1968. (Abstr.)
- Maguire, M. P. Evidence on the stage of heat induced crossover effect in maize. *Genetics* 60:353-362. 1968.

- Maleckij, S. I., V. I. Semenov, and V. K. Sumnyj. The use of gametophytic gene Ga^S for the production of an incompatibility barrier between diploid and tetraploid maize. *Genetika, Moskva* 4:98-103. 1968.
- Mangelsdorf, P. C., H. W. Dick and J. Camara-Hernandez. Bat cave revisited. *Bot. Mus. Leafl. Harv.* 22:1-31. 1967.
- Maršálek, L. and V. Voříšek. Analysis of some physiologically active substances in maize inbreds and single crosses in relation to the heterosis effect. *Z. für Pflanzenzuchtung* 59:259-272. 1968.
- Marziani, G. and M. Orsenigo. Observations on the fine structure of the plastids in a "lycopinic" mutant of maize. A preliminary report. *Atti V Cong. Ital. Microscop. Elettron.* 11:158-159. 1965.
- Matsumura, S. and T. Mabuchi. Photoreactivation of an ultraviolet light induced mutation in maize pollen. *Rep. Kihara Inst. Biol. Res.* 1966 (18):1-5. 1966.
- McClintock, B. Genetic systems regulating gene expression during development. *Developmental Biology Suppl.* 1:84-112. 1967.
- McDaniel, R. G. and I. V. Sarkissian. Mitochondrial heterosis in maize. *Genetics* 59:465-475. 1968.
- Mescerjakova, P. I. Differences in resistance to smut in reciprocal crosses of maize. *Sci. Not. Har'kov Univ.* 141:146-149. 1963.
- Nelson, O. E. The waxy locus in maize. II. The location of the controlling element alleles. *Genetics* 60:507-524. 1968.
- Nelson, O. E. A mutant gene affecting protein synthesis in the maize endosperm. *Genetica Agraria* 21:209-230. 1967.
- Neuffer, M. G. and E. H. Coe, Jr. Linkage map and annotated list of genetic markers in maize (*Zea mays* L.). In Handbook of Biochemistry, H. Sober, ed. Chemical Rubber Co., Cleveland, Ohio. 1968.
- Neuffer, M. G., L. Jones, and M. S. Zuber. The Mutants of Maize. Crop Science Soc. of Amer., Madison, Wis. 1968.
- Nickerson, N. H. Dooryard agriculture of Choco Indians in Eastern Panama. *Agricultural Ecology*, Part 10, Vol. III- Trip Reports, J. F. Gamble, S. C. Snedaker and Associates, University of Florida, Gainesville, pp. 66-74. 1968.
- Noble, S. W. Sampling of heterogeneous testers and the comparison of a double cross with parental and non-parental single crosses as testers for the evaluation of corn lines. *Diss. Abstr.* 27:3740-3741. 1967. (Abstr.)

- Orel, L. I. A cytological study of maize pollen with cytoplasmic male sterility. *Genetika Moskva* 1967 (12):3-11. 1967.
- Paez, A. V., J. L. Helm, and M. S. Zuber. Quantitative measurement of light transmission through corn endosperm. *Cereal Chem.* 45:595-599. 1968.
- Parodi, L. R. La agricultura aborigen argentina. Editorial Universitaria de Buenos Aires. 48 pp. 1966.
- Penny, L. H. Selection-induced differences among strains of a synthetic variety of maize. *Crop Sci.* 8:167-168. 1968.
- Peterson, P. A. The origin of an unstable locus in maize. *Genetics* 59:391-398. 1968.
- Peterson, P. A. Mutable genes in maize and Callan's chromosome model of serially repeated gene copies. *Nature* 221:480-482. 1968.
- Petrov, D. F., N. I. Belousova, and U. N. Yurchikov. Cytogenetic study of hybrids between tetraploid maize forms and Tripsacum. *Genetika, Moskva* 4:58-71. 1968.
- Phillips, R. L. Cytogenetic location of the gene for purple plant color in maize. *J. Heredity* 59:56. 1968.
- Reddy, G. M. Amino acid and protein content studies of some Indian maize varieties. 12th Int. Cong. Genet., Tokyo. 1968.
- Reddy, G. M. and B. V. Ramasastri. Amino acid composition of Indian maize varieties. *Indian Jour. Genet. and Pl. Breeding* 27(3):461-464. 1967.
- Robertson, D. S., M. D. Bachmann, and I. C. Anderson. The effect of modifier genes on the plastid fine structure of albino mutants in maize. *Genetics* 60:216. 1968. (Abstr.)
- Roos, E. E. and I. V. Sarkissian. Isocitric dehydrogenase and isocitric lyase in heterotic and nonheterotic maize (Zea mays L.). *Crop Sci.* 8:683-686. 1968.
- Russell, W. A. Testcrosses of one- and two-ear types of Corn Belt maize inbreds. I. Performance at four plant stand densities. *Crop Sci.* 8:244-247. 1968.
- Russell, W. A. and S. A. Eberhart. Testcrosses of one- and two-ear types of Corn Belt maize inbreds. II. Stability of performance in different environments. *Crop Sci.* 8:248-251. 1968.
- Russell, W. A. and A. R. Hallauer. Multiple-eared corn may give farmers more stable yields. *Iowa Farm Sci.* 23:3-7. 1968.

- Sahbazov, V. G. and L. V. Kotenko. The relation of heterosis to the bioelectric potentials of the cells. *News Acad. Sci. USSR Ser. Biol.* 1967 (6):891-894. 1967.
- Salamini, F. Mendelian variability in Italian maize varieties. *Maydica*, Bergamo 13:1-45. 1968.
- Sandstedt R. M., B. D. Hites and H. Schroeder. Genetic variations in maize: effects on the properties of the starches. *Cereal Sci. Today* 13:82-85. 1968.
- Santini, G. S. and V. Ulrich. Comparison of inbred and hybrid cytoplasmic RNA's on the basis of their nucleotide compositions. *Genetics* 60:220. 1968. (Abstr.)
- Sarkissian, I. V. and H. R. Srivastava. Mitochondrial polymorphism in maize. II. Further evidence of correlation of mitochondrial complementation and heterosis. *Genetics* 57:843-850. 1967.
- Saxena, K. M. S. and A. L. Hooker. On the structure of a gene for disease resistance in maize. *P. N. A. S.* 61:1300-1306. 1968.
- Scandalios, J. G. Genetic control of alcohol dehydrogenase isozymes in maize. *Biochem. Genet.* 1:1-8. 1967.
- Scandalios, J. G., E. H. Liu, and G. P. Longo. Genetic, developmental and subcellular localization studies of catalase enzymes. *Genetics* 60:221. 1968. (Abstr.)
- Sehgal, O. P., J. H. Jean, and M. S. Zuber. Sorghum hybrids as local hosts for maize dwarf mosaic virus. *Phytopath.* 58:1708-1709. 1968.
- Shlyk, A. A., L. I. Fradkin, G. E. Savchenko and A. Faludi-Daniel. Synthesis of plastid pigments in corn mutants of increased light sensitivity. *Photosynthetica*, Praha 1:241-248. 1967.
- Shortess, D. K. A genetic and physiological study of a lutescent chlorophyll-deficient mutant in maize (*Zea mays* L.). *Diss. Abstr.* 27:2994. 1967. (Abstr.)
- Shortess, D. K. A low temperature chlorophyll deficiency in maize inbred line Oh51A. *Genetics* 60:225. 1968. (Abstr.)
- Shortess, D. K., W. D. Bell and J. E. Wright. The lutescent mutant in maize: II. Nitrogen metabolism. *Genetics* 58:237-248. 1968.
- Shortess, D. K., J. E. Wright, and W. D. Bell. The lutescent mutant in maize: I. Inheritance patterns and environmental effects. *Genetics* 58:227-235. 1968.

- Siddiq, E. A. and M. S. Swaminathan. Superior radioresistance of polyploids--a tool for the preferential elimination of diploid cells in a colchicine-induced mixoploid tissue. *Curr. Sci.* 36: 307-308. 1967.
- Singh, A. and J. R. Laughnan. Mutation of the S cytoplasmic element for male sterility in maize. *Genetics* 60:226. 1968. (Abstr.)
- Singh, M. Cytoplasmic effects on agronomic characters in maize. *Indian J. Genet.* 26:386-390. 1966.
- Sinha, S. K. Chemical induction of asynapsis in maize. *Cytologia* 32: 317-319. 1967.
- Sinha, S. K. and A. P. Rao. An analysis of factors controlling chiasmata in maize. *Cytologia* 32:343-345. 1967.
- Snope, A. J. Cytogenetic studies with abnormal chromosome 10 in autotetraploid and haploid maize. *Diss. Abstr.* 27:3408-3409. 1967. (Abstr.)
- Snyder, R. J. The relationship of silk balling, husk length, husk tightness and blank tip to earworm and sap beetle resistance in maize. *Proc. Amer. Soc. Hort. Sci.* 91:454-461. 1967.
- Sprague, G. F. International outlook for hybrid corn. *Proc. Ann. Hybrid Corn Industry Res. Conf.* 22:65-70. 1968.
- Sprague, G. F. and J. H. McGahen. Influence of alien cytoplasm on yield of grain in Zea mays L. *Crop Sci.* 8:163-164. 1968.
- Sprague, G. F. and W. I. Thomas. Further evidence of epistasis in single and three-way cross yields of maize (Zea mays L.). *Crop Sci.* 7:355-356. 1967.
- Sreenath, P. R. and S. K. Sinha. An analysis of chromosomal behavior during meiosis in asynaptic maize. I. Distribution of bivalents. *Cytologia* 33:69-72. 1968.
- Stauber, S., M. S. Zuber, and W. L. Decker. Estimation of tasseling date of corn (Zea mays L.). *Agron. Jour.* 60:432-434. 1968.
- Storey, H. H. and A. K. Howland. Resistance in maize to a third East African race of Puccinia polysora Underw. *Ann. Appl. Biol.* 60: 297-303. 1967.
- Tantravahi, R. V. Cytology and crossability relationships of Tripsacum. 123 pp. The Bussey Institution of Harvard Univ. 1968.
- Ting, Y. C. Chromosome polymorphism of teosinte. *Genetics* 60:230-231. 1968. (Abstr.)

- Troyer, A. F. Yield as influenced by maturity and population. Proc. Ann. Hybrid Corn Industry Res. Conf. 22:91-98. 1968.
- Troyer, A. F. and A. R. Hallauer. Analysis of a diallel set of early flint varieties of maize. Crop Sci. 8:581-584. 1968.
- Tsai, C.-Y. and O. E. Nelson. Phosphorylases I and II of maize endosperm. Plant Physiol. 43:103-112. 1968.
- Tsangarakis, C. Z. and A. A. Fleming. Polyethylene versus glassine shoot bags in pollination of corn (Zea mays L.). Crop Sci. 8:126-128. 1968.
- Ullstrup, A. J. and M. P. Britton. An unusual epiphytotic of common corn smut in Indiana and Illinois. Plant Disease Reporter 52(12). 1968.
- Ullstrup, A. J. and A. F. Troyer. A lethal leaf spot of maize. Phytopath. 57:1282-1283. 1968.
- Ulrich, V. and R. S. Snell. The relative efficiency of ear-to-row and convergent improvement in increasing disease resistance of Zea mays. Proc. W. Va. Acad. Sci. 39:135-142. 1967.
- Van der Walt, W. J. Paramutation of R alleles of diverse geographic origin. Diss. Abstr. 28:796-797. 1967. (Abstr.)
- Van Dyke, C. G. and A. L. Hooker. The Z reaction in corn to Puccinia sorghi. Phytopath. 59:33-36. 1969.
- Vetturini, M. Cytological analysis of pure lines derived from Italian maize populations. Maydica, Bergamo 12:117-125. 1967.
- Walters, M. S. Ribonucleoprotein structures in meiotic prophase of Zea mays. Heredity 23:39-47. 1968.
- Webel, O. D. and J. H. Lonnquist. An evaluation of modified ear-to-row selection in a population of corn (Zea mays L.). Crop Sci. 7:651-655. 1967.
- Weber, D. F. A test for nonhomologous recombination in Zea mays. Genetics 60:235. 1968. (Abstr.)
- Wilkinson, D. R. and A. L. Hooker. Genetics of reaction to Puccinia sorghi in ten corn inbred lines from Africa and Europe. Phytopath. 58:605-608. 1968.
- Williams, B. R. and R. G. Creech. The ultrastructure of genetically modified Zea mays L. endosperm cells. Genetics 60:237-238. 1968. (Abstr.)

Williams, T. V., R. S. Snell and C. E. Cress. Inheritance of drought tolerance in sweet corn. Crop Sci. 9:19-23. 1969.

Wright, J. A. Estimation of components of genetic variance in an open-pollinated variety of maize using single and three-way crosses among random inbred lines. Diss. Abstr. 27:3729. 1967. (Abstr.)



