MAIZE GENETICS COOPERATION

NEWS LETTER

45

April 15, 1971

Department of Botany
Indiana University
Bloomington, Indiana

140

TABLE OF CONTENTS

I. FOREWORD

The increased size of the 1971 Maize Genetics Cooperation News Letter has added to the labors of Miss Ellen Dempsey who, once again as in the past, has been solely responsible for the editing and supervisory work entailed in publishing the volume. I am confident that I speak for all who profit from these informal research reports when I express our gratitude and appreciation for her dedicated and efficient services. Hers is a demanding task and her critical reading of manuscripts has saved more than one contributor from embarrassing errors.

The cost of publishing the 1971 News Letter has been met from a grant by the National Science Foundation to the Maize Genetics Stock Center at the University of Illinois. There could be no News Letter without this financial support, so our thanks are heartfelt.

One of the virtues of the Maize News Letter is that no contribution has been subjected to editorial review before publishing. We have accepted all reports which have been submitted, relying upon the judgment of the contributors as to their worth. Admittedly, some reports have been more verbose than seemed necessary and occasionally one is of marginal genetic interest, but on the whole the policy has worked well. It may well be that our increasing publishing costs will force us to limit the size of the News Letter and that some editorial evaluation may occasionally be necessary before accepting a contribution. I hope this never happens, for if it does it will be a policy foreign to the past tradition of the News Letter and at variance with the uninhibited exchange of information between students of maize.

M. M. Rhoades

II. ANNOUNCEMENT

Barley Genetics II (Proceedings of the Second International Barley Genetics Symposium) will be published in late winter of 1970-71. It contains 69 papers under the following main headings: Germ Plasm Sources and World Collections of Genes; Origin and Evolution; Chromosome Aberrations and Their Utilization; Mutations - Induction and Utilization; Linkage Data and Chromosome Mapping; Genetic Fine Structure Analyses; New Phenomena Important to Barley Genetics and Breeding; Breeding Techniques; Hybrid Barley; Genetics of Yield; Genetics of Hardiness, Growth Habit and Aluminum Reaction of Winter Barley; Population Genetics; Genetics of Disease and Insect Resistance; and Genetics of Feed and Malt Quality. It also includes reports from committees concerned with: genetic marker stocks, nomenclature and gene symbols; origin, phylogeny and exploration; chromosome aberration stocks; and barley literature.

The volume of xvi plus 622 pages will cost $20. Orders for this book should be addressed to the Washington State University Press, Washington State University, Pullman, Washington, 99163.

R. A. Nilan, President
Third International
Barley Genetics Symposium

III. REPORTS FROM COOPERATORS

AGRICULTURAL ALUMNI SEED IMPROVEMENT ASSOCIATION, INC.
Romney, Indiana

1. Mini-plant, a dominant anther-eared dwarf.

During 1963 through 1965 a synthetic was built up containing the following eight lines, C103 cms^{T} Rf Rf, C123, H14, Mo17, Oh40B, Oh41, Va20 and Va36. Each of these lines traces wholly or partially to the Pennsylvania variety Lancaster Surecrop and we have designated the synthetic as our Lancaster Synthetic.

In 1966 the synthetic was grown in an isolated plot about one acre in size and seed ears saved from 214 selected plants. Seed from these ears was mixed for a planting in 1967. As C103 cms^{T} Rf Rf had been the ultimate seed parent in producing the synthetic, the plantings in 1967 contained both fertile and male sterile plants. About 300 desirable fertile plants were self fertilized and about 75 desirable sterile plants were pollinated with pollen from desirable fertile plants.

In 1968 ear-to-row progenies were grown from 245 of the selfs and 68 of the sterile x fertile sib crosses. One of the sib-crossed progenies, row 5670, contained 13 normal (tall) plants and 12 short plants. The short plants were vigorous, 5 to 6 feet tall and produced good sized ears. At harvest it was observed that all of the ears from the short plants were anther-eared.

One of the short plants in row 5670 was selfed and the progeny grown from it contained 84 short plants and 28 normal plants, a perfect 3:1 ratio. Eight normal plants from this progeny were selfed and the progenies grown from them contained only normal plants. Fifteen of the short plants were crossed on plants of a normal single cross as a tester. Progenies from four of these testcrosses contained only short plants indicating the parent was homozygous for the gene concerned. The remaining 11 progenies segregated in 1:1 ratios for short and normal plants. The data from these testcrosses are reported in Table 1.

Table 1

Data on crosses of short plants on a normal single cross tester

Pedigree	Tall	Short	Total
(Oh28 x M14) x Short 1	17	10	27
(" ") x " 2	16	16	32
(" ") x " 3	16	12	28
(" ") x " 5	9	20	29
(" ") x " 6	16	14	30
(" ") x " 7	16	8	24
(" ") x " 8	19	10	29
(" ") x " 9	11	15	26
(" ") x " 11	11	18	29
(" ") x " 14	14	13	27
(" ") x " 15	14	15	29
Total	159	151	310

The data indicate that the character is controlled by a monogenic dominant. Anther-eared plants have not been observed in any of the eight lines used as parents of the synthetic or in the synthetic itself. The ear which furnished the seed for row 5670 did not have anthers. This leads to the conclusion that the plant furnishing pollen for the sib-cross must have been heterozygous for the gene involved and the mutation may have occurred in one of parental gametes of this plant.

Mini-plant is suggested as a timely name for this character with a temporary designation as D5670 (pending allele tests with other dwarfs). Heterozygous mini-plants have been vigorous and productive. Homozygous mini-plants are much reduced in vigor and many are only 2 to 3 feet tall.

Seed carrying the D5670 gene has been supplied to the Maize Genetics Cooperation. Being a dominant the character should be useful as a genetic marker. Although some progenies have been a little difficult to classify on the basis of plant height, the character is readily and definitely classified at silking time or later by the occurrence of anthers on the ears.

M. T. Jenkins

THE BIRLA INSTITUTE OF SCIENTIFIC RESEARCH
Rupar, Punjab, India
Department of Agricultural Research

1. Studies in the development of "Notched leaf of corn".

In the 1969 volume of the Maize Genetics Cooperation News Letter, Bhat and Pande reported the occurrence of a new leaf character in Caribbean Flint Composite Corn. The new character has subsequently been assigned the name "Notched leaf of corn" and symbolized with Nt. Reported observations, then, were confined to the positioning of Nt and its external morphological details. During the summer growing seasons of 1969 and 1970, Nt was studied at great length for its development. This communication is based on those studies.

In the 1970 population of 7600 plants, in different self or hybrid generations, the first Nt appeared in a self family on the forty-fifth day from sowing. In a homozygous population, Nt appeared on different plants at varying time intervals but all within ten days. In an individual plant bearing Nt on more than one leaf, the character appeared more or less simultaneously on all the leaves, but exceptions have frequently been recorded where Nt appeared on lower leaves seven days prior to upper leaves.

In initial stages of development Nt appears as a single or a series of parallel or in many cases coalescing whitish streaks or bands. These bands differ considerably in their longitudinal spread. From less than one centimeter in some cases, it may extend through the entire length of the leaf. In later cases fully developed Nt appears to have either a solitary comet like "tail" or a number of diverging "tails". Against light, streaks and bands look very bright white against a green leafy background.

The second step in the development of Nt consists of the centrally oriented fusion of streaks and the appearance of a groove at the broad point in the band. Within forty-eight hours, one or more deep indentations (= Notch) appear on the leaf. A notch usually bears one or two deep indents but as many as five in a series have also been noticed. In forty percent of the cases, bands or streaks develop only imperceptible

grooves or none at all. In later types Nt is represented by faint or sharply defined streaks or bands. In many leaves, thus, well defined Nt can be seen side by side with a Nt recognizable only when the leaf is viewed against light.

Towards maturity soft, silky bristles develop on both dorsal and ventral surfaces of Nt. These bristles are thicker, longer and denser than those normally found on the leaf surface. Before drying of the plant blotches of pink colour appear on the Nt. These blotches turn brownish with drying.

Though the spatial position of a Nt on the leaf, from its inception to maturity, is stationary, in scattered cases Nt seems to "shift" upward towards the apex. It appears that Nt is carried upward with developing tissue. In a closely observed case a break in the band occurred at the point of initiation and Nt appeared well above toward the apex. A connection between the two was maintained by a thread-thin streak. It, therefore, is cogent to conclude that only a specified tissue zone is transformed to Nt and that the process of development is pre-defined.

Mohan C. Pande

2. Character association of "Notched leaf of corn".

While investigating large populations for Nt, the frequency of some other plant characters in modified expressive forms was found to be significantly high. These characters, however, do not show a close linking with "visible" Nt. Four characters studied in detail are discussed below.

(A) Pollen production and sterility: Very poor pollen production in Nt populations was noted in the summer of 1968. Partial sterility of the pollen has also been noticed. Anthers, though well developed, fail to dehisce. Pollination with crushed anthers produced very few or no grains. In continuous self progenies the loss in pollen production has increased significantly. A tendency of "more notching - less pollen" is discernible in these families. A very poor seed set is obtained in hybridisation attempts. In hybrids involving Nt as a parent, the pollen production is normal.

(B) Barren culms: A very high proportion of barren culms has been observed in Nt populations. The frequency of occurrence ranges between twenty to fifty percent and is equally distributed among notched and notchless derivatives of original Nt. In isolated self families the frequency of barren stalks is as high as eighty percent. Over the years the barren population in self families has increased many fold. A tendency of "more notching - less female fertility" is seen. In cross section the stalk looks circular and the characteristic concave groove is absent.

(C) Dwarf culms: All inbred lines derived from original Nt, either by continuous selfing or sibbing, have produced plants one half to one fourth the normal height. This dwarfism is accompanied by a thickening of the lower stem, a reduction in the internodal distance (the number of nodes remaining the same), a lower placement of the ear (in some cases right at the soil level) and a hardening of the entire culm. Leaves become very dark in colour, shorter, very wide, stiff and wrinkled in most families. Hybrids with Nt parents, however, are normal and sometimes taller than either parents.

(D) Anthocyanin pigmentation: From the start it has been observed that derivative lines from parental Nt stocks produce deep purple coloured stalks, leaf sheaths, ear husk and leaf margins. The pigmentation becomes deeper toward maturity. In a 1970 summer hybrid family (F_1 normal ♀ backcrossed to Nt ♂) purple pigmentation was so widespread and intense that the entire plant was purple at maturity. Calculations indicate that about seventy-five percent of the total plant surface area is purple in this family. In notchless derivatives the pigmentation is normal.

Mohan C. Pande

3. Inheritance of "Notched leaf of corn": Some considerations.

Originally starting from two 1967 Nt plants, some 200 self and 75 hybrid lines have been developed and field investigated. The segregation pattern available from these studies does not permit a definite theorisation. In Table 1, the behaviour of self families derived from one of the original Nt plants is shown. Table 2 gives the hybrid behaviour of the same.

Table 1

Segregation of self progenies for the Nt factor

1967 (S1)	1968 (S2)	1969 (S3)	1970 (S4)
2 Nt : 240 Normal	17 Nt : 49 Normal	52 Nt : 17 Normal	30 Nt : 31 Normal
		105 Nt : 36 Normal	15 Nt : 10 Normal
		51 Nt : 31 Normal	31 Nt : 8 Normal
		27 Nt : 6 Normal	54 Nt : 19 Normal
			85 Nt : 1 Normal
			46 Nt : 26 Normal

At the present level of investigations two possibilities can be considered to explain the nature of Nt: first, a gene based mechanism and second, a chromosomal aberration theory.

If a gene based mechanism is considered, then it is apparent that the gene or genes involved have a very weak phenotypic stability. With advancing generations, however, the expression of Nt is becoming stronger (as is evidenced by comparative visual studies of Nt in the last three years). The poor and incomplete penetrance may also be due to a complex manipulation of the character by a major gene and some modifiers so that phenotypic manifestation of the Nt depends on the modifying actions of the latter.

An alternative possibility is that a chromosomal aberration (probably a deletion) is responsible for Nt. Bridges (1917) and Mohr (1917) investigated a similar deletion in Drosophila melanogaster. Also called "Notch," the mutant produced a notched margin of the wings. In the female, the mutant was inherited as a sex linked dominant while in the male it was lethal. A number of recessive genes in the deleted chromosome were also observed to show pseudo dominance. In the "Notched leaf of corn" a parallel condition can be observed. A survey of Table 1 indicates that in many families Nt behaves like a dominant factor. Off ratios, however, are very frequently recorded. Table 1 includes such ratios. It is possible that pollen sterility and barren culm factors contribute toward this ratio disturbance. In 1970 summer,

Table 2

Behaviour of Nt in hybrids

Cross	F_1 Segregation	⊗ Class or Cross	F_2 Segregation	⊗ Class or Cross	F_3 Segregation
BM 178 x Nt	1 Nt : 239 Normal	Nt	15 Nt : 79 Normal	Nt	15 Nt : 49 Normal
				Normal	0 Nt : 52 Normal
				Normal Nt	3 Nt : 22 Normal
		Normal	6 Nt : 172 Normal	Normal	0 Nt : 53 Normal
Nt x BM 408	0 Nt : 121 Normal	Normal	0 Nt : 51 Normal		
Nt x BM 404	0 Nt : 320 Normal	Normal	0 Nt : 107 Normal		
BM 408 x Nt	7 Nt : 626 Normal	Nt - 1	17 Nt : 72 Normal		
		Nt - 2	27 Nt : 143 Normal		
		Nt - 3	10 Nt : 94 Normal		
		Normal bc Nt	13 Nt : 64 Normal		
BM 404 x Nt	6 Nt : 226 Normal	Nt - 1	10 Nt : 74 Normal		
		Nt - 2	5 Nt : 103 Normal		
		Normal - 1	0 Nt : 86 Normal		
		Normal - 2	0 Nt : 74 Normal		

BM 170, BM 404 and BM 408 are inbred lines of the Institute.

one family with a homozygous genotype for Nt has been isolated.

The behaviour of Nt in hybrids gives another indication of chromosomal deletion. A survey of Table 2 shows that a very low frequency of character transmission to F_1 progenies is shown by Nt. Further, Nt appears in F_1's only when transmitted through the pollen. Differential transmission of the deletion in the male and female gametophytes may be the cause of the disturbance in the observed phenotypic ratios.

The very low frequency of Nt in open populations and hybrids and its stabilization pattern in some continuous self families compare well with several studies on chromosomal aberrations. Norman Giles (1940) showed that in Tradescantia spontaneous chromosome aberrations are produced in very low frequencies and are caused by very low natural radiation doses. Sax (1940, 1938) also states that the frequency of spontaneous or induced deletions shows an approximately linear relation with radiation doses and hence they have varying levels of stability and perpetuation.

In 1967 summer only two plants in a population of 240 produced Nt. Seed for this population came from a five year old stock stored in natural conditions of temperature and humidity. It has been demonstrated by some workers (Peto, 1933 and Navashin, 1933) that seedlings grown from aged seed show a pronounced increase in the frequency of spontaneous chromosome structural changes. Physiological conditions attendant on aging may be responsible for the increase in such structural changes. Storage factors coupled with natural sources of stress (radiations, etc.) might have been responsible for Nt.

Studies with the character association of Nt suggest that a segment of the third chromosome may be involved. This, however, is a tentative proposition and needs further strengthening.

References

Giles, Norman. 1940 Spontaneous chromosome aberrations in Tradescantia. Genetics 25:69-87.

Sax, K. 1940 An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics 25:41-68.

Sax, K. 1938 Chromosome aberrations induced by X-rays. Genetics 23: 494-516.

Navashin, M. 1933 Origin of spontaneous mutations. Nature 131:436-438.

Peto, F. N. 1933 The effect of aging and heat on the chromosomal mutation rates in maize and barley. Can. J. Research 9:261-264.

Mohan C. Pande

4. Does "Notch" represent an altered biochemical sequence?

While conducting some anatomical studies with Nt leaves, a few leaf portions were inadvertently put in a jar containing strongly acetified solution of ferric acetate in absolute alcohol. Within a fortnight's time while green, chlorophyll containing parts of the leaf turned colourless, the notched area and streaks emanating from it turned brown-black.

This indicates a chemical reaction in the Nt area. Several possibilities exist to explain this phenomenon. The absence of chlorophyll from the notched area indicates the presence of either an altered form of the pigment or some other chemical not to be found in other tissues of the leaf. This altered pigment or chemical could be reacting with ferric or acetate ions in the solution. The altered chemical may be a carbohydrate or a protein.

If a gene based mechanism is accepted, then the reaction will have to be viewed either as a primary or a secondary product of the gene action. Further studies are necessary before a final theory is accepted.

Mohan C. Pande

BOSTON COLLEGE
Chestnut Hill, Massachusetts 02167
Department of Biology

1. Further studies on the synaptonemal complex of haploid maize.

Microsporocyte divisions of haploid maize were studied with electron microscopy. From zygonema to early pachynema, the central element of synaptonemal complexes was not present, while the two lateral elements were consistently observed. At middle pachynema, all of the

three elements of the complexes were clearly shown. However, as soon as the divisions reached late pachynema and early diplonema, the central element became diffused. At middle diplonema, the two lateral elements also disappeared, and the chromatin areas of the nuclei were apparently devoid of any structure. In parallel with this, investigations with light microscopy were likewise carried out. It was found that during microsporocyte divisions of haploid maize, chromosomes behave unconventionally. For instance, from zygonema to early pachynema, most of the chromosomes formed nonhomologous pairings of the foldback type. Most of these pairings seemed loose. As the divisions advanced to middle pachynema, the pairings became complete and close. However, from late pachynema to early diplonema, they gradually dissociated themselves. At middle diplonema, all of the chromosomes straightened out and became single. This study supports my previous theory that synaptonemal complexes are the product of chromosome pairings, either homologously or nonhomologously. Therefore whenever the chromosome pairings disappear, as at the middle diplonema of haploid maize, the complexes cease to exist.

Y. C. Ting

2. Staining maize chromosomes with a DNA-binding fluorescent agent.

In order to examine the genetic organization of maize chromosomes, a fluorescent agent, quinarine mustard dihydrochloride, was used to label pachytene chromosomes of maize. The techniques followed were about the same as those employed by Caspersson et al. (1970) to identify metaphase chromosomes of animals and plants, and also humans. It was found that the maize chromosomes were selectively labeled along their length. The heterochromatic regions, such as knobs, were strongly fluorescent. This demonstrates the genetic difference between heterochromatin and euchromatin of maize chromosomes. Through this study it is hoped that an insight into the genetical relationships among different races of maize as well as between maize and its relatives will be provided.

Y. C. Ting

3. The effects of X-radiation on maize pollen.

Before crossing, pollen grains of the male parent in a cross of Wilbur's Flint X tester, having the genotype $\underline{A_1}$ $\underline{C}$ $\underline{R}$ $\underline{pr}$ $\underline{su}$, were subjected to acute X-radiation with a dose of 1500r. Among the F_1 individuals, one of the plants which was shown to contain aberrations was self pollinated. All of the 51 seeds from this plant were grown last summer. Forty-one of them germinated and grew into mature plants. Twenty-three plants were available for chromosome studies. The inflorescences were collected and fixed and the anthers were squashed by following standard acetocarmine squash techniques. Of the 23 plants studied, one was found to contain a fragment. This fragment was measured at pachytene and found to be approximately 29.5u. Another plant frequently showed a bridge at anaphase I, but no fragment accompanying the bridge was observed. A further study is in progress to trace the fragment through to the quartet stage.

Lorraine Sartori

BROOKHAVEN NATIONAL LABORATORY*
Upton, New York
Biology Department

and

FUNK BROTHERS SEED COMPANY**
Bloomington, Illinois
Research Department

1. Studies on induction of cytoplasmic male sterility with ethyl methanesulfonate.

"Apparently EMS can be used to produce cytoplasmic mutants in plants . . . and may be useful to produce cytoplasmic sterility in maize . . . probably more important, cytoplasmic sterility may be produced in other species . . ." (1). This statement was made based on research started at Brookhaven National Laboratory in 1967. At that

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

**Present address.

time the induction of cytoplasmic male sterility in maize was more academic than pragmatic. Following the discovery that corn with Texas male sterile cytoplasm is more susceptible to yellow leaf blight (Phyllosticta sp) and southern leaf blight (Helminthosporium maydis, Race T) than normal cytoplasm, research on detection of a new source of cytoplasmic male sterility is timely. Research on using EMS to induce cytoplasmic male sterility was started at Brookhaven National Laboratory during 1967 and has continued here at Funk Bros. Seed Co.

Initially, the inbred M14 was used and since 1969 W22 was used in these studies. To date, 18,087 plants have been examined to detect cytoplasmic male sterility, including treated and control populations. The total was made up of M14 treated with various doses of EMS, 3,045; W22 treated with various doses of EMS, 8,746; W22 control, 6,296 (Table 1).

In the first generation after EMS treatment, the plants were self pollinated. In the next generation, the self pollinated material was planted ear-to-row. Sterile plants in these progeny rows were crossed with the untreated inbred parent. Out of 818 of these progeny rows, 49 had from 1 to 5 male sterile plants. The male sterile plants, after being crossed with the untreated inbred parent, were grown in a subsequent generation. If any of this material was sterile, it was again crossed with the untreated inbred parent; this happened in three cases. One of the cases (2 ears) will be checked in the next generation to determine if it will remain male sterile. This is in a control population.

Material that was sterile after the first self pollination could be sterile due to a recessive or dominant gene or cytoplasmic factor. Plants remaining sterile in the next generation could be due to a dominant gene or cytoplasmic sterility factor. Plants sterile and not segregating in the next generation would be due to cytoplasmic factors. Therefore, to date no cytoplasmic sterile plants were induced by EMS and one control population remains to be checked in the next generation for the presence of cytoplasmic male sterility. Treatment procedures and further references to this work can be found in previous Maize Newsletters (1, 2).

Table 1

Ethyl methanesulfonate treatments (10 hours at 25° C.)
and progenies with sterile plants

Inbred	Mutagen Treatment	Nr. M_1 Rows	Nr. M_2 Plants	Nr. M_1 Rows With Steriles
M14	1	45	675	0
M14	2	63	945	3
M14	3	64	960	1
M14	4	26	390	1
M14	5	5	75	0
W22	3	308	8,009	22
W22	4	47	737	6*
W22	Control	260	6,296	16**
		818	18,087	49

1 - 0.005M 10 hrs. @ 25° C., planted wet.

2 - 0.01M 10 hrs. @ 25° C., planted wet.

3 - 0.005M 10 hrs. @ 25° C., post-soaked 3° C. 48 hrs., dried 72 hrs. at 60% R.H.

4 - 0.0075M 10 hrs. @ 25° C., post-soaked 3° C. 48 hrs., dried 72 hrs. at 60% R.H.

5 - 0.01 M 10 hrs. @ 25° C., post-soaked 3° C. 48 hrs., dried 72 hrs. at 60% R.H.

* - 2 with sterile plants in next generation but fertile in following generation.

** - 1 with sterile plants in next generation, remains to be checked in following generation.

EMS has been amply demonstrated to be a good mutagen of nuclear genes. Hence it might be a good cytoplasmic mutagen if the genetic material is similar for both methods of inheritance. There are a few reports of the induction of cytoplasmic mutants in plants. Dulieu (3) used EMS to induce chlorophyll deficient mutations in Nicotiana that were maternally inherited. Favret and Ryan (4) have induced cytoplasmic male sterile mutants in barley with x-rays and with EMS. Also, Lysikov et al. (5) reported that cytoplasmic male sterility has been induced in maize by chemical and physical mutagens.

Failure to detect cytoplasmic male sterility in the EMS treated material may be due to relatively small populations used; also, cytoplasmic male sterility may occur at a very low frequency.

References

(1) Briggs, Robert W. (1969) Maize Genetics Newsletter 43:23-31.

(2) Briggs, Robert W. (1970) Maize Genetics Newsletter 44:11-17.

(3) Dulieu, H. (1967) Mutation Research 4:177-189 (In French).

(4) Favret, E. A. and G. S. Ryan (1966) Mutations in Plant Breeding, pp. 49-61. International Atomic Energy Commission, Vienna.

(5) Lysikov, V. N., A. N. Konotop, O. V. Bljandor and S. G. Byrka. (1970) Plant Breeding Abstracts. Vol. 40.

Robert W. Briggs

DEFIANCE COLLEGE
Defiance, Ohio

1. The effect of red and far-red light interruptions on paramutant R expression.

In MGCNL Vol. 43 we reported that the level of aleurone pigment produced by R' (paramutated R) was directly related to the number of dark periods administered to seedlings at an early stage of tassel initiation. We have been concerned with defining more closely those periods of development when R' is most sensitive to genetic "instruction" as well as finding more effective treatments for making heritable changes in R' expression.

Since much physiological work over the past 20 years has indicated that many plant systems are responsive to red and far-red light, it was necessary to find ways of testing the paramutational system of $\underline{RR}^{st}$ for its responses to both red and far-red. This report is concerned with changes in $\underline{R}'$ expression following red and far-red light interruptions of tassel-inducing dark periods.

Inbred W22 seedlings, heterozygous for $\underline{RR}^{st}$, were sown at growth chamber temperatures of 26-27° C. under continuous light supplied by 14 200W cool white fluorescent lamps 80 cm. from the surface of the soil. At 12 days of age, seedlings were subjected to six cycles of 12 hours of light and 12 hours of darkness (12:12,L:D) for six successive days. Each dark period was interrupted for one hour with red, far-red or white light during the 3rd, 5th, 7th or 9th hours--making a total of 12 different treatments.

Red light was provided by filtering with one eighth inch red plastic (Rhom and Haas) with a transmission maximum between 650 and 675 nanometers. Far-red light was supplied by filtering with combined red and blue plastic where the blue filter showed a transmission maximum between 450 and 475 nanometers. The light source for the above filters was provided by one 150W incandescent bulb with built-in reflector. The bulb was positioned 60 cm. above the tops of the plants. White light interruption was supplied by two of the above incandescent bulbs 60 cm. from the tops of plants.

Following L:D treatments, plants were held in constant light until the 20th day at which time all seedlings were transplanted to field conditions for maturation. Testcrosses of each of the above series of treated plants were made on Inbred W22 tester, $\underline{rr}$, females. 50 $\underline{R}'$ kernels from each testcross ear were scored for aleurone pigment level by matching each against a set of standard kernels ranging from colorless, assigned a value of 0, to completely pigmented, assigned a value of 22. Kernel means for each testcross ear are reported along with treatment means and their variance. Results of t-test comparisons are reported.

Table 1 shows that treatment means for $\underline{R}'$ expression from seedlings which received red light interruptions at the 3rd and 5th hour of the dark period differ significantly from means of $\underline{R}'$ kernels

representing plants whose dark periods were interrupted with far-red either the 7th or the 9th hours ($P =< .01$). It can be noted that the R' scores of seedlings interrupted with white light lie between scores of those seedlings which received red and far-red light interruptions. Among those seedlings interrupted with red light, plants treated the 3rd hour of the dark period produced R' scores different from those treated the 7th or the 9th hours ($P =< .05$ and $P =< .02$, respectively).

Table 1

Comparison of R' expressions following light interruptions of dark period. Treatment means of R' kernels from testcrosses of RR^{st} plants which had six dark periods interrupted by one hour of light beginning at the designated times.

Color of Light Interruption	Hour of Dark Period for Light Interruption (each of six nights)			
	3	5	7	9
Red	n = 8	n = 10	n = 13	n = 9
	12.70	13.35	13.99	14.79
Variance	.80	1.64	2.03	3.88
Far-Red	n = 11	n = 10	n = 8	n = 8
	14.85	14.69	15.10	15.64
Variance	3.47	1.34	.71	.85
White	n = 9	n = 10	n = 11	n = 9
	13.75	13.97	14.76	13.45
Variance	1.35	1.07	1.79	3.09

Table 2

Ear means of R' expression from RR^{st} plants which received light interruptions of their dark periods.

These data are summarized in Table 1.

Color of Light Interruption	Hour of Dark Period for Light Interruption			
	3	5	7	9
Red	11.92	15.08	12.66	15.28
	11.29	14.69	14.90	13.36
	13.24	12.80	15.18	15.16
	13.70	14.82	15.53	17.66
	12.94	13.30	12.96	12.88
	12.22	12.82	14.70	16.40
	12.44	12.74	14.00	16.82
	13.86	11.26	13.46	11.80
		11.92	10.26	13.76
		14.02	13.84	
			14.38	
			15.04	
			14.96	
Far-Red	13.32	13.72	15.58	14.18
	16.92	14.96	14.53	16.72
	13.08	14.84	14.82	16.88
	16.02	13.42	14.98	15.02
	14.58	13.98	14.02	15.24
	11.68	13.94	15.00	15.24
	14.12	17.18	16.86	15.52
	15.82	15.82	14.98	16.30
	18.02	15.18		
	13.92	13.89		
	15.90			
White	15.56	14.59	13.10	16.69
	13.82	14.84	14.86	12.12
	13.88	15.86	15.37	10.62
	14.10	12.60	13.74	12.16
	11.56	13.54	13.64	13.28
	13.20	14.80	16.00	13.82
	14.18	13.66	16.46	14.00
	14.74	13.42	16.96	14.98
	12.71	12.70	15.00	13.39
		13.64	13.12	
			14.10	

A variance increase can be noted for plants which received red light interruptions toward the end of the dark period whereas score deviations are greatest for plants which received far-red interruptions early in the dark period. The data suggest red light, applied early in the dark period, has a greater probability of conditioning the R' expression to a lower level of expression in the W22 Inbred. Far-red has the opposite effect of red light and is most effective when applied toward the latter part of the dark period.

The difference in effect of red and far-red on R' expression is of that reported for various L:D treatments at early stages of development.

Bernard C. Mikula
Jordan Christiansen

2. Heritable differences in paramutant R expression after one growing season in different latitudes of the United States.

In previous reports, MGCNL Vols. 41, 42, 43, we have shown that heritable levels of R' (paramutated R) expression are conditioned by light-dark cycles applied early in development. It seemed likely, therefore, that different levels of R' expression could be conditioned by natural environments if RR^{st} plants were grown out one season at different latitudes. Such latitudinally induced heritable differences in gene expression would pose a very interesting challenge to existing biological dogma regarding sources of genetic variation for evolution. To test the hypothesis that one growing season in a different environment can make a heritable difference in a gene expression, a common lot of seed was divided and distributed to each of six locations across the latitude of the United States.

Seeds of Inbred W22 RR^{st}, from a cross involving a single pollen parent of RR to $R^{st}R^{st}$ female sib plants, were distributed to Wisconsin, Illinois, Iowa, Texas, and a final sample was grown in Ohio (a Missouri sample was lost because of adverse weather conditions). At each of the localities named above the Inbred W22 RR^{st} plants were testcrossed to Inbred W23 rr. The hybrid seed (W22 and W23) was then returned to Defiance, Ohio to be grown out and testcrossed to assay the level of R' expression.

Seeds of six ears returned from each geographic area were pooled and replicate field plantings were made for each of the five areas. The R'r hybrid plants were testcrossed to highly Inbred W22 rr; six testcross ears from each replicate planting were scored (total of 12 ears representing each geographic locality). 50 R'r kernels were removed from each testcross ear and compared against a set of standard kernels ranging from colorless, score of 0, to completely pigmented, scored 22. A mean kernel score for each ear was determined and from this a mean ear score for each geographic locality was computed and compared, by means of the t-test, with each of the scores from other localities.

Analysis of the results of Table 3 shows that a significant difference between R' expression from Texas and Wisconsin exists, $P < .001$. A significant difference was also found when plants from seeds produced in Texas were compared with those from Iowa, Illinois or Ohio, $P < .01$. Scores of R' expression from seeds derived from Wisconsin approach the margin of significance when compared with Iowa, or Illinois, $P < .02$ and $< .06$ respectively.

It is remarkable, therefore, that one season at a given latitude within the United States can make a measurable, heritable, input into the expression of a single gene. It is known that native plant populations across the latitude of the United States can differ morphologically, physiologically and genetically. Accounting for the source of such variation, however, can make very stringent demands of a biological system, experimentally. Few systems can satisfy the demands for accounting for sources of variation and, as a result, it is commonly assumed that the latitudinal differences observed in native plant populations are the result of long periods of time. The experimental results reported here suggest that certain gene combinations, active at critical periods of development, could have much greater flexibility and responsiveness to environmental conditions than existing interpretations of experimental evidence permit. From the information on the R locus, it appears that genes or gene complexes with the capabilities of paramutant combination, RR^{st}, could show all the necessary requirements for data processing for evolutionary purposes--such as gene complex could receive, store, sum

Table 3

Comparison of R' expression from RR^{st} plants raised in different areas. Ear mean scores of R' from testcrosses of R'r hybrid plants raised the previous year in five states across the latitude of the United States.

Wisconsin	Ohio	Illinois	Iowa	Texas
20.02	19.88	17.02	17.68	15.32
19.98	21.10	18.90	18.96	15.18
18.52	19.48	18.56	17.68	17.48
19.02	18.77	19.50	19.24	17.46
18.50	18.84	18.41	19.88	15.82
19.72	17.98	18.88	19.18	17.52
19.53	21.20	19.64	18.72	15.66
18.68	19.44	16.87	19.70	16.02
20.94	16.82	16.76	19.22	17.30
19.66	18.42	19.10	19.13	18.86
20.54	17.12	21.48	18.08	18.76
21.18	19.66	19.62	17.14	17.96
19.69	19.06	18.73	19.24	16.95
			18.76	

and feed back information--in short, it could have all the important qualities of a "modern", "responsive" genetic system worthy of a higher plant.

This study was made possible through the assistance and cooperation of the following geneticists who provided their time and laboratory facilities for the production of the seeds reported on in this study:

Edward H. Coe, Jr.	Univ. of Missouri, Columbia
Jerry L. Kermicle	Univ. of Wisconsin, Madison
John R. Laughnan	Univ. of Illinois, Urbana
Marjorie P. Maguire	Univ. of Texas, Austin
Peter A. Peterson	Univ. of Iowa, Ames

Special acknowledgment is made of the help of the Charles F. Kettering Foundation and the Center for the Biology of Natural Systems, Washington University, St. Louis, under Public Health Service Grant No. ES-00139-01.

Bernard C. Mikula
John Trautman

DEKALB-ITALIANA S.p.A.
Mestre, Italy

and

DEKALB AGRESEARCH, INC.
Dekalb, Illinois

1. Helminthosporium maydis in Italy.*

The fungus Helminthosporium maydis Nishikado and Miyake has been noted to occur in isolated fields of maize in Italy (Goidanich, 1964). Symptoms of an infection by the fungus were observed on leaves, stalks and ears of maize. No economic significance was attached to these occurrences.

Photographic records of symptoms resembling those commonly associated with H. maydis infection were obtained by Cinzio De Carli of the "Ispettorato Agrario" of the province of Brescia in 1956-1958. These photographic records did not specifically cite the localities in which they were made.

Following the outbreak of the T-race epidemic of H. maydis in the U.S.A. in 1970, the authors undertook a survey to detect the presence of the fungus in Italy during the 1970 season. Symptoms of H. maydis were observed in a farm at Vacarrino, Padova originally located by Dr. Grancini of the Istituto Sperimentale per la Cerealicoltura, Bergamo, on September 15. Infected tissue collections were made on this site and in an additional field near Brescia on September 16.

The above collected samples were used as a source of inoculum for infecting normal (N) and Texas sterile (T) carrying counterparts of maize inbred lines. The inoculations were made under greenhouse conditions and according to procedures outlined by Hooker et al. (1970). The results of the inoculations are shown in Table 1.

---

*Edited by Dr. Basil Tsotsis, Dekalb Agresearch, Inc.

Table 1

Degree of infection of maize seedlings five days after inoculation with H. maydis

	(a) mean	(a) range
WF9 N	5.7	5-7
WF9 T	7.7*	7-9
B14 N	3.7	3-5
B14 T	8.3**	7-9
A239 N	3.0	3
A239 T	7.7**	7-9
W64A N	3.0	1-5
W64A T	7.0**	7

(a) mean of 9 seedlings and possible range from 1 (free of symptoms) to 9 (completely susceptible).

* and ** indicate significance at the .05 and .01 levels for the difference between means of same inbred.

The data appear to be similar to those obtained with comparable material in the U.S.A. following inoculations with the T race of H. maydis and suggest that the same (T) or a similar race of the fungus were present in Italy. On the basis of the severity of symptom expression and economic loss under field conditions, however, the infections were considerably milder than those reported from the U.S. with race T. Had it not been for the U.S. epidemic with race T, the level and scale of infection in Italy would have been considered similar to those reported earlier in Italy and for periods before the Texas T cytoplasm was used for hybrid making. On this basis, it appears to the authors that if the presence of the T race is further confirmed in 1971 in Italy, its damaging effects may not be as significant as those reported from the U.S. Further, since H. maydis has only been observed in the Po Valley, local inoculum appears to be the likely source of infection. Movement of inoculum from areas in the South in a manner analogous to the U.S. experience does not appear to be plausible.

References

Goidanich, G. (1964) Manuale di Patologia Vegetale. Vol. II: 1150. Edizioni Agricole Bologna.

Hooker, A. L. et al. (1970) Reaction of corn seedlings with male sterile cytoplasm to Helminthosporium maydis. Plant Disease Reptr. 54 708-712.

P. Grandi
L. Costa-Rodrigues

HEBREW UNIVERSITY OF JERUSALEM
Rehovot, Israel

1. Tissue differences in the relative activity of alcohol dehydrogenase isozymes in maize in crosses with a newly discovered high activity line.

Efron (Science 170:751,1970) described an inbred line of maize with low activity of alcohol dehydrogenase (ADH) in the scutellum of the mature seed. This line is homozygous $\underline{Adh_1}^S/\underline{Adh_1}^S$, having a slow migrating isozyme band. In a genetic study, the above line was crossed with a homozygous $\underline{Adh_1}^F/\underline{Adh_1}^F$ line having a fast migrating isozyme band and a normal activity of ADH. Based on the results obtained in the F_1, F_2 and B.C. generations, it was concluded that the activity level of ADH in the scutellum of maize is controlled by the gene $\underline{Adh_r}$ which is located about 17 crossover units from the $\underline{Adh_1}$ gene. The $\underline{Adh_r}^N$ allele specifies equal activity of both the $\underline{Adh_1}^F$ and $\underline{Adh_1}^S$ products. The $\underline{Adh_r}^L$ allele gives lower activity of the $\underline{Adh_1}^S$ products only and is dominant over the $\underline{Adh_r}^N$ allele.

In the present study, the ($\underline{Adh_1}^S/\underline{Adh_1}^S$; $\underline{Adh_r}^L/\underline{Adh_r}^L$) line (7) was crossed with six different homozygous $\underline{Adh_1}^F/\underline{Adh_1}^F$ inbred lines. Line 1 was of known activity (Efron, Science 170:751, 1970) whereas the other lines were of unknown activity. The scutella of the mature F_1 seeds from each cross were tested by means of starch gel electrophoresis and the intensities of the FF, FS and SS isozyme bands were measured with a densitometer. The results measured as the percent activity contributed by the $\underline{Adh_1}^S$ allele are summarized in Table 1.

Table 1

Relative activity contributed by the $\underline{Adh_1}^S$ allele in the scutellum and the pollen of $\underline{Adh_1}^F/\underline{Adh_1}^S$ heterozygotes in crosses between line 7 and six different $\underline{Adh_1}^F/\underline{Adh_1}^F$ lines

Cross	Activity in the parental lines	Percent activity contributed by the $\underline{Adh_1}^S$ allele	
		In scutellum	In pollen
1 x 7	Normal x Low	41.5 ± 2.0	39.6 ± 5.2
10 x 7	Normal x Low	41.1 ± 4.2	38.7 ± 4.1
11 x 7	Normal x Low	39.2 ± 3.3	39.6 ± 4.9
20 x 7	Normal x Low	41.0 ± 4.6	39.4 ± 4.6
45 x 7	Normal x Low	41.9 ± 2.1	38.3 ± 3.5
19 x 7	High x Low	25.7 ± 2.3	63.3 ± 4.3

In ($\underline{Adh_1}^F/\underline{Adh_1}^S$; $\underline{Adh_r}^N/\underline{Adh_r}^N$) scutella (equal activity of the two parental lines) the percent activity contributed by the $\underline{Adh_1}^S$ allele is 49.6 and in ($\underline{Adh_1}^F/\underline{Adh_1}^S$; $\underline{Adh_r}^L/\underline{Adh_r}^N$) scutella the $\underline{Adh_1}^S$ allele contributed only 39.9 percent (Efron, Science 170:751, 1970). Five out of the six crosses, including line 1, showed very similar results indicating that the five parental $\underline{Adh_1}^F/\underline{Adh_1}^F$ lines have equal activity of ADH and that the effect of the $\underline{Adh_r}^L$ allele is similar in different genetic backgrounds. On the other hand, in the cross 19 x 7 the percent activity contributed by the $\underline{Adh_1}^S$ allele was significantly lower.

There are two possible explanations for this result: (1) line 19 has high activity levels of ADH, or, (2) the effect of the $\underline{Adh_r}^L$ allele is stronger in the genetic background of line 19. A test for the activity levels in three parental lines showed clearly that line 19 had higher activity than line 7 (low) and line 1 (normal):

Parental line	:	19	7	1
Activity units	:	234.3 ± 35.6	79.2 ± 7.89	123.9 ± 15.0
Percent activity	:	100.0	33.8	52.9

Based on these results, the expected contribution of the $\underline{Adh_1}^S$ allele in 19 x 7 and 1 x 7 F_1 scutella is 25.3 and 39.0 percent, respectively. The actual values obtained in the F_1 hybrids (Table 1) are very similar to the above expected values.

ADH is active also in the mature pollen of maize. Only FF and SS isozyme bands are found in $\underline{Adh_1}^F/\underline{Adh_1}^S$ heterozygotes. This indicates that the ADH which is present in the mature pollen was formed after meiosis. When the percent activity contributed by the $\underline{Adh_1}^S$ allele was tested in pollen collected from the various F_1 plants (Table 1), the results were similar to those obtained for the scutellum in the first five crosses. However, in the cross 19 x 7 the results were markedly different. In the scutellum the relative contribution of the $\underline{Adh_1}^S$ allele was lower, while in the pollen it was higher than in the other crosses. The possibility of any deviation in the number of $\underline{Adh_1}^F$ and $\underline{Adh_1}^S$ pollen grains produced by this hybrid was ruled out by testing the F_2 generation. Among 220 F_2 seeds tested, the ratio of the $\underline{Adh_1}^F/\underline{Adh_1}^F$: $\underline{Adh_1}^F/\underline{Adh_1}^S$: $\underline{Adh_1}^S/\underline{Adh_1}^S$ was 51:113:56, respectively, which is in good agreement (P = .8 - .9) with the 1:2:1 ratio expected.

Unfortunately, the level of activity in the pollen of the parental lines has not been tested. Therefore, it is too early to hypothesize an explanation for the difference between the two tissues. Also, it is not known at the present whether the high activity of ADH in line 19 is controlled by the $\underline{Adh_r}$ gene.

Yoel Efron

2. <u>Relative activity of AP_1 controlled acid phosphatase isozymes in pollen of hybrids between inbred lines of maize differing in activity level</u>.

Efron (Biochem. Genetics 5:33, 1971) presented evidence that the $\underline{AP_1}$ controlled acid phosphatase activity in maize is variable in different inbred lines. Five distinct groups of activity were found among 23 inbred lines tested.

Two major difficulties arise in the attempt to study the genetic control of the variation in specific enzyme activity: (1) the presence

of other phosphatases and (2) conventional biochemical methods do not distinguish between the activities contributed by each of the two parental genomes. Three alleles, $\underline{AP_1}^F$, $\underline{AP_1}^I$ and $\underline{AP_1}^S$, which specify acid phosphatase isozymes with fast, intermediate and slow migration rates have been described for the $\underline{AP_1}$ locus (Efron, Genetics 65:575, 1970). The above mentioned limitations were overcome in the present study by crossing two lines with known activity levels but having different $\underline{AP_1}$ alleles.

Seven homozygous $\underline{AP_1}^S/\underline{AP_1}^S$ inbred lines with different activity levels of acid phosphatase were crossed in almost all possible combinations with six homozygous $\underline{AP_1}^F/\underline{AP_1}^F$ inbred lines representing five groups of activity. All the F_1 hybrids were grown in the field; samples of the mature fresh pollen were collected from six different plants of each hybrid and stored at -20°C. Three different extracts from each pollen sample were electrophoresed separately by means of starch gel electrophoresis and stained specifically for acid phosphatase. Then, the intensity ratio of the color developed at the position of the $\underline{AP_1}^S$ and $\underline{AP_1}^F$ isozyme bands was compared visually in each gel by seven unbiased persons; the intensity of the $\underline{AP_1}^F$ band was used as a reference (activity value of 1.0). The average intensities of the $\underline{AP_1}^S$ bands in relation to the $\underline{AP_1}^F$ bands for the different F_1 hybrids are summarized below:

$\underline{AP_1}^S$ parental line	Group of activity#	K-4 1	CL-31A 2	B-53 3	B-57 3	Oh-7 4	B-50 5	$\underline{AP_1}^F$ parental line Group of activity#
N-6	1	3.48*	2.13	3.32*	2.23	2.61*	.55*	
B-55	1	3.66*	3.43	3.49	3.80	2.65	1.96	
B-56	2	3.00	2.30	3.00	--	3.30	1.00	
Hy	3	2.85*	--	2.80	2.12*	2.50	--	
E_1^S/E_1^S	3	2.02*	--	1.90*	1.91*	1.79*	.49*	
C-103	4	.51*	--	.59*	.57*	--	--	
M-14	4	2.16*	--	--	1.86*	1.30*	1.12	

#Group 1 = highest activity and Group 5 = lowest activity (Efron, Biochem. Genetics 5:33, 1971).

*Mean values of two reciprocal crosses.

Two general conclusions might be drawn from these results: (1) the intensity ratios of the $\underline{AP_1}^S$ and the $\underline{AP_1}^F$ bands in the pollen collected from the F_1 hybrids are not correlated with the activity levels of the parental lines and (2) equal intensity of the two isozyme bands (a value of about 1.0) was found only in three out of 33 crosses. The best explanation for these results may be based on the "Limited Factor" hypothesis for regulation of gene activity in higher organisms proposed by D. Schwartz (Genetics, in press). Schwartz proposed that the number of enzyme molecules which are synthesized in a certain tissue at a given time is controlled by the presence of a limited factor, its effect being on the gene level. He also showed that there is competition, and differences in competitive ability, between alleles for the limited factor.

Following this hypothesis, it is assumed that the parental inbred lines differ in the activity level of the $\underline{AP_1}$ controlled acid phosphatase since they have different amounts of the limited factor. Also, the $\underline{AP_1}$ alleles from different inbred lines may vary in their competitive ability for the limited factor. Thus, both N-6 and K-4 had high activity levels; however, in the cross N-6 x K-4 the $\underline{AP_1}^S$ allele contributed by N-6 was a much better competitor than the $\underline{AP_1}^F$ allele contributed by K-4. On the other hand, N-6 had a higher activity level than B-50, but in the N-6 x B-50 cross the $\underline{AP_1}^F$ band was more intense than the $\underline{AP_1}^S$ band. From this we concluded that the $\underline{AP_1}^F$ allele contributed by B-50 was a better competitor than the $\underline{AP_1}^S$ allele contributed by N-6.

Both the $\underline{AP_1}^F$ and $\underline{AP_1}^S$ alleles may vary in their competitive ability. Among the $\underline{AP_1}^S$ parents, B-55 and C-103 showed the highest and lowest competitive ability, respectively. Among the $\underline{AP_1}^F$ parental lines, B-50 was the best competitor while K-4 and B-53 had the lowest competitive ability.

Yoel Efron

UNIVERSITY OF ILLINOIS
Urbana, Illinois
Department of Botany

1. Reversions to fertility in S male-sterile cytoplasm in corn.

A number of cases involving changes of the S cytoplasmic sterile condition to the normal or fertile state have been identified. Several strains carrying the S type cytoplasm exhibit surprisingly high frequencies of change from sterile to fertile and to semifertile condition and these represent changes at the cytoplasmic rather than the nuclear level. Singh and Laughnan (Genetics 60:226, 1968) reported the initial studies of this phenomenon as we know it. Exceptional male-fertile offspring appeared among the progeny of an M825S male-sterile plant crossed by inbred line R138TR, carrying no S restorer. While the fertility of the exceptional F_1 individuals was not transmissible through the pollen, it was transmitted efficiently through the egg. The results are consistent with the hypothesis that mutation of the S cytoplasmic element is involved.

Singh and Laughnan investigated the exceptional products of a single event involving somatic change of the S cytoplasmic element. A search has been carried out for additional cases of S element changes in the male-sterile versions of inbred lines R839, R851, R853, R825, M825 and E1. It was soon discovered that the M825 male-sterile line not infrequently produces fertile tassel sectors and occasionally produces plants with entirely fertile tassels. While the M825 line shows the strongest tendency to "go fertile" in this way, similar events have been identified in the other S male-sterile inbred lines.

Upon encountering an exceptional male-fertile plant with an entirely fertile tassel, or with a fertile sector in the tassel, we have introduced and, wherever possible, abided by, a routine crossing procedure:

(1) The exceptional plant is either self pollinated or, as has more often been the case, is crossed as the pistillate parent by a nonrestoring maintainer plant from a defined inbred line source.

(2) The exceptional plant is employed as the pollen parent in crosses with S male-sterile plants from the same defined inbred source as indicated in (1) above.

(3) The exceptional plant is employed as the pollen parent in a cross with a sibling male-sterile plant.

(4) A male-sterile sibling of the exceptional plant is crossed with a nonrestoring maintainer plant from the same defined source as indicated in (1) and (2) above.

Step (2) above is the most crucial one as it determines whether the male fertility of the exceptional plant is assignable to mutation of the S cytoplasmic factor or to mutation at the nuclear restorer locus. So far all such exceptions, of which hundreds have been analyzed, fail to transmit through the pollen and as such are assignable to changes in the S element at the cytoplasmic level.

The crossing procedure outlined above affords the opportunity to make certain other interesting comparisons, and at least one of these appears to be significant. Both steps (3) and (4) involve sibling male-sterile plants as pistillate parents; in the first case, the pollinator is the exceptional sibling individual while, in the second, the pollinator is an inbred line maintainer source whose male-sterile counterpart has no immediate record of mutation to male-fertility. When we registered the frequencies of offspring, from these crosses, with fertile tassel sectors we found that the pollinator sources differed significantly in regard to their contribution. Among the progeny of sibling male-sterile plants crossed with exceptional plants whose tassels were entirely fertile, 12 per cent had fertile sectors in the tassel while only two per cent of the offspring from crosses of sibling male-sterile plants, in the same families, with maintainer pollen had fertile tassel sectors. Similar results were obtained, 14 per cent and two per cent, respectively, in those cases where the exceptional parents carried fertile tassel sectors. We are inclined, at this point, to regard this contribution from the pollen parent as nuclear, or genotypic, preferring to believe that mutations of the S element are governed by both the plasmon and the nucleus. Nevertheless, we have not excluded the possibility that a cytoplasmic contribution, transmitted through the male gametophyte, is involved in the phenomenon.

J. R. Laughnan
Susan J. Gabay

UNIVERSITY OF ILLINOIS
Urbana, Illinois
Departments of Agronomy and Plant Pathology

1. Cytoplasmic susceptibility to Helminthosporium maydis in the U.S.

In 1970, southern corn blight caused by Helminthosporium maydis developed to a major disease of the U.S. corn crop. This was due to the appearance and widespread distribution of a new race of H. maydis which has been named race T.

Race T is unique in that it produces a pathotoxin that is highly specific for the T cms (Texas) cytoplasm widely used in seed production in the U.S. Race T reproduces rapidly and attacks the leaf, leaf sheath, husk, shank, ear, seedling, and sometimes stalk tissue of the plant. It spread from Florida west to Texas and north to Canada during the summer of 1970.

All "normal" cytoplasms (not male-sterile) are resistant to race T. The same is true for the S cms, C cms, and a number of other cytoplasms for male-sterility. The same nuclear genes for resistance to the old race O of H. maydis condition partial resistance to race T when interacting with T cms cytoplasm.

With the exception of symptom expression, the U.S. situation is similar to the experience in the Philippine Islands reported in 1961 and subsequent years.

A. L. Hooker

ILLINOIS STATE UNIVERSITY
Normal, Illinois
Department of Biological Sciences

1. The use of monosomy to detect genes altering recombination in Zea mays.*

The $\frac{r}{x-1}$ deficiency in maize induces an extremely high frequency of monosomes in Zea mays (Satyanarayana, unpublished). With this system, I have obtained at least three confirmed cases of monosomy for 8 of the

*Partially supported by A.E.C. Contract No. AT(11-1)-2121.

10 chromosomes (all possible monosomes except monosomes for chromosomes 3 and 5). A report on this system which produces monosomes will be published in the near future. Since monosome 3 has been produced through the interaction of a knob on chromosome 3 with a B chromosome (Rhoades, Dempsey, and Ghidoni, 1967, Proc. Nat. Acad. Sci. 57: 1626-32), monosomy for 9 of the 10 chromsomes in maize has been confirmed. This is the first time that a series of this type has been obtained in a higher diploid organism.

In a monosomic plant, an entire chromosome is present in the hemizygous condition. Plants monosomic for a specific chromosome can be compared with their diploid sibs. If a gene on this chromosome exhibits dosage effect, a difference will be found between these two types for the trait being analyzed. Thus, one can screen <u>all</u> of the genes on a given chromosome at the same time by comparing monosomic and disomic sibling plants. Given 10 monosomic plants, it is possible to screen the entire genome. This is a new method for screening the genome, and it analyzes all genes on a given chromosome at the same time. One can in this way observe gene loci without inducing mutations!

The above approach is being utilized to study several different classes of genes. Of special interest are genes affecting recombination. Monosomic plants are being generated utilizing the $\underline{r}_{x-1}$ deficiency which are heterozygous for linked genes in chromosome 2 and the amount of recombination in the monosomes is being compared with recombination in diploid sibs.

One especially vigorous monosomic 8 plant was used both as a male and female parent in crosses this past summer. The amount of recombination in this plant and in diploid sibling plants is presented in the following table:

Comparison of Recombination in Monosomic 8 and Diploid Sibs

Female Parent	Male Parent	Population	% Recombination
$\underline{ws}_3\ \underline{gl}_2$	Monosome 8		
	$\underline{Ws}_3\ \underline{Gl}_2/\underline{ws}_3\ \underline{gl}_2$	1696	28.07
ws gl	Diploid Sibs		
	Ws Gl/ws gl		
	Plant 1	500	23.3*
	Plant 2	580	23.8*
	Plant 3	149	19.5*
	Plant 4	393	21.9*
	Total Diploid Sibs	1622	22.75**
Monosome 8, Ws Gl/ws gl	ws gl	94	29.8
Diploid Sib Ws Gl/ws gl	ws gl	295	28.1

X^2 Contingency tests of the above using Yates correction factor.

*Significantly different from monosome 8 used as a male at the 5% level.

**Significantly different from monosome 8 used as a male at the 0.5% level.

It can clearly be seen that recombination in the monosomic 8 plant is distinctly higher than in diploid sibling control plants when they were used as males. No difference was detected when the plants were used as maternal parents, but only a limited amount of data is available from the monosomic plant as a maternal parent. The difference in recombination values between the monosomic 8 plant and its diploid siblings might be attributed to two causes:

A. A gene or series of genes are present on chromosome 8 which in one dose allows more recombination than in two doses.

B. The difference could be due to an interchromosomal effect, i.e., more recombination occurs in chromosome 2 in the absence of recombination in chromosome 8. To distinguish between these two

alternatives, similar tests will be conducted with additional monosomes 8 and other monosomes this coming summer.

A relaxation of recombination inhibition might be of tremendous economic importance since an increase in recombination would permit breaking up of linkage groups and allow more variability in the plants.

David F. Weber

2. The use of monosomy to detect genes for lipid biosynthesis in Zea mays.*

Monosomes generated by the $\underline{r}_{x-1}$ deficiency are being used in a study to detect major genes or gene complexes for lipid biosynthesis in Zea mays embryos. This paper describes the procedure of study. In monosomes generated by the $\underline{r}_{x-1}$ deficiency, there is a non-correspondence between the chromosome numbers of the embryo and endosperm, thus monosome generation must take place in the post-meiotic megaspore divisions of the embryo sac. In other words, a monosomic embryo is not accompanied by monosomic endosperm.

The line carrying the $\underline{r}_{x-1}$ deficiency in this study has been maintained in inbred W22 for many generations. $\underline{R}/\underline{r}_{x-1}$ heterozygotes in inbred W22 were crossed by a second inbred, Mangelsdorf's multiple chromosome tester ($\underline{bm}_2$; $\underline{lg}_1$; $\underline{a}_1$; $\underline{su}_1$; $\underline{pr}$; $\underline{y}_1$; $\underline{gl}_1$; $\underline{j}_1$; $\underline{wx}$; $\underline{g}_1$). The F_1 kernels carrying the $\underline{r}_{x-1}$ deficiency were dried until the water content of the kernel was below 4.5 per cent. The lipid content of each kernel was determined with wide line nuclear magnetic resonance spectroscopy. The NMR analysis was conducted as described by Alexander et al. (1967, J. Am. Oil Chem. Soc. 44:555-558). Although the chromosome number of the endosperm and embryo do not correspond, this would not alter the results because virtually all of the free lipid is concentrated in the embryo of the kernel (Brunson, Earle, and Curtis, 1948, Agron. J. 40:180-185).

Over 2,300 kernels have been scanned individually with the NMR to date. Since it is impossible to detect monosomic kernels, all of the kernels had to be scanned by the NMR and then planted. The monosomic

*Partially supported by A.E.C. Contract No. AT(11-1)-2121.

individuals (about 11 per cent of the $\underline{r}_{x-1}$ heterozygotes) were detected at the seedling stage by appropriate genetic markers. Lipid content of at least 11 plants monosomic for each of 5 different chromosomes (monosomes 2, 6, 7, 8, and 10) were compared with their diploid sibs. If a gene or gene complex exists on a specific chromosome that is involved in lipid biosynthesis, and if this gene exhibits dosage effects, then one would find a lowering of lipid content in a specific monosome.

Kernels monosomic for chromosomes 2 or 6 have a lower free lipid content than their diploid sibs, whereas those monosomic for chromosomes 7 or 8 appear similar to their diploid sibs. Thus, it appears that major lipid biosynthetic genes or gene complexes which have a dosage effect are located on chromosomes 2 and 6.

Alternatively, Manglesdorf's tester might carry recessive genes on chromosomes 2 and 6 for decreased lipid biosynthesis which were uncovered in the respective monosomes. This alternative would seem unlikely since the authors are not aware of any single recessive gene which has a substantial effect on lipid content in the corn kernel. Tests are in progress to determine if this possibility has credence.

The decrease in lipid content might be due to two factors, the specific monosomes may have a lower concentration of lipids than their diploid sibs or monosomy might induce a smaller embryo size. To distinguish between these two alternatives, it was necessary to measure embryo volumes in the monosomes and diploid sibs. Since the reduction in lipid content in monosome 2 is not accompanied by a corresponding decrease in embryo size, it appears that the difference is due to lipid concentration. Similar measurements are not available for monosome 6.

We would like to express our appreciation to Dr. D. E. Alexander for the use of the N.M.R. at the Agronomy Department, University of Illinois.

Michael E. Plewa
David F. Weber

INDIAN AGRICULTURAL RESEARCH INSTITUTE
New Delhi, India
Division of Genetics

1. Differentiation of evolved varieties and primitive races of maize of Himalayan and Latin American distribution.

It has been known for a long time that Assam and various other Eastern Himalayan states including Sikkim have a wide range of maize varieties many of which possess primitive characteristics (Stonor and Anderson, 1949). Two of these primitive varieties SP 1 (MNL 38: 69) and SP 2, two primitive Mexican races, Chapalote and Nal-Tel (Yucatan 7), one primitive Colombian race, Pollo Segregaciones and two highly evolved commercial types, Kanpur type 41 from India and Mexican June, have been compared for a number of their economic characters. Observations recorded in the course of a yield trial conducted at Delhi during 1969 in the form of a Randomized Block Design are presented in Table 1.

It will be seen that the primitive types are, in general, later in maturity than the evolved types. The Himalayan primitives are extremely late in maturity, silking about a month later than the evolved types. They are also much shorter in height, lower in yield, having smaller ears in their length and diameter, with fewer kernel rows and lower kernel weight compared to the evolved types. The American primitives, on the other hand, do not differ so markedly from the evolved commercial types.

Observations were also recorded on the pollen size (Table 2) of various primitive and evolved varieties. A total of 75 pollen grains from several plants were measured for each variety. It is observed that the Himalayan primitive varieties, especially SP 1, have a very low pollen diameter compared to the other maize varieties. An increase in pollen size during evolution has been clearly demonstrated by Galinat (1961).

The characters described above as well as the emergence of ears from the upper joints of the stalks, reduced internode length, and the occurrence of male and female flowers in the same inflorescence in the case of the Himalayan primitive varieties show that these varieties are closer to the progenitor corn plant reconstructed by Mangelsdorf (1958)

Table 1

Observed means for various primitive and evolved varieties for various quantitative characters

Variety	Days to 75% silking ± S.E.	Plant height (cms) ± S.E.	Grain yield per plant (gms) ± S.E.	Ear length (cms) ± S.E.
Himalayan primitives				
SP 1	85.50 ± 1.34	105.86 ± 5.44	8.25 ± 5.21	7.30 ± 0.53
SP 2	93.50 ± 1.34	93.10 ± 5.44	7.50 ± 5.21	5.67 ± 0.53
American primitives				
Pollo Segregaciones	81.75 ± 1.34	211.35 ± 5.44	21.36 ± 5.21	10.95 ± 0.53
Chapalote	78.50 ± 1.34	181.35 ± 5.44	27.90 ± 5.21	12.17 ± 0.53
Nal-Tel (Yucatan 7)	64.75 ± 1.34	169.00 ± 5.44	27.92 ± 5.21	10.21 ± 0.53
Evolved varieties				
KT 41	52.50 ± 1.34	148.55 ± 5.44	40.55 ± 5.21	13.50 ± 0.53
Mexican June	68.00 ± 1.34	180.90 ± 5.44	50.66 ± 5.21	11.63 ± 0.53

Variety	Ear diameter (cms) ± S.E.	Kernel rows ± S.E.	1000 grain weight (gms) ± S.E.	Seed density ± S.E.
Himalayan primitives				
SP 1	1.64 ± 0.09	8.86 ± 0.32	90.38 ± 9.77	1.22 ± 0.07
SP 2	1.77 ± 0.09	8.63 ± 0.32	72.84 ± 9.77	1.18 ± 0.07
American primitives				
Pollo Segregaciones	2.39 ± 0.09	10.92 ± 0.32	157.69 ± 9.77	1.17 ± 0.07
Chapalote	2.57 ± 0.09	11.02 ± 0.32	133.03 ± 9.77	1.16 ± 0.07
Nal-Tel (Yucatan 7)	2.71 ± 0.09	10.77 ± 0.32	134.77 ± 9.77	1.16 ± 0.07
Evolved varieties				
KT 41	3.00 ± 0.09	10.92 ± 0.32	155.88 ± 9.77	1.20 ± 0.07
Mexican June	3.73 ± 0.09	13.50 ± 0.32	174.25 ± 9.77	1.16 ± 0.07

Table 2

Mean pollen size of various primitive and evolved varieties

Variety	Mean pollen diameter (μ) ± S.E.
SP 1	66.09 ± 1.23
SP 2	78.88 ± 1.23
Palomero Toluqueno	85.31 ± 1.23
KT 41	85.05 ± 1.23
Mexican June	83.48 ± 1.23

than such American races as Chapalote, Nal-Tel and Palomero Toluqueno, which are believed to be direct descendants of the wild corn.

References

Galinat, W. C. (1961) Corn's evolution and its significance for breeding. Econ. Bot. 15:320-325.

Mangelsdorf, P. C. (1958) Reconstructing the ancestor of corn. Science 128:1313-1320.

Stonor, C. R. and E. Anderson. (1949) Maize among hill people of Assam. Ann. Missouri Botan. Gard. 36:355-404.

D. Gupta
H. K. Jain

2. Cytoplasmic differentiation between evolved and primitive types of maize.

A number of primitive varieties of maize of Himalayan and Latin American distribution were crossed reciprocally with two commercial varieties of maize, KT 41 (an Indian variety) and Mexican June. Observations on hybrid plants from these crosses have shown significant differences between the reciprocal crosses for a number of characters including maturity index, plant height and yield components such as ear length, number of kernel rows and 1000 grain weight (Table 1).

Table 1

Crosses and the respective characters showing significant reciprocal differences

Cross	Character	Cytoplasm favoring better expression
KT 41 X SP 1	Ear length	Primitive
KT 41 X SP 2	Plant height	Evolved
	Ear length	Evolved
KT 41 X Pollo Segregaciones	Kernel rows	Evolved
KT 41 X Chapalote	Ear length	Primitive
	1000 grain weight	Evolved
KT 41 X Nal-Tel (Yucatan 7)	--	--
Mexican June X SP 1	--	--
Mexican June X SP 2	Ear length	Primitive
Mexican June X Pollo Segregaciones	Days to silk	Evolved
Mexican June X Chapalote	Ear length	Primitive
	Kernel rows	Evolved
Mexican June X Nal-Tel (Y.7)	--	--

It will be seen that except for the character of ear length the evolved cytoplasm favors better expression of various characters. Bhat and Dhawan (1969) have also found evidence of cytoplasmic effects in such crosses of maize.

Thus, the analysis clearly demonstrates that some differentiation has taken place at a cytoplasmic level between the primitive and the evolved varieties of maize. There are not many examples available in the literature where cytoplasm appears to have played an important role in the differentiation of populations of a species in the course of its evolution. The evidence from maize is, thus, of particularly great interest, and it may stimulate studies of a similar nature in other important crop plants.

Reference

Bhat, B. K. and N. L. Dhawan (1969) Effect of cytoplasm on quantitative characters of maize. Indian J. Genet. 29:321-326.

D. Gupta
H. K. Jain

3. Dominant genes in the evolution of maize.

Observations on a series of crosses involving primitive and evolved varieties of maize including SP 1 and SP 2 (Himalayan primitives), Chapalote, Nal-Tel (Yucatan 7), Pollo Segregaciones (Latin American primitives), KT 41 and Mexican June (evolved types) have shown that the genes determining early maturity, increased plant height, grain yield, ear length and the 1000 grain weight show a partial or complete expression of dominance in the first generation hybrids. The dominant genes were found to be contributed by the evolved varieties. It is generally believed that the primitive populations of a species show a greater concentration of wild type genes, and in the course of their evolution, they produce a large number of mutant alleles. Thus, the recessive genes are expected to show a greater preponderance in the evolved types. In the case of maize, many mutant genes having dominance effects have also been produced in the course of evolution. These mutant genes were obviously of great value and have been unconsciously or consciously selected by the early farmers and the present day plant breeders.

D. Gupta
H. K. Jain

4. Primitive and evolved varieties of maize and generation of heterosis.

A number of crosses between the primitive varieties of Himalayan and American distribution on the one hand, and highly evolved maize on the other, have provided evidence of a heterotic response. In the crosses between the primitive and evolved types, significant negative heterosis was more commonly generated when the Himalayan primitive varieties were involved as one of the parents. A significant positive heterosis for various characters of economic value was observed only in a few of the crosses, as shown in Table 1.

Table 1

Crosses showing significant positive heterosis for various characters

Cross	Character	Percent increase over better parent
KT 41 X SP 1	--	--
KT 41 X SP 2	--	--
KT 41 X Pollo Segregaciones	Kernel rows	14.0
KT 41 X Chapalote	--	--
KT 41 X Nal-Tel (Yucatan 7)	--	--
Mexican June X SP 1	Ear length	14.0
Mexican June X SP 2	Days to silk	5.9*
Mexican June X Pollo Segregaciones	--	--
Mexican June X Chapalote	Days to silk Plant height Ear length	5.9* 10.6 19.0
Mexican June X Nal-Tel (Yucatan 7)	--	--

*This indicates earliness of the hybrid.

D. Gupta
H. K. Jain

5. Expression of the opaque-2 and floury-2 genes in the genetic background of the primitive and evolved maize varieties.

In order to assess the usefulness of the primitive varieties from the point of view of nutritional improvement of maize, an attempt has been made to study the expressivity of opaque-2 and floury-2 mutant genes in the genetic background of some of the primitive and evolved varieties. Crosses were made to incorporate the two mutant genes o_2 and fl_2 separately in SP 1 and SP 2 (Himalayan primitive varieties), Chapalote and Nal-Tel (Yucatan 7) (Mexican primitive varieties) and the evolved variety KT 41. After the first backcross with the wild type parent, the progeny was selfed and the mutant and non-mutant seeds sorted out from each ear. Protein, tryptophan and methionine was estimated in these kernels.

An estimate of nitrogen was made using the microkjeldahl method and multiplied by a factor 6.25 to obtain the protein content in corn (A.O.A.C., 1965). Tryptophan was analysed as per the method described by Hernandez and Bates (1969) after papain hydrolysis of the defatted corn flour. Methionine estimation was carried out colorimetrically as described by McCarthy and Pailler (1959) after extraction with 2N HCl adopting the procedure given by Gupta and Das (1954). The observations are presented in Tables 1 and 2.

The observations in Table 1 show that the opaque-2 segregates from the different crosses differ in their protein content. In general, the segregates coming from crosses involving the primitive varieties as the non-opaque parent have a higher protein content compared to those in which the non-opaque parent is an improved variety. As regards the tryptophan content, the variation between segregates from different crosses, is not very marked. It is, however, observed that most of the segregates from crosses involving the primitive varieties have as much or more content of tryptophan than the segregates from crosses involving a non-opaque evolved variety. It can be concluded that the opaque-2 gene, when placed in the genetic background of the primitive varieties, expresses itself as well as or even better than when placed in the background of evolved types. It should, however, be emphasized that the mutant gene was not placed in a completely primitive or evolved background.

The observations presented in Table 2 show that the protein content of floury-2 segregates is slightly higher compared to the protein content of non-floury segregates obtained from the same ear. It is further observed that the methionine content in the floury-2 segregates from crosses involving the primitive varieties is, in general, higher than in similar segregates from crosses involving the evolved types. Thus, the floury-2 gene seems to express itself better, as far as methionine content is concerned, in the genetic background of primitive varieties. However, as far as the tryptophan content is concerned, the floury-2 segregates from different types of crosses do not differ much from each other.

Table 1

Protein and tryptophan content in opaque-2 segregates of primitive and evolved varieties, together with their content in non-opaque segregates from the same ear

Cross	Segregates	Protein (%)	Tryptophan g/100 g protein
[(SP 1 X o_2o_2) X SP 1] ⊗	o_2	14.52	0.78
	+	14.98	0.50
[(o_2o_2 X SP 1) X SP 1] ⊗	o_2	13.96	0.89
	+	13.58	0.45
[(SP 2 X o_2o_2) X SP 2] ⊗	o_2	16.71	0.88
	+	16.45	0.55
[(o_2o_2 X SP 2) X SP 2] ⊗	o_2	14.48	0.89
	+	14.22	0.42
[(Chapalote X o_2o_2) X Chapalote] ⊗	o_2	13.47	0.82
	+	13.82	0.57
[(o_2o_2 X Chapalote) X Chapalote] ⊗	o_2	13.17	0.87
	+	13.27	0.56
[(Nal-Tel Y.7 X o_2o_2) X Nal-Tel Y.7] ⊗	o_2	11.68	0.95
	+	11.82	0.44
[(o_2o_2 X Nal-Tel Y.7) X Nal-Tel Y.7] ⊗	o_2	13.31	0.66
	+	13.94	0.36
[(KT 41 X o_2o_2) X KT 41] ⊗	o_2	13.41	0.83
	+	13.46	0.41
[(o_2o_2 X KT 41) X KT 41] ⊗	o_2	12.66	0.82
	+	13.30	0.31

Table 2

Protein, methionine and tryptophan content in floury-2 segregates of the primitive and evolved varieties, together with their content in non-floury segregates from the same ear

Cross	Segregates	Protein (%)	Methionine g/100 g protein	Tryptophan g/100 g protein
[($\underline{fl_2}\underline{fl_2}$ X SP 1) X SP 1] ⊗	$\underline{fl_2}$	15.31	2.69	0.58
	+	13.49	2.00	0.52
[($\underline{fl_2}\underline{fl_2}$ X SP 2) X SP 2] ⊗	$\underline{fl_2}$	14.24	2.79	0.73
	+	13.05	2.19	0.59
[($\underline{fl_2}\underline{fl_2}$ X Chapalote) X Chapalote] ⊗				
	$\underline{fl_2}$	16.68	2.85	0.83
	+	14.23	2.35	0.62
[($\underline{fl_2}\underline{fl_2}$ X Nal-Tel Y.7) X Nal-Tel Y.7] ⊗				
	$\underline{fl_2}$	13.84	2.91	0.75
	+	13.01	2.08	0.65
[($\underline{fl_2}\underline{fl_2}$ X KT 41) X KT 41] ⊗	$\underline{fl_2}$	14.45	2.53	0.76
	+	13.62	1.89	0.40

References

A.O.A.C. (1965) Official methods of analysis. 10th Edition. Association of the Official Agricultural Chemists, Washington, U.S.A.

Gupta, Y. P. and N. B. Das (1954) Estimation of methionine in wheat. Sci. Cult. 20:90-91.

Hernandez, H. H. and L. S. Bates (1969) A modified method for rapid tryptophan analysis of maize. Res. Bull. 13. International Center for the Improvement of Wheat and Maize, Chapingo, Edo de Mexico.

McCarthy, T. E. and M. M. Paille (1959) A rapid determination of methionine in crude protein. Biochem. Biophys. Res. Commun. 1:29-31.

D. Gupta
H. K. Jain

6. Amino acid profile of an Himalayan primitive corn variety SP 2.

An attempt has been made to find whether the amino acid profile of a typical primitive corn variety like SP 2 (a variety from Himalayas) differs from that of the evolved maize. SP 2 corn flour was defatted and 5 mg of kernel protein hydrolyzed at 110°C for 24 hours by refluxing with 20 ml of 6 N HCl. One milligram portions of hydrolyzed protein were placed on the short and long columns of an automatic amino acid analyzer. Norleucine was used as an internal standard. This profile for the variety SP 2 is presented in Table 1. For the sake of comparison, similar observations on whole corn flour of two of the commercial maize hybrids have been included in this Table. These data have been presented by Prakash and his collaborators (Prakash et al., 1970) and Mertz and his associates (Mertz et al., 1965). The results (Table 1) do not show any marked differences between SP 2 and the two evolved types Ganga-3 and Indiana 453, for most of the amino acids. The only observation of some interest is that SP 2 has a higher content of aspartic acid and a lower content of proline compared to the two evolved commercial hybrids. It may be added that the protein content in SP 2 is considerably higher than in the two improved hybrids.

References

Mertz, E. T., O. A. Veron, L. S. Bates and O. E. Nelson (1965) Growth of rats fed on opaque-2 maize. Science 148:1741-1742.

Prakash, V., J. Singh and M. S. Naik (1970) Improvement of amino acid balance of protein of maize and sorghum. Indian J. Genet. 30: In press.

Table 1

Amino acid composition of the Himalayan primitive corn variety SP 2 analyzed from defatted whole corn flour (expressed as gm/100gm protein)

Amino acid	SP 2 (Mean of two different samples)	Ganga-3* (After Prakash et al., 1970)	Indiana hybrid 453 (After Mertz et al., 1965)
Aspartic acid	8.52	6.85	6.7
Threonine	3.13	3.16	3.6
Serine	5.25	5.21	4.8
Glutamic acid	22.01	23.29	20.8
Proline	7.21	10.00	10.0
Glycine	2.67	2.67	3.8
Alanine	8.64	9.30	7.9
Valine	5.45	5.36	5.0
Cysteine	1.55	1.84	-
Methionine	1.49	1.49	2.0
Isoleucine	3.72	3.90	4.0
Leucine	14.29	17.24	13.9
Tyrosine	5.04	4.91	4.0
Phenylalanine	5.47	5.40	5.2
Ammonia	2.59	2.30	3.4
Lysine	2.45	1.64	2.8
Histidine	2.49	2.54	3.0
Arginine	3.99	3.26	4.8
Cystine	-	-	1.2
Protein (%)	14.50	10.27	10.5

*Data reported with permission from the authors.

D. Gupta
H. K. Jain

7. Karyotypic studies on Himalayan primitive varieties of maize.

SP 1 and SP 2, two varieties of maize collected from Sikkim, are known to show some of the most primitive characters associated with this plant (MNL 38:69). The two varieties were studied for their karyotype and the observations are presented on knob forming sites (Fig. 1). The

first observation of interest is that the pachytene chromosomes in SP 1 and SP 2 are somewhat smaller (Table 1) in size than chromosomes of many of the American varieties of maize as reported by Longley (1939). Evidence will be presented in another report which shows that the total DNA content of the Himalayan primitive varieties is more than that in the chromosomes of the evolved varieties of American and Indian distribution. It would, thus, appear that the reduced size of chromosomes in SP 1 and SP 2 is due to a greater degree of condensation, which, however, is not uniform in all the chromosomes.

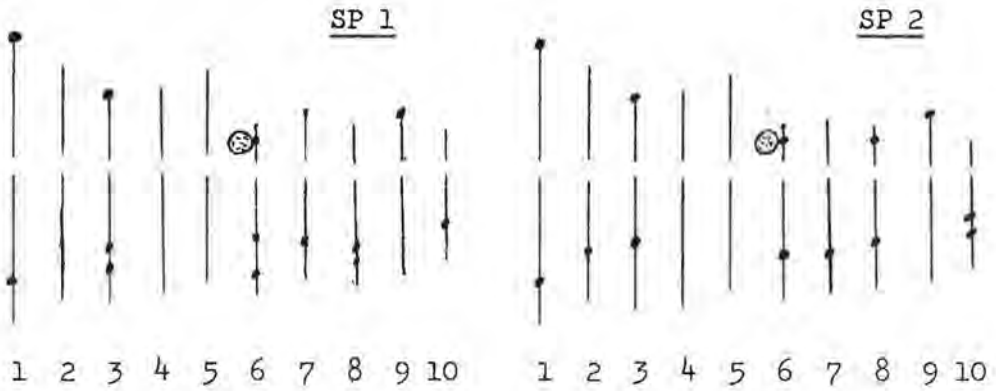


Fig. 1 - Knob sites of Sikkim primitive strains SP 1 and SP 2.

This conclusion is also supported by the observation that the arm ratio of the different chromosomes in the two primitive varieties and the evolved types is almost the same.

A second observation of interest is that while the number of knobs in the chromosomes of the primitive varieties is not very different from that reported in many of the evolved types, the Himalayan primitive varieties show a characteristic position of the knobs in the chromosomes. Thus, four of the knobs 7L, 8S, 8L and 10La, all observed in SP 2, and 10La observed in SP 1, also, appear to indicate new knob forming sites not earlier reported from the extensive observations of a large number of authors on American varieties of maize.

The knobs in the Himalayan primitive varieties SP 1 and SP 2 were found to be larger in size compared to those reported for most of the evolved varieties.

Table 1

Individual chromosome length and arm ratios at mid-pachytene in the Himalayan primitive varieties

Chromosome No.	SP 1		SP 2		Mean data after Longley (1939)	
	Total length (μ)	Arm ratio	Total length (μ)	Arm ratio	Total length (μ)	Arm ratio
1.	61.50	1.19	54.00	1.32	82.40	1.30
2.	48.75	1.26	38.00	1.23	66.50	1.25
3.	48.00	2.08	42.50	2.00	62.00	2.02
4.	53.50	1.60	52.25	1.69	58.78	1.60
5.	43.75	1.17	33.50	1.08	59.82	1.10
6.	36.50	3.18	22.50	2.85	48.73	3.10
7.	33.50	2.53	31.50	2.64	46.78	2.80
8.	44.25	3.07	31.50	3.07	47.48	3.20
9.	33.00	1.89	26.00	2.07	43.24	1.84
10.	24.50	2.73	18.50	2.75	36.93	2.80
Total	427.25		350.25		552.66	

The above observations taken as a whole would suggest that the Himalayan primitive varieties are quite distinct from the present day evolved types in their chromosome structure.

Reference

Longley, A. E. (1939) Knob positions on corn chromosomes. Jour. Agr. Res. 59:475-490.

H. K. Jain
D. Gupta

8. Observations on chiasma frequency in primitive and evolved varieties of maize and their hybrids.

Observations have been recorded on chiasma formation in 155 plants of maize belonging to 6 primitive varieties, 3 evolved types and 18 hybrids obtained by crossing the primitive and evolved types. The number of chiasmata were counted at metaphase I in a minimum of 10 pollen mother cells (Table 1).

It will be seen from Table 1 that the primitive varieties, as a group, show a significantly higher chiasma frequency than the evolved varieties. This observation can have several explanations. It is clear that these varieties have been grown for a very long time under primitive conditions of management. In other words, these varieties have been subjected to conditions of natural selection to a greater extent than the present day commercial varieties like KT 41, which have been relatively free from pressures of natural selection. In view of these differential selection pressures in the two groups of materials, it is obvious that a greater potential for genetic recombination would be of greater value in the case of primitive varieties. This would be particularly so, if the primitive varieties showed some amount of self-pollination, as has been suggested on the basis of occurrences of inflorescences having both male and female flowers such as those found in SP 1. The observations on chiasma frequency, thus, support the suggestion that the primitive varieties, especially varieties like SP 2, are quite distinct from the present day varieties of commerce. The two would appear to have been separated from each other over a much longer period than the concept of a wholly post-Columbian introduction of maize in Asia would suggest.

Secondly, the present studies have also provided evidence of a heterotic response for chiasma formation in the crosses between the primitive and the evolved types. The heterosis is significant in one of the crosses, that is, Palomero Toluqueno X Kanpur type 41, where the hybrid forms a significantly higher number of chiasmata than either of the parents as will be seen below.

Table 1

Mean chiasmata per cell per plant in the primitive and evolved varieties and their hybrids

S.No.	Variety or cross	Plants analyzed	Mean chiasmata ± S.E.
A.	Primitive varieties		
1.	SP 1	9	30.54 ± 0.86
2.	SP 2	8	33.79 ± 0.91
3.	Chapalote	4	29.96 ± 1.30
4.	Chapalote Sinaloa	4	31.36 ± 1.29
5.	Nal-Tel (Yucatan 7)	6	31.63 ± 1.05
6.	Palomero Toluqueno	8	30.91 ± 0.91
	Mean over primitives	-	31.36 ± 0.41
B.	Evolved varieties		
7.	Kanpur type 41	7	29.83 ± 0.98
8.	Basi	7	28.40 ± 0.98
9.	Mexican June	7	30.43 ± 0.98
	Mean over evolved varieties	-	29.55 ± 0.56
C.	Hybrids		
10.	SP 1 X KT 41	5	31.88 ± 1.15
11.	SP 1 X Basi	6	33.05 ± 1.05
12.	SP 1 X Mexican June	6	31.59 ± 1.05
13.	SP 2 X KT 41	8	33.50 ± 0.91
14.	SP 2 X Basi	2	33.65 ± 1.82
15.	SP 2 X Mexican June	6	33.75 ± 1.05
16.	Chapalote X KT 41	5	33.28 ± 1.15
17.	Chapalote X Basi	4	30.85 ± 1.29
18.	Chapalote X Mexican June	5	32.12 ± 1.15
19.	Chapalote Sinaloa X KT 41	6	31.47 ± 1.05
20.	Chapalote Sinaloa X Basi	5	31.98 ± 1.15
21.	Chapalote Sinaloa X Mexican June	6	31.84 ± 1.05
22.	Nal-Tel (Yucatan 7) X KT 41	6	33.51 ± 1.05
23.	Nal-Tel (Yucatan 7) X Basi	5	33.62 ± 1.15
24.	Nal-Tel (Yucatan 7) X Mexican June	6	32.40 ± 1.05
25.	Palomero Toluqueno X KT 41	5	35.66 ± 1.15
26.	Palomero Toluqueno X Basi	6	31.23 ± 1.05
27.	Palomero Toluqueno X Mexican June	3	31.23 ± 1.49
	Mean over hybrids	-	32.59 ± 0.26
Total plants analyzed		155	
Overall mean			31.98

	Hybrid (Pal. Tol. X KT 41)	Palomero Toluqueno	KT 41
Mean chiasmata per cell	35.66	30.91	29.83

It is known that chiasma frequency is genetically determined. Rees has also found evidence for the occurrence of heterosis for this character in rye when completely different inbred lines were crossed (Rees, 1955). Thus, we find that the primitive and the evolved races of maize show genetic differentiation in respect of chiasma formation.

Reference

Rees, H. (1955) Heterosis in chromosome behaviour. Proc. Roy. Soc. London, B, 144:150-159.

H. K. Jain
D. Gupta

9. Effect of knobs on chiasma number in a cell.

We have observed that the varieties and hybrids of maize having greater knob volume (measured in arbitrary scores) form more chiasmata at metaphase I. A highly significant correlation has been obtained ($r = 0.628^{**}$) with the regression equation, $Y = 27.66 + 0.257\ X$, where Y = chiasma number in a cell, and X = units of knob volume in the cell. When a consideration is made of the knob number and chiasma frequency together, a highly significant negative correlation is obtained ($r = -0.704^{**}$) with the regression equation, $Y = 37.83 - 0.609\ X$, where Y = chiasma number in a cell, and X = number of knobs in the cell. These results demonstrate that a higher number of knobs is associated with fewer chiasmata per cell, whereas the greater knob volume increases the chiasma frequency in a cell.

Thus, the knobs, which are supposed to be made up of heterochromatin, appear to have a regulatory function with regard to crossing over. It may be explained that the heterochromatic B-chromosomes have also been found to have a similar regulatory function with regard to crossing over (MNL 42:63, 79; 43:54).

D. Gupta
H. K. Jain

10. Duration of mitotic cycle in evolved and primitive varieties of maize.

Techniques for the determination of the duration of the nuclear cycle and some of its component phases, such as the inter- and the mitotic phases, have become available in recent years with the development of the tritium labelling technique of chromosomes. Not only does this technique give an estimate of the length of interphase, it also makes it possible to determine the length of its sub-phases such as G_1 (the pre-DNA synthesis period of interphase), S (the stage during which the chromosomes replicate their DNA) and G_2 (the post-DNA synthetic stage of interphase). In recent years it has been found from observations of a wide variety of materials that the length of the S-phase is directly correlated with the DNA content of the cell. Thus, the S-phase is longer in those materials which have relatively more DNA (Van't Hof, 1965). In this way the length of the S phase provides a measurement of the DNA content of a cell, and if the different varieties or species show varying lengths of the S phase in their cells, it can be concluded that they show a corresponding variation in their DNA content. Two of the primitive varieties SP 2 (Himalayan) and Palomero Toluqueno (Mexican) and two of the improved varieties, KT 41 (Indian) and Mexican June, were analyzed for the length of their nuclear cycle.

Seeds of the four varieties were germinated and incubated at 26 $\pm$ 1°C on filter paper sheets kept moist with distilled water in petri dishes. The germinated kernels with roots at least 1 cm in length were placed at the same temperature for 0.5 hours in a solution of ^{3}H-thymidine (1 µc/ml; specific activity 15.0 c/mM). After the treatment the roots were transferred to a solution of cold thymidine to stop further incorporation of the label, washed thoroughly with distilled water and incubated further on moist filter paper at the same temperature. Roots were fixed subsequently at 2 hour intervals in 1:3 aceto-alcohol solution for 15 minutes and stored in 70% ethanol in a deep freeze until use.

Root-tips were hydrolyzed with 1 N HCl at 60°C for 8 minutes, stained in Feulgen for 2 hours and treated with a 2% pectinase solution for 2 hours. The meristematic portion of the secondary root tips was squashed on gelatinized slides in 0.5% aceto-orcein. Cover glasses were subsequently removed over dry ice and the slides transferred to 95% ethanol kept in a freezer. Autoradiographs were prepared from these slides using stripping film technique on Kodak A.R. 10 emulsions giving an exposure of 25 days in a refrigerator at 4 to 6°C temperature. Nuclei having grains sufficiently distinct from the background grains were taken as labelled. At least 3 roots were examined for each fixation time. The percentage of labelled mitotic cells was recorded at different durations after pulse-labelling of the root-tips and is shown graphically in Figure 1 for each of the four varieties studied. A total of 7237 mitotic cells were scored from the four varieties. The observations presented in Figure 1, together with the observations on mitotic index (Table 1), help to estimate the various parameters of the nuclear cycle (Matagne, 1968). The estimates of the G_1, S, G_2 and the mitotic phase of the different varieties are summarized in Table 2.

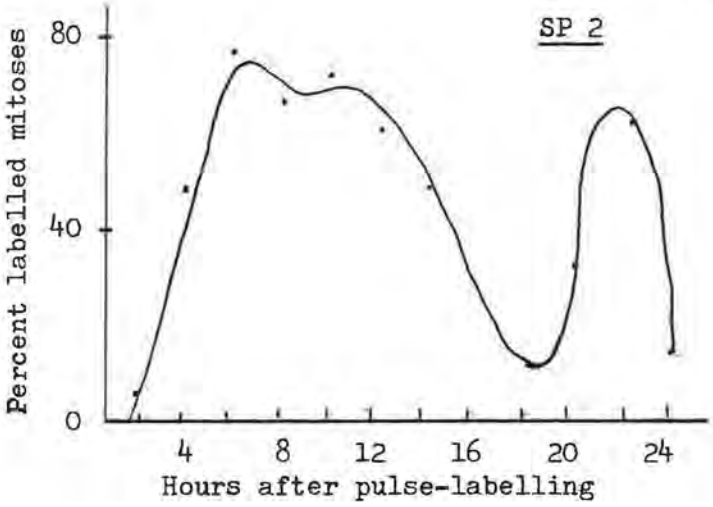
Table 1

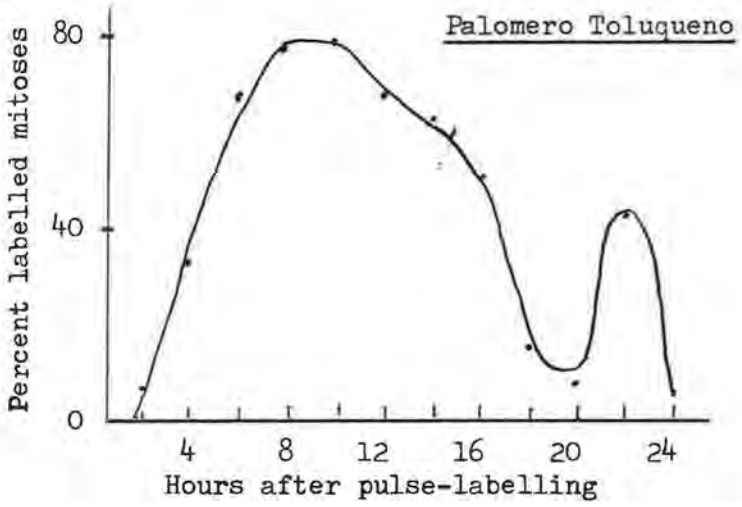
Observations on mitotic index of different varieties obtained from early hours of fixations

Variety	Total cells scored	Cells in mitosis	Mitotic index
SP 2	2112	255	12.07
Palomero Toluqueno	2369	324	13.68
KT 41	2138	297	13.89
Mexican June	2035	250	12.29

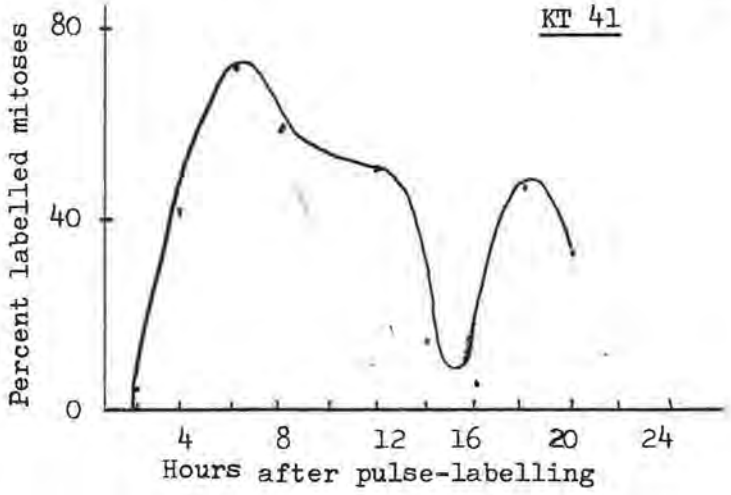
It will be seen that both the primitive varieties show a longer nuclear cycle compared to the improved varieties. It is further observed that the S phase, which represents the period during which DNA is synthesized in the chromosomes, is longer in the two primitive varieties

Primitive varieties





Evolved varieties



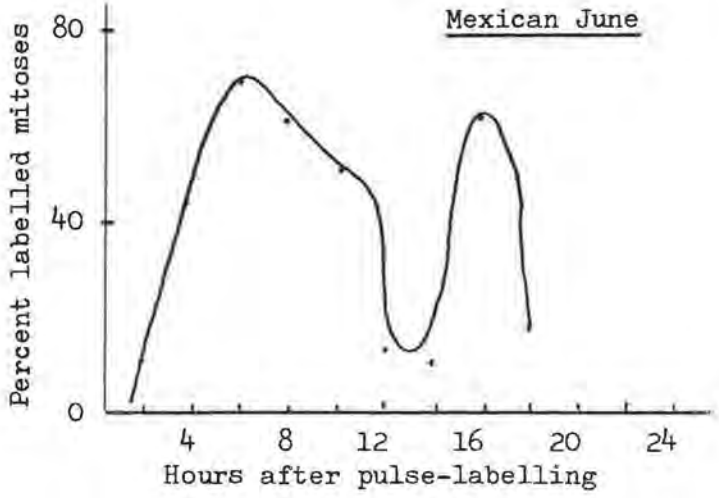


Fig. 1. Graphs showing curves of percent labelled mitosis plotted against duration after pulse labelling in primitive and evolved maize types.

Table 2

Duration of cell cycle in the root-tips of primitive and evolved varieties at 26 ± 1°C.

Phase	Calculated duration (Hours)			
	Primitive varieties		Evolved varieties	
	SP 2	Palomero Toluqueno	KT 41	Mexican June
Interphase				
G_1	0.65	0.01	-0.13	-0.76
S	9.82	10.93	8.35	6.80
G_2	2.87	3.03	2.74	3.09
Sub-total	13.34	13.97	10.96	9.13
Mitosis	2.63	3.17	2.53	1.83
Total	15.97	17.14	13.49	10.96

compared to the improved types. In fact, the longer nuclear cycle of the primitive types is largely accounted for by their longer S phase. These observations, thus, suggest that the two primitive varieties have a greater content of DNA in their cells compared to the two evolved varieties.

It may be added that during the last three years a number of reports have appeared on the duration of the nuclear cycle and its component phases in maize roots grown at different temperatures. It may be observed that the estimates presented on the "Seneca 60" hybrid by Douglas (MNL 42:175) obtained from roots kept at 28°C (which is close to the temperature used in the course of the present studies) show a close correspondence with the values obtained in the present studies with the two evolved varieties, particularly the "Mexican June." Thus, there is evidence that the two primitive varities used in the present studies show a longer nuclear cycle compared to the evolved maize, in general.

This finding is in agreement with the well known observation that the present day commercial varieties of maize show a faster rate

of growth and are more vigorous than the primitive varieties. This fact has been commented upon among others by Stonor and Anderson (1949). The finding is of value in that it helps to establish an important characteristic of the primitive strains. As has been pointed out earlier, it is well known that a longer nuclear cycle corresponds with a greater DNA content. It, therefore, follows that there is more DNA in the chromosomes of the primitive varieties of maize compared to the evolved types. It would appear that in our search for fast growing varieties, suited to agricultural needs, we have selected variation in which there has been some loss of the DNA. It is not very clear at this stage, what the function of the lost DNA was in the primitive varieties. It is possible that some of it did not have any particular genetic information. On the other hand, the possibility cannot be ruled out that some important attribute of the maize plant was lost as a result of the loss of this DNA. Thus, the very prolific nature of the maize plant in which we are now so much interested, and which is commonly found to be present in the primitive varieties, may be a function of this additional DNA.

References

Matagne, R. (1968) Duration of mitotic cycle and patterns of DNA replication in chromosomes of Allium cepa. Caryologia 21:209-224.

Stonor, C. R. and E. Anderson (1949) Maize among hill people of Assam. Ann. Missouri Botan. Gard. 36:355-404.

H. K. Jain
D. Gupta*

*Present Address:
Agricultural Research Institute
Hungarian Academy of Sciences
Martonvasar, Hungary

INDIANA UNIVERSITY
Bloomington, Indiana

1. Location of knobs on the genetic maps of chromosomes 6 and 3.

The long arm of chromosome 6 contains four cytological markers, including a prominent chromomere and three knobs. Some stocks carrying the y allele possess a medium sized knob at the most proximal knob site and plants of this type were crossed with a homozygous Y pg_{11} tester having no knob in the proximal position. The heterozygotes $\frac{y\ Pg_{11}\ K}{Y\ pg_{11}\ k}$ were backcrossed to a y pg_{11} k stock and 38 plants of various phenotypes were examined for knob constitution. All plants of the backcross progeny were homozygous for pg_{12} on chromosome 9, which is necessary for expression of pg_{11}. The following data were obtained:

(0) Y pg_{11} k	12	The relative frequencies of noncrossovers
(0) y Pg_{11} K	6	and crossovers are meaningless since
(1) Y Pg_{11} K	10	plants with crossover phenotypes were
(1) y pg_{11} k	6	purposely selected. The knob is more
(2) Y pg_{11} K	3	closely linked to Pg_{11} than to Y. It
(2) y Pg_{11} k	1	cannot lie between the two loci because
		the postulated singles in (2) would then

become doubles. The data are best interpreted as indicating the order: centromere - Y - Pg_{11} - K.

The knob on the long arm of chromosome 3 has been placed at 3L.6 and falls between Na and A. In order to obtain a more precise location of the knob with respect to these markers, backcrosses were made as follows:

$$\frac{Lg\ Na\ K\ A}{lg\ na\ k\ a} \quad K10\ K10\ ♀\ X \quad lg\ na\ k\ a\ ♂$$

In a population of 566, there was 36.0% recombination between Na and A and 2.5% between Lg and Na. The Lg Na a crossovers were classified for presence or absence of the knob by observing preferential segregation of Lg in testcross progenies derived from the cross:

$$\frac{Lg\ Na\ (K)\ a}{lg\ na\ k\ a} \quad K10\ N10\ ♀\ X \quad lg\ na\ k\ a\ ♂$$

Backcross progenies were obtained from 54 Lg Na a plants. Six of the

progenies gave 1:1 ratios for Lg:lg while the remainder gave frequencies of Lg varying from 57-72%, but all significantly different from a 1:1. The six progenies with 1:1 ratios are presumably derived from plants of $\frac{\text{Lg Na k a}}{\text{lg na k a}}$ constitution resulting from a crossover between Na and K. Progenies with excess Lg came from $\frac{\text{Lg Na K a}}{\text{lg na k a}}$ plants resulting from a crossover between K and A. If all six of the 1:1 progenies trace to Na k crossovers, the frequency of Na-K recombination in the total population would be 36% X 11% or about 4% and the frequency of K-A recombination would be 32%. The presence of K10 K10 in the original backcross may have increased these frequencies above their normal levels. Two of the six 1:1 progenies were borderline cases, whose occurrence would be expected with probabilities of 20% and 30%; if these are eliminated, Na-K recombination becomes 2.5% and K-A would be 33.5%.

The variation in frequencies of preferential segregation of Lg (from 57% to 72%) in the second backcross was greater than expected, even though the 54 populations sampled generally did not exceed 200 individuals. K10 is heterozygous in all cases and the same knob is present; some unidentified factor apparently influenced the rate of preferential segregation. Since preferential segregation occurs only after a crossover between the knob and its centromere, variations in crossing over may be responsible.

E. Dempsey

2. The effect of K10 on chromosome breakage and recombination.

In structurally normal bivalents, the enhancement of recombination produced by abnormal chromosome 10 (K10) is restricted to the proximal regions adjacent to the centromere. In general, these regions consist of heterochromatic, deeply staining chromomeres. Crossing over in k10 k10 plants within segments adjacent to centric regions is much lower per unit of pachytene length than in more distally situated euchromatic regions. It was suggested (Rhoades and Dempsey, 1966) that the proximal heterochromatic regions were not as tightly coiled in K10 plants and that this relaxation in coiling facilitated the intimate pairing

essential for exchanges to occur. If crossing over takes place by breakage and reciprocal reunion, might not the enhancement induced by K10 be caused by breaks occurring more readily in the relatively uncoiled proximal chromonemata of K10 plants than in the more contracted chromatin of k10 plants? Cytogenetic studies of inversion heterozygotes provide data which are pertinent to the above question.

Progenies were obtained from sib plants heterozygous for In3a and for the Gl Lg and A markers in the long arm of 3 which were testcrossed. The sibs differed by the presence and absence of K10. Both the N and the In chromosome possessed the large knob at position 0.6 in the long arm. Consequently, preferential segregation due to knob heterozygosity is not a complicating factor in interpreting the recombination data.

$\frac{\text{Gl A K Lg In3a}}{\text{gl lg K a N3}}$ heterozygotes were used as female parents in backcrosses. The crossover regions are indicated in the leftmost diagram in Figure 1 and the constitution of the anaphase I bridge following a crossover between A and Lg is shown on the right. The backcross data from K10 N10 and N10 N10 sibs are given below (Families 29736-29753):

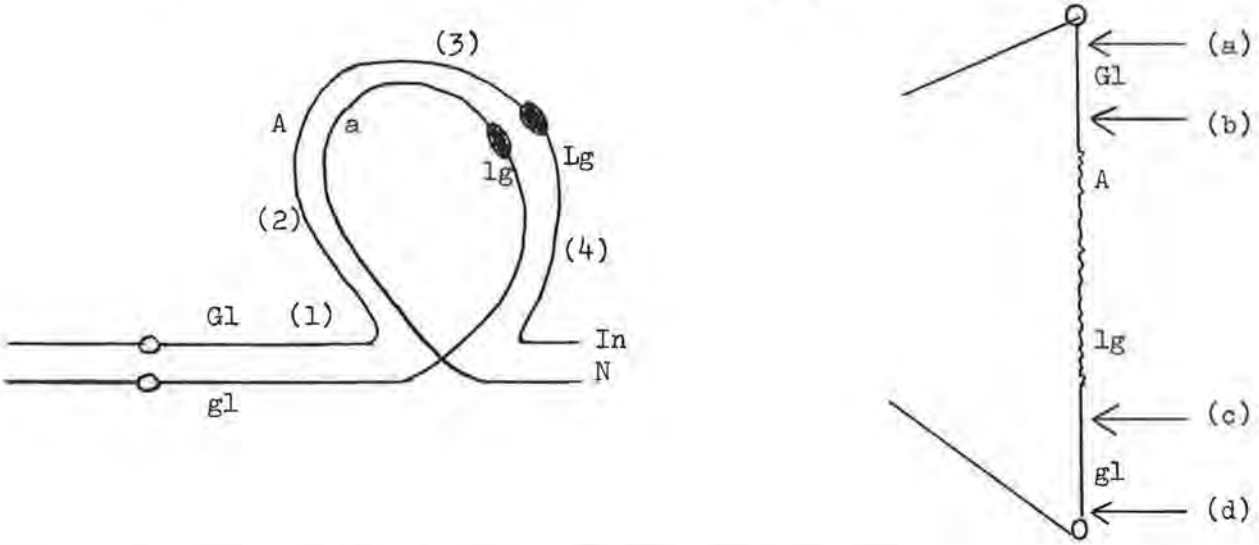
	(0)	(3-4)*	(2-4) (1)	(2-3)*	(2-3)	(2-4)** (1)	(3-4)	(0)	
	Gl Lg A	Gl lg A	gl Lg A	gl lg A	Gl Lg a	Gl lg a	gl Lg a	gl lg a	Σ
K10	1678	160	407	66	54	415	92	1549	4421
N10	3316	182	453	91	34	458	78	3282	7894

*Dp Df chromosomes from (3) and (1-3) exchanges included

**Dp Df chromosomes from (2) included

In the K10 data there are 160 Gl lg A and 92 gl Lg a chromosomes. The gl Lg a arise from (3-4) double exchanges and an equal number of Gl lg A are expected from the same doubles. The difference (160 - 92 = 68) is attributed to Gl lg A chromosomes which are Dp Df strands arising from singles in (3) and doubles in (1-3). They may come from breaks in regions represented by arrows (a), (c) and (d) in the diagram of the anaphase I bridge (Figure 1). The fraction stemming from breaks between gl and the In (region c) can be estimated, if region c and region b breaks occur with the same frequency, by comparing the frequency of gl

Figure 1. Pachytene pairing and anaphase I bridge configuration (following a single crossover in region (3)) in $\frac{Gl\ A\ K\ Lg\ In3a}{gl\ lg\ K\ a\ N3}$ heterozygotes.



Bridge breakage at AI between the Gl locus and the centromere is indicated by arrows (a) and (d). Breaks between Gl and the inversion occur in regions (b) and (c). A wavy line indicates the inverted segment. Breaks in (a) or (d) give Dp Df chromosomes with both the Gl and gl alleles while breaks in regions (b) and (c) give Dp Df chromosomes that are not redundant for the Gl locus.

Bridge breakage at (a) gives a N Dp Df gl lg A Gl chromosome
" " " (b) gives a N Dp Df gl lg A "
" " " (c) gives an In Dp Df Gl A lg "
" " " (d) gives an In Dp Df Gl A lg gl "

---

lg A and Gl Lg a classes. The 54 Gl Lg a chromosomes are derived solely from (2-3) doubles and an equal number of gl lg A is expected from the same doubles. The difference between 66 and 54 or 12 represents those Dp Df gl lg A chromosomes coming from bridges following single exchanges in region (3) or (1-3) doubles and they are formed only when the bridge breaks between the Gl locus and the inversion breakpoint (arrow b in the anaphase diagram). An equal number of Dp Df Gl A lg should come

following breaks at arrow c. It follows that 56 (68 - 12) Dp Df chromosomes arose from breaks in the proximal segments between Gl and the centromere. The ratio of breaks in the Gl-centromere regions (a and d) to those in the Gl-In regions (b and c) is 56 : 24 or 2.3 : 1.0. When a similar analysis is made of the N10 N10 data the calculations give 47 cases of breaks in the a and d regions and 114 in the b and c segments, a ratio of 0.4 : 1.0. It appears that, when breaks occur in an anaphase bridge, the chances of a proximal break (at a or d) are approximately six times greater in K10 than in N10 plants. In the above calculations the number of Dp Df chromosomes is an indirect estimate based on the assumption that complementary crossovers from double exchanges occur with equal frequencies in the population. Moreover, sampling errors would be high because of the small number of Dp Df chromosomes. However, the validity of the conclusions reached regarding a greater tendency for breaks to occur in segments a and d in K10 plants than in N10 plants was supported by an analysis of proven Dp Df chromosomes derived from both K10 and N10 parents. Among the Dp Df chromosomes from K10 plants there were twelve arising from breaks in the a or d regions and six from breaks in the b and c regions. The Dp Df chromosomes from N10 parents consisted of ten coming from breaks in the a and d regions and 58 from breaks in the b and c regions. The ratio of the Dp Df chromosomes coming from a and d breaks to those from b and c breaks is much higher in K10 than in N10 plants. The data are in agreement with the original conclusion.

It may be of more than passing interest to find that the K10 chromosome renders the proximal heterochromatin more susceptible to breakage and also enhances the frequency of recombination within the same chromosomal segment. If crossing over occurs by breakage and reciprocal reunion a greater susceptibility to breakage might be correlated with increased crossing over. Such appears to be the case although it should be kept in mind that enzyme induced breaks are presumably involved in crossing over while rupturing of the dicentric bridge is due to tension. Incidentally, all of the inversion heterozygotes in these experiments had several B chromosomes so any differential effect of B's on transmission or recovery of Dp Df chromosomes

(see Rinehart, MNL 1970) is not a factor.

An estimate of the amount of crossing over within the inversion loop in K10 and N10 plants can be calculated from the frequencies of Gl Lg a (2-3) and gl Lg a (3-4) double crossover chromosomes. These two recombinant types contain no Dp Df chromosomes, although their complementary crossover classes do. In K10 plants there were 146 such doubles in a population of 4421, or 3.3%. This value should be doubled (6.6%) to allow for the equal number of gl lg A and Gl lg A double crossover chromatids. The percentage of double crossover chromatids calculated in a similar manner is 2.8% in N10 plants. If the frequency of double crossover chromatids truly reflects the amount of recombination within the loop in K10 and N10 plants, there is 2.36 times as much inversion crossing over in K10 as in N10 sibs. This finding is in agreement with our earlier studies where K10 was shown to markedly increase crossing over in structural heterozygotes. Since the frequency of Dp Df chromosomes from dicentric bridges produced following a crossover between Lg and A is 2% in N10 plants (161 ÷ 7,894), the percentage of Dp Df chromosomes recovered from K10 plants should be 2% x 2.36 or 4.72%, if there is no preferential segregation to the functional megaspore of the intact member of a dyad consisting of a normal and a broken chromatid. The observed percentage of Dp Df chromosomes from K10 plants is only 1.8% (80 ÷ 4421) even though more frequent breaks in the proximal segments of the dicentric bridge in K10 plants might be expected to enhance the number of viable Dp Df chromosomes. These data support the hypothesis of Rhoades and Dempsey (1966) that a knobbed intact chromatid is preferentially recovered from deficient dyads in K10 plants and that random segregation of the intact and deficient chromatids occurs in N10 plants.

M. M. Rhoades
E. Dempsey

3. Evidence that ameiotic results in a substitution rather than an elimination of meiosis.

Preliminary evidence suggested to us that in the ameiotic plants meiosis did not occur and that it was not replaced by a modified form

of meiosis (MNL, 1970). However, we also reported last year that a higher frequency of dividing cells was found in the premeiotic mitosis from ameiotic plants than from normal plants. To resolve the discrepancy, anthers of different lengths were collected from normal and ameiotic tassels and the developmental stage determined by chromosome squashes for one of the three anthers in the flower. The remaining two anthers were carried through the tertiary butyl alcohol dehydration series and embedded in paraffin. Longitudinal sections 10μ and 20μ in thickness were cut with a rotary microtome and stained with iron-alum hematoxylin.

The data presented in Tables 1 and 2 indicate that the number of cells per locule in 2.2 mm. ameiotic anthers, which have completed the mitotic division observed in 1.9 mm. anthers, is twice that found in normal anthers of the same length and presumably therefore of comparable developmental stage. While the number of cells in 1.0 mm. anthers from ameiotic and normal plants was about the same, it is evident that ameiotic anthers of 2.2 mm. length contain more cells than those from the normal sibs.

The following conclusions can be drawn regarding the effect of the am gene on the course of meiosis. Counts of cell number in longitudinal sections of fertile and sterile anthers of similar length reveal that the number of sporogenous cells formed by the end of the premeiotic divisions is approximately the same in the two types--i.e., the am gene has no effect on the premeiotic divisions insofar as the number and character of the mitoses are concerned. However, a profound difference arises in anthers approximately 1.9 - 2.0 mm. long. In normal anthers, the archesporial cells have entered the extended meiotic prophase while in ameiotic anthers cells of comparable age are in a somatic mitosis. We shall call this the ameiotic mitosis since it is a substitute for the first meiotic division. The ameiotic mitosis, with rare exceptions, proceeds rapidly and is completed in 2.2 mm. anthers, while in fertile anthers of this length all cells are still in early meiotic prophase. Significantly the number of cells in 2.2 mm. anthers, which have undergone the ameiotic mitosis, is approximately twice the number of pollen mother cells in fertile anthers

Table 1

Average number of cells observed in a microscopic field at 430 X. Values in parentheses represent number of determinations. In several examples two anthers from the same flower were used for sectioning, one at 10μ, the other at 20μ (e.g. 3-11). Cells undergoing mitosis were counted twice.

Anther length (mm.)	Normal			Ameiotic		
	Plant no.	Section thickness		Plant no.	Section thickness	
		10μ	20μ		10μ	20μ
1.0	3- 1	31(6)				
1.0	16- 9	31(6)		15- 7	30(6)	
1.1				15- 8	30(6)	
1.9	3-11	23(4)	23(14)	6- 3		45(7)
1.9				6- 5	47(4)	48(7)
2.0	3-14		24(6)	12-10	46(4)	49(4)
2.0	3-17	24(7)	24(9)			
2.0	3-19	24(7)	25(10)			
2.2				6- 1	48(4)	49(6)

Table 2

Total number of cells observed in longitudinal sections of anthers. Values in parentheses represent number of determinations. In several examples two anthers from the same flower were used for sectioning, one at 10μ, the other at 20μ (e.g. 3-11). Cells undergoing mitosis were counted twice.

Anther length (mm.)	Normal			Ameiotic		
	Plant no.	Section thickness		Plant no.	Section thickness	
		10μ	20μ		10μ	20μ
1.0	3- 1	52(3)				
1.0	16- 9	57(3)		15- 7	49(3)	
1.1				15- 8	57(3)	
1.9	3-11	85(2)	91(3)	6- 3		194(1)
1.9				6- 5	197(2)	204(3)
2.0	3-14		88(2)	12-10		194(1)
2.0	3-17					
2.0	3-19	101(2)	110(2)			
2.2				6- 1		196(3)

which are in leptonema-zygonema. It is the doubled cell number at this developmental stage which offers the most cogent evidence that a mitotic division replaces meiosis. The occurrence of a second ameiotic division (corresponding to the second meiotic division) is doubtful because degeneration of the archesporial cells is evident in anthers 2.2 mm. and longer in length and by the time the quartet stage is reached in fertile anthers, there is only disorganized cellular debris in ameiotic anthers.

The final number of sporogenous cells in both fertile and ameiotic anthers is attained in anthers approximately 2.0 mm. long. Since the number of archesporial cells in younger anthers is considerably less than the number reached when the ultimate premeiotic mitosis is completed, it follows that the increase in cell number arises from somatic mitosis. At any given time the number of nuclei in mitosis in the young anthers is small, most of the cells being in interphase--i.e., there is no indication of synchronized mitosis in the developing anthers. However, in those locules where the ameiotic mitosis is occurring, the majority of the cells are dividing. The synchronization observed in this division is comparable to that in true meiosis and is in contrast to the sporadic mitosis observed in the sporogenous cells of premeiotic anthers.

From our study of microsporogenesis in ameiotic plants we conclude that in ameiotic anthers meiosis is replaced by a somatic mitosis. This mitosis has been called the ameiotic mitosis.

Reid G. Palmer*

*Present Address

Agronomy Department
USDA CRD ARS
Iowa State University
Ames, Iowa 50010

INSTITUTO FITOTECNICO DE SANTA CATALINA
Llavallol, F. N. G. R., Argentina

1. Studies on the resistance of corn to Helminthosporium maydis.

During the last few years, a project has been underway with the purpose of finding resistant genes to Helminthosporium maydis Nisik and Miyake in pure corn lines, using a collection of 350 lines (S8-S34) and one made up of lines selected from populations derived from various Latin American countries.

On account of such studies, this Institute now possesses a number of lines resistant or very resistant to H. maydis. Some of them have already been distributed among private and official Institutions devoted to corn plant breeding projects.

At present our research work attempts to find a relationship among T cytoplasm and some other cytoplasms with various genotypes resistant to Helminthosporium maydis Nisik and Miyake.

Elisa Hirschhorn de Mazoti
Josefa A. Calvo

2. Euchlaena perennis Hitch. X Zea mays L.

Studies on Euchlaena perennis Hitch. (2 or 4N = 40) X Zea mays L. (2n = 20) were carried out by R. A. Emerson and G. W. Beadle in 1930 (Amer. Nat. LXIV: 190-192) and by D. S. Shaver in 1963 (Maize News Letter 37:8-11). In 1964, we carried out crosses between Euchlaena perennis Hitch. and Zea mays L. getting a perennial F_1 with very strong plants, with abundant tillers and with inflorescences similar to Euchlaena. A cytological study of the F_1 plants showed in diakinesis trivalents, bivalents, and monovalents; these characteristics agree with Emerson's and Beadle's studies. Only one F_2 kernel was formed in every 100 flowers.

The F_2 plants showed a segregation of 75% annual plants and 25% biennials or perennials, with a pollen fertility of about 0-50% in 85% of all plants and with a fertility of about 85-95% in 15% of all plants.

After six generations of mass and genealogical selection, we got a perennial population with 80% fertility. This population has some

characteristics of forage plants and they may be added to maize (by means of chromosomes made up structurally of segments of Euchlaena and Zea): (1) prolificness, (2) endurance to drought due to their strongly developed radical system and (3) heterosis factors from F_1 vigor.

At the present time, the cytogenetics of inbred lines from this population is being studied.

L. B. Mazoti
Pedro Rimieri

INSTITUTO FITOTECNICO DE SANTA CATALINA
Llavallol, F. N. G. R., Argentina

and

DEKALB COMPANY, ARGENTINA

1. Behavior of inbred lines in Texas cytoplasm.

The behavior of 375 corn lines (selfed S8-S34) was studied in relation to cytoplasmic androsterility conditioned by Texas cytoplasm. This work was carried out at two different localities: Salto (Prov. Buenos Aires) for visual observations, and Llavallol (Prov. Buenos Aires) for visual and microscopic observations.

As a result of this study we made the following conclusions: 26 (6.9%) of the inbred lines showed 100% restoration in both localities; 70 (18.6%) of the lines showed no restoration in both localities; and 279 (74%) of the inbred lines showed an undetermined condition.

Luis B. Mazoti
M. Hernandez
Juan E. Aguilar

INSTITUTO FITOTECNICO DE SANTA CATALINA
Llavallol, F. N. G. R., Argentina

and

PIONEER HI-BRED CORN COMPANY
Johnston, Iowa

1. The fl^a gene and its percentage of lysine.

The gene fl^a (allele of fl_1) differs from fl_1 in that it is recessive in two doses in the endosperm (Maize News Letter 41:86-87). As a result of preliminary studies by Dr. Alix V. Paez, fl^a appears to be similar to the gene opaque-2 (o_2) in its percentage of lysine content.

Luis B. Mazoti

UNIVERSITY OF IOWA
Iowa City, Iowa
Department of Botany

1. A survey of B^9 instability in the sporophyte.

The B^9 chromosome is less stable in both the endosperm and sporophyte than members of the regular (A) complement (1). The instability is observed as a loss of the dominant alleles present on the B^9 chromosome and appearance of recessive sectors. Two general types of instability have been observed which produce either a fractional or a mosaic pattern of gene loss. (Fractional refers to the appearance of a single recessive sector; mosaic indicates a pattern of repeated loss of the dominant allele.) Both types of sectoring have been studied with TB-9b (2,3). Concurrent investigations of mosaic kernels have been reported with TB-4a (4). The indication is that fractional loss represents formation of an isochromosome of the B^9 during development of the endosperm or sporophyte, and the mosaic pattern results from transmission of a ring B^9 chromosome by the male parent. However, patterns of loss are not easily separated into fractionals and mosaics. Many intermediate types are seen that, for example, may have two sectored losses rather than one. Whether this is a mosaic pattern, or a fractional pattern with a re-arrangement of embryonic cells is open to

question. In addition, some unusual plants have recessive areas that cover more than one-half of the plant or endosperm, but appear to be single events. Therefore, a survey of sectored plants found in one set of TB-9b crosses is being made. The parental cross was: yg sh bz wx X 9^{wx} 9^{BWx} $B9^{Yg\ Sh\ Bz}$ $B9^{Yg\ Sh\ Bz}$. The Sh Bz Wx progeny were selected, planted, and the Yg-yg sectored seedlings kept for classification of root tips. Of twenty-five sectored plants, the chromosomes of fourteen have been analyzed. Despite the wide range of patterns found, only two chromosome changes seem to have occurred; isochromosome formation and ring formation. Below are listed the sector types and the abnormality responsible:

	Fractional Plant (½-¼ yg)	Mosaic Plant	Intermediate between Fractional and Mosaic	Plant more than ½ yg
Number of Plants	7	3	3	1
Classification	all isochromosome	2-ring 1-isochromosome	2-isochromosome 1-ring	isochromosome

(The classification of the ring chromosome is done with some reservation. The chromosomes are very small and rounded. They are much smaller than a normal B^9 and must be reduced derivatives. However, the chromosomes are too small in present preparations to visualize the ring structure. Classification during meiosis may yield more positive identification.)

Apparently two types of chromosome rearrangement are responsible for all B^9 instability in the sporophyte. It is possible, in addition, that these two chromosomal changes have a common origin: centromere misdivision at the second pollen mitosis. B-type chromosomes undergo nondisjunction at a high frequency in the second pollen mitosis. This nondisjunction probably consists of two steps: centromere fixation, and unipolar migration. If the second step fails to occur, misdivision of the centromere is likely, and an unstable telocentric can result. In the case of the isochromosome, this telocentric would then form an isochromosome during early development of the embryo. (An alternate suggestion is that centromere misdivision occurs in the sporophyte and

gives rise directly to the isochromosome--reference 2). The origin of ring chromosomes is more difficult to explain. However, the first step in ring formation may also be centromere misdivision at the second pollen mitosis. This would provide one of the two broken ends required for ring formation, and eliminate the need to postulate high rates of breakage in the minute short arm of the chromosome. (Origin of the second break in the long arm is not understood). Consistent with this idea is the fact that the Yg-yg mosaics identified were derived from seeds with a stable Bz and Sh phenotype. Therefore, the sperm that fertilized the egg differed in stability from the one that fertilized the polar nuclei, suggesting an origin of the ring at the second pollen mitosis. However, the previous report on B^9 mosaicism (3) gave contrary results. In the previous experiment, selection of endosperm mosaics and self pollination of the resultant plant often resulted in transmission of the mosaic character, indicating that the B^9's of both sperm were unstable. The main difference between the two experiments was the presence or absence of the 9^B chromosome. The implications for centromere misdivision are several:

1. In the absence of 9^B, the B^9 does not undergo nondisjunction at the second pollen mitosis (5). It is unlikely, therefore, that misdivision would occur at this time in the absence of 9^B.
2. The 9^B B^9 pollen type can tolerate gene deficiencies of the B^9 only in the sperm, and ring formation must, therefore, be restricted to the second pollen mitosis. On the other hand, the 9 B^9 pollen type is duplicate for genes on the B^9 and may gain in viability by loss of duplicate genes (ring formation) prior to the second pollen mitosis.
3. In the 9 9^B B^9 B^9 plants, the B^9's have pairing partners and should divide properly in meiosis. In 9 9 B^9 plants, the B^9 may be excluded from pairing. Centromere misdivision may then occur.

The factors listed suggest that ring formation in the absence of the 9^B may begin with centromere misdivision in meiosis. In conclusion,

centromere misdivision may be the primary factor in production of derivatives of the B^9 chromosome. Experiments are underway to test the validity of this idea.

Bibliography

1. Bianchi, A., Bellini, G., Contin, M., Ottaviano, E.: Nondisjunction in presence of interchanges involving B-type chromosomes in maize and some phenotypical consequences of meaning in maize breeding. Z. Vererbungsl. 92, 213-232 (1961).

2. Carlson, Wayne R.: Nondisjunction and isochromosome formation in the B-chromosome of maize. Chromosoma (Berl.) 30, 356-365 (1970).

3. Carlson, Wayne R.: Unstable derivatives of the B^9 chromosome. Maize News Letter 43: 78-79 (1969).

4. Ghidoni, Achille: Inheritable instability of the B^4 chromosome. Maize News Letter 44: 142-144 (1970).

5. Carlson, Wayne R.: A test of homology between the B chromosome of maize and abnormal chromosome 10, involving the control of non-disjunction in B's. Molec. Gen. Genetics 104, 59-65 (1969).

Wayne Carlson

IOWA STATE UNIVERSITY
Ames, Iowa 50010
Department of Agronomy

1. The effect of B chromosomes on a chromosome translocation during endosperm development.

In MNL 43: p. 70 it was reported that a chromosome segment of a 1-3 translocation (1L.95; 3L.35) was lost during endosperm growth. This could be detected by following a marked segment ($\underline{A_1}\,\underline{Sh_2}$) on the translocated arm.

In testcrosses of this translocation $\underline{TA_1}\,\underline{Sh_2}/\underline{a_1}\,\underline{sh_2}$ not all the colored-round progeny were sectored since both sectored and non-sectored colored kernels were observed. It was assumed that a second factor was necessary for sectoring behavior and that, from the frequency of sectored and non-sectored kernels, this second factor was assorting independently of the translocation. It was suspected that B chromosomes represented the second factor. This was tested by crossing non-sectored kernels on

the $\underline{a_1 sh_2}$ testers containing B chromosomes. It is seen from Table 1 that sectoring ear cultures were more frequent among B chromosome containing crosses.

Table 1

Effect of B chromosomes on sectoring behavior among crosses of $\underline{a_1 sh_2}$ testers with and without B chromosomes by plants with the translocation from non-sectoring kernels

Number of B's	Number of ear cultures with colored round kernels with	
	Sectors**	No sectors
0 B's*	8	20
1-3	41	6
Control (without B's)	3	9

*These are sibs of B containing stocks that were segregating without B's.

**An ear culture was designated as sectored when one kernel showed a sector.

Additional findings from this study:

- the frequency of sectoring is undifferentiated in crosses with male and female originated B chromosomes.
- a dosage effect of B chromosomes was not evident.
- kernels with a sectoring potential (the translocation plus B's) do not always show sectors.

The chromosome segment leading to the sector is limited to the distal portion of the T1-3 chromosome indicating that the sector is a result of a breakage loss rather than of non-disjunction of the whole chromosome.

It is likely that the B chromosomes interact with the knob on the chromosome 3 portion of the T1-3 chromosome.

Sunee Ruktanonchai
Peter A. Peterson

2. Effect of segment relocation on intra-genic recombination.

In MNL 44: 79-80, we reported on the intra-genic waxy recombination of heteroallelic combinations following changes in the position of this locus in the maize genome. The wx recombination frequencies of three heteroallelic combinations involving two translocated segments were reported. Here, data are presented (Table 2) for six translocated segments that include five heteroallelic combinations. Four of the translocations have breakpoints proximal to wx and two, distal.

The sampling included a test of over 1/2 million pollen grains in each instance. As indicated in the table, the values for the standard chromosome changed in comparison to our 1969 values; three increased and three decreased. The 1970 results are in better agreement with Nelson's preliminary map (1959, Sci 130: 795) which itself was not completely consistent with respect to the additivity of distances.

All recombination values from the proximal translocation series are lower than the 1969 values. Within this series, there are some inconsistencies. When two different heteroallelic combinations involving the same translocation are compared, it is found that the recombination values differ even though the recombination frequencies of these same combinations are very similar in the control series. For example, the C/H21 combinations (lines 9 and 10) show more recombination than the C/B (lines 7 and 8) but the control values (lines 1 and 3) are very similar.

The B/H21 heteroallelic combination shows a similar rate in two different translocations that have similar breakpoints with respect to the centromere, at 26.72 μ and 24.10μ.

For the B/90 combination (proximal series) with a longer distance from the wx locus to the centromere, a higher recombination value resulted.

	distance wx-centromere	recombination value
proximal B/90	5.16μ (line 12 - table 2)	0.73
"	31.83μ (" 13 - ")	1.23
	distance wx-distal tip	
distal C/90	38.07μ (line 19 - table 2)	95.98
"	18.23μ (" 18 - ")	56.72

Table 2

Intracistron recombination values among heteroallelic combinations involving standard and relocated waxy segments

	Line	Approx. distance of wx to centromere (μ)	The heteroallelic combination	Wx frequency x 10-5	Compared** to 1969	Year#
A	1	7.72	C/B	74.64	+	1970
	2	"	C/90	111.45	+	"
	3	"	C/H21	74.80	+	"
	4	"	B/90	0.85	-	"
	5	"	B/H21	54.15	-	"
	6	"	90/H21	30.54	-	"
B	7	24.10	T 5-9 4871; C/B	36.52		1969
	8	"	" " ; "	28.33	-	1970
	9	"	" " ; C/H21	48.17		1969
	10	"	" " ; "	43.08	-	1970
	11	5.16	T 8-9 5391; B/90	1.02		1969
	12	"	" " ; "	0.73	-	1970
	13	31.83	T 8-9 5300; "	1.23		"
	14	24.10	T 5-9 4871; B/H21	47.79		1969
	15	"	" " ; "	30.35	-	1970
	16	26.72	T 5-9a ; "	41.41		1969
	17	"	" ; "	30.67	-	1970
C	18	18.23*	T 3-9F ; C/90	56.72		1970
	19	38.07*	T 7-9 7074; "	95.98		"

*Distance between wx locus and the end of chromosome arm.

**+ = increase; - = decrease.

A - Standard; B - Proximal translocation; C - Distal translocation.

- designates year collected.

A comparison can also be made in the distal series with the C/90 combination. A longer distal segment results in more recombination than that found with the shorter distal segment.

Ming-Hung Yu
Peter A. Peterson

3. Control of the $a_2^{m(r\text{-}pa\text{-}pu)}$ allele.

From $a_2^{1\ 1511}$, an unstable allele (En system) at the a_2 locus (Peterson, 1968 Genetics), a number of derivatives are obtained. One phenotype, a solid pale, is designated $a_2^{m(r\text{-}pa\text{-}pu)}$. This allele is stable pale in the absence of En; in the presence of En, it is colorless and mutates, expressing both pale and deep purple sectors. These conclusions on the nature of the allele are based on the following series of crosses.

If mutable kernels heterozygous for this allele and a standard a_2 are testcrossed (Cross #1), approximately 1/2 of the Bt progeny are mutable and 1/2 are stable pale.

Cross #1--$a_2^{m(r\text{-}pa\text{-}pu)}$ Bt/a_2 bt x a_2 bt/a_2 bt

	Bt				
	(A)	(B)	(C)	(D)	(E)
	colored	pale-stable	purple & pale dots on colorless bkgd	colorless	A+B+C%
9 2143-4	0	52	53	6	50.4
9 2150-2	3	156	142	8	47.1

The colored Bt progeny in column (A) have been shown to be germinal mutations of the $a_2^{m(r\text{-}pa\text{-}pu)}$ allele and the colorless Bt progeny in column (D) are assumed to result from crossing over between a_2 and Bt.

The original $a_2^{m\ 1\ 1511}$ mutable allele contained En. In testing for the presence of En among the progeny of cross #1, the $a_2$bt/$a_2$bt segregants from Cross #1 were crossed by an En tester - $a_2^{m(r)}$. In the progeny of these crosses approximately 1/2 of these $a_2$bt/$a_2$bt segregants contained En. Therefore one En was segregating independently of the a_2 locus in Cross #1.

Stable pale kernels from column (B) were also tested by crossing to the same En tester and these were found not to contain En. The critical cross on the identify of this allele was made when these same solid pale kernels were further tested for response to a known En by crossing to an En containing stock ($a_2$bt/$a_2$bt En). From this cross, $a_2^{m(r\text{-}pa\text{-}pu)}$ Bt/$a_2$bt x $a_2$bt/$a_2$bt En, approximately 1/2 of the plump seeded progeny (Bt) were stable pale and 1/2 were mutable, the latter identical in phenotype to the kernels in column (C) in Cross #1 - i.e. having a colorless background with pale and purple dots.

This series of crosses is evidence for the presence of some activity at this allele in the absence of En. Further, in the presence of En, it responds giving pale and purple sectors--thus the designation r-pa-pu.

Robert Fowler
Peter A. Peterson

4. Attempted intergeneric crosses involving maize and sorghum.*

Sixteen maize cultivars (Table 1) and 16 sorghum cultivars (Table 2) were planted at the Agronomy Field Research Center, Ames, Iowa, on three dates (delayed approximately 10 days) during the growing season of 1970. A total of 1,667 control pollinations were made (891 maize x male-fertile sorghum and 796 male-sterile sorghum x maize). The maize cultivar Gangtok-Sikkim matured too late to nick with any male-sterile sorghum and the maize cultivar Pollo was barren; therefore, Gangtok-Sikkim was excluded from all crosses and Pollo was used only as a pollen parent.

Silks were shortened on approximately 1/2 the maize ear shoots pollinated; several ears with shortened silks were self-pollinated and served as checks of damage caused by the cutting procedure. Also, some ears with silks generally considered "too mature" to pollinate were pollinated.

Several male-sterile sorghum heads were bi-pollinated (approximately 48 hours between pollinations) and a few heads were tri-pollinated (approximately 24 hours between pollinations).

Eight ear shoots from each of the maize cultivars (N28 x Mo17), (A619 x A632), and A619 were pollinated by Tx7078 sorghum. Silks were shortened on four of the plants and were left uncut on the remaining four plants. Two ear shoots of each type (shortened and normal silks) were treated on two consecutive days (beginning 24 hours after pollination) with 0.8 ml gibberellic acid solution (45 ppm GA in 0.05% Tween 20) and two ear shoots were untreated. Similarly, eight sorghum heads each of male-sterile Wheatland, Martin, and Kafir 60 were pollinated by the maize

*Research supported by the Rockfeller Foundation.

Table 1

Maize cultivars used in 1970 intergeneric crossing nursery

Adapted	Unadapted
Crows #1 (O.P. 7-45-3-2-1)*	Zapalote Chico**
B14A*	Maiz Chapalote**
A619*	Japanese Hulless Popcorn
MS214*	Argentine Popcorn**
B57*	Northern Flint**
(A619 x A632)*	Early Teosinte**
(B37 x B70)*	Gangtok-Sikkim**
(N28 x Mo17)*	Pollo**

*Seed obtained from Dr. W. A. Russell, Agronomy Department, Iowa State University, Ames, Iowa.

**Seed obtained from Dr. Wm. L. Brown, Vice-President and Research Director, Pioneer Hi-Bred Corn Company, Des Moines, Iowa.

Table 2

Sorghum cultivars used in 1970 intergeneric crossing nursery

Male-sterile	Male-fertile	
	Adapted types	Basic types
Wheatland*	Plainsman*	Durra**
Martin*	Tx7078*	Kaoliang*
Kafir 60*	Redbine*	Feterita*
Redlan*	Caprock*	Hegari*
	Kafir 60*	Shallu**
	Sooner milo*	Sorghum virgatum**

*Seed obtained from Dr. R. E. Atkins, Agronomy Department, Iowa State University, Ames, Iowa.

**Seed obtained from Dr. Wm. L. Brown, Vice-President and Research Director, Pioneer Hi-Bred Corn Company, Des Moines, Iowa.

cultivar B57. Four of the eight heads were treated with gibberellic acid and four were untreated.

An abnormal amount of seed abortion (especially on maize ears) was observed, but 148 potential hybrid seeds (139 from maize ♀ and nine from sorghum ♀) were recovered and are being germinated on artificial nutrient media. Approximately 50% of these seeds have germinated and several plants are in the 2-to-3-leaf stage and have been transplanted into clay pots and transferred to a growth chamber. Root tip chromosome analyses are being made on all potential hybrid plants.

One "potential" plant (MS214 ♀ x Feterita ♂) has reached maturity and has a maize phenotype. It was partially male-sterile, however, and, therefore, it has been saved and self-pollinated. The somatic chromosome number of this plant is 20. No further analysis of it has been made.

No treatment appreciably enhanced potential hybrid seedset (Table 3).

Table 3

Potential hybrid seeds produced by intergeneric crosses receiving various treatments

Treatment	Number seeds	% of total
Shortened silks	36	25.8
Old silks	2	1.4
Gibberellic acid	0	0.0
Multiple pollinations	0	0.0

Additional research on the production of maize x sorghum hybrids is being conducted.

James J. Mock
Wayne J. Dahmen

IOWA STATE UNIVERSITY
Ames, Iowa
Department of Genetics

1. A possible locus on chromosome 10 responsible for a high mutation rate.

In last year's News Letter (MGCNL 44:81-84, 1970), I reported on the genetics of a new white endosperm mutant ($\underline{y}_9$). Since homozygous $\underline{y}_9$ plants are sometimes not very vigorous, linkage tests were made by crossing F_1 plants heterozygous for $\underline{y}_9$ and the marker genes as males to stocks homozygous for the marker (in the case of $\underline{g}_1$ and $\underline{r}$) or to standard lines (M14/W22) (in the case of $\underline{bf}_2$ and T9-10b). These crosses were then self-pollinated to check for the presence of $\underline{y}_9$ (and $\underline{bf}_2$, in crosses involving this gene). All selfed ears were seedling tested. In the seedling tests an unusually large number of new seedling mutants was observed. We test seeds from thousands of self-pollinated ears each year and have observed occasional ears that will segregate for a different mutant than the one under test. Such ears are probably the result of spontaneous mutations or contamination during outcrossing. In the $\underline{y}_9$ crosses these "new mutants" seemed to be occurring at an unexpectedly high frequency. To determine if this were so, all of the seedling tests of self-pollinated ears from outcrossed heterozygous $\underline{y}_9$ plants for the years 1965 to 1968 were checked. The number and types of "new mutants" observed in these crosses are shown in Table 1. Table 2 shows the number of "new mutants" observed in the selfs of outcrosses in linkage studies for several other genes tested in 1968. In Tables 1 and 2 families grouped together by the + sign are replicated plantings from the same outcross ear. It is obvious that stocks carrying $\underline{y}_9$ are relatively mutagenic. The crosses recorded in Table 1 represent many different F_1 genotypes (7) and outcrosses (15). Thus the high mutation rate is not the result of the non-$\underline{y}_9$ F_1 parents or the outcross parents but must be the result of the presence of the chromosome 10 which carries $\underline{y}_9$. In all the crosses listed in Table 1, the F_1 parents did not segregate for any of the mutants observed in the outcross.

Table 1

Spontaneous mutants observed in crosses carrying y_9

Family	Total No. of Plants Selfed	Seedling Mutants												Total No. of Mutants	No. of Diff. Mutants
		Yellow-green		Luteus		Pale Yellow		Necrotic		Albino		Pale Green			
		homo +	seg y_9	homo +	seg y_9	homo +	seg y_9	homo +	seg y_9	homo +	seg y_9	homo +	seg y_9		
68-7001+7004	84	6**	1	4	3	1								15	3
68-7002+7005	93		1	1		1						1		4	4
68-7003+7006	96	4	2	8	4	3				1	1			23	4
68-7007+7008	94													0	0
68-7009	42													0	0
68-7010	45				1	1	2							4	2
68-7011	46						1							1	1
68-7012	42			1	1	1								3	2
68-1001	9													0	0
68-1051	10													0	0
67-5001+66-7001	82			24*	19*									43	1
67-5002+66-7002	94									1	1		1	3	2
67-5002+66-7003	91			4	4	1	1			17*	21*			48	3
67-5004+66-7004	87	4		2+(1)	1***		1			1	1			11	4
67-5005+5006	85	17*	18*											35	1
67-5007+5008	88	22*	26*							3	2			53	2
67-3001	6													0	0
67-3061	9													0	0
67-1001	2													0	0
67-1002	8			(2)										2	1

Table 1 (Continued)

Family	Total No. of Plants Selfed	Seedling Mutants												Total No. of Mutants	No. of Diff. Mutants
		Yellow-green		Luteus		Pale Yellow		Necrotic		Albino		Pale Green			
		homo +	seg Y_9	homo +	seg Y_9	homo +	seg Y_9	homo +	seg Y_9	homo +	seg Y_9	homo +	seg Y_9		
67-1003	7					1								1	1
67-1004	10													0	0
67-1005	7													0	0
67-1006	6													0	0
66-7005+8001	41													0	0
66-7006+8002	44						2							2	1
66-7007+8003	35		1			1	2	(1)						5	3
65-1001	9													0	0
65-1002	3										1			1	1
65-1003	4													0	0
65-1004	9						1			2*	3*			6	2
65-5003	9													0	0
65-5004	7											1		1	1
65-5006	7	1+(1)												2	1
65-5007	7												1	1	1
65-5008	10	2	2											4	1
65-5009	9	3					1							4	2
65-5010	9	2	1											3	1
65-5011	10													0	0
65-5012	10										5*			5	1

Table 1 (Continued)

Family	Total No. of Plants Selfed	Seedling Mutants												Total No. of Mutants	No. of Diff. Mutants
		Yellow-green		Luteus		Pale Yellow		Necrotic		Albino		Pale Green			
		homo +	seg y_9	homo +	seg y_9	homo +	seg y_9	homo +	seg y_9	homo +	seg y_9	homo +	seg y_9		
65-5013	8													0	0
65-5014	39						1			1			1	3	3
65-5015	39					1								1	1
65-5016	43	1	1			1								3	2
65-5017	42			1		1								2	2
65-5018	41	1	2				1							4	2
65-5019	44													0	0
65-5020	41		1	1		2	2							6	3
Total	1663	64	56	49	33	15	15	1	0	26	35	2	3	299	59
Total - *		25	12	25	14	15	15	1	0	7	6	2	3	125	53

*Mutant possibly contributed by female parent.

**2 mutable.

***mutable.

Since the data in Table 1 are subject to more than one interpretation, there are several possible ways to calculate the mutation rate. For example, in many instances mutants with the same phenotype segregated in several plants from the same outcross family. Are these the result of separate mutational events or did a mutation take place in a somatic cell that gave rise to a cluster of microsporocytes all of which carried the mutation? In some families nearly one half of the plants segregated for a given mutant. Is this due to the mutational event occurring early in the development of the sporophyte so that the whole tassel carried the mutant allele or are these the result of a mutant contributed by the female parent of the outcross? No allele tests have been made between similar mutants in the same family.

If all the segregating ears are counted as resulting from independent mutational events, the mutation rate is 17.98% (299 ÷ 1663). However, if all mutants with the same phenotype from a given family are considered to be the result of a single mutation, the mutation rate is 3.55% (59 ÷ 1663). If those families in which nearly one half of the ears segregated for a mutant of a given phenotype are considered to be the result of a mutation in the female parent and thus are not counted in the totals, the two mutation rates indicated above become 7.52% (125 ÷ 1663) and 3.19% (53 ÷ 1663), respectively. The most conservative estimate of the mutation rate (3.19%) is nearly 15 times the corresponding rate observed for the control population (4 ÷ 1763 = .23%). This is certainly a minimal figure since it is only the rate of seedling color mutations. Seed and mature plant mutations have also been observed in these outcross families. Sixteen of the families listed in Table 1 were observed to be segregating for defective seeds, one family for shrunken seeds and one for brittle seeds. Families of ten seeds each from the self of four outcross ears were planted. Two families did not segregate for any mature plant traits but one family produced a tassel seed plant with thick tassel branches and a short brachytic-like plant and another family produced several plants with small crinkled leaves. Thus, if seed and mature plant mutations were included the mutation rate would be considerably higher than 3.19%.

Table 2

Spontaneous mutants observed in crosses not carrying y_9

Family	Total No. of Plants Selfed	Albino	Pale Yellow	Total No. of Mutants	Total No. of Diff. Mutants
68-7026+7027	87				
68-7028+7029	92				
68-7030+7031	91				
68-7037+7038 +7039+7040	131				
68-7041+7042	90				
68-7043+7044	91				
68-7053+7054 +7056 +7057+7058	198				
68-7090	48				
68-7091	44	2		2	1
68-7092	46				
68-7093+7094	94				
68-7095+7096	92				
68-7097+7098	88	1		1	1
68-7099+7100	67				
68-7127+7128	85				
68-7129+7130	85		4	4	1
68-7131+7132	85		1	1	1
68-7133+7134	78				
68-7229	44				
68-7230	42				
68-7231	39				
68-7232	46				
Total	1763	3	5	8	4

From Table 1 it can be seen that mutants occur in ears that are not segregating for $\underline{y}_9$ and those that segregate for this gene. Thus, the mutations are probably not confined to the chromosome 10 carrying $\underline{y}_9$ or the homologous ten. None of the mutants in Table 1 have been located to chromosome although a mutant found in a $\underline{y}_9$ line in 1963 turned out to be allelic to $\underline{w}_3$ on chromosome 2. This would suggest that the mutants are not necessarily confined to chromosome 10. If the mutator locus on chromosome ten affects any locus and if it functioned in the sporocyte before microsporogenesis, it would explain the presences of possibly more than one outcross ear segregating for the same mutation in an outcross family and the occurrence of the mutations on ears with and without $\underline{y}_9$.

Whether the mutator locus is the same as $\underline{y}_9$ or just closely linked to it cannot be determined from the present information. Most of the data considered in this report were obtained incidentally in experiments designed to give information on the location of $\underline{y}_9$. Experiments designed to clarify the apparent mutagenic nature of this stock are planned.

Donald S. Robertson

2. Linkage studies involving $\underline{o}_5$.

In 1967 (MGNL 41:94-95) I reported on a new opaque mutant $\underline{o}_5$. This mutant has opaque shrunken seeds that produce pale green seedlings which grow into mature plants a little less vigorous than normals. In our previous report, preliminary linkage tests with only 70 plants had indicated this mutant was on chromosome 7, 12.9 units from the breakpoint of 7-9a (7L.63). Additional linkage information involving 7-9a are given in Table 1 which are in close agreement with the previously reported data. The results of linkage tests with $\underline{gl}_1$ are given in Table 2.

Table 1

Linkage data from the testcross of 7-9a/o_5

Family No.	Genotype of F_1 Gametes				Total	%CO
	T +	+ o_5	T o_5	+ +		
67-5094	19	14	2	5	40	17.5
67-5095	7	17	2	7	33	27.3
67-5096	16	15	2	1	34	8.8
67-5097	17	17	3	3	40	15.0
67-5098	17	18	0	3	38	7.9
67-5099	17	18	0	3	38	7.9
67-5100	8	13	1	2	24	12.5
67-5101	17	15	0	1	33	3.0
Total	118	127	10	25	280	12.5

Table 2

Linkage data from the testcross of gl_1/o_5 plants

Family No.	Genotype of F_1 Gametes				Total	%CO
	gl +	+ o_5	gl o_5	+ +		
69-5141	27	14	0	1	42	2.4
69-5142	30	17	0	0	47	0
69-5143	25	19	0	0	44	0
69-5144	17	22	0	0	39	0
69-5145	17	24	0	0	41	0
69-5146	27	20	0	0	47	0
Total	143	116	0	1	260	0.4

These data indicate that o_5 is very close to gl_1. Crosses with TB-7b (7L.3) confirm this location, since this translocation which uncovers gl_1 also uncovers o_5.

Donald S. Robertson

ISTITUTO DI GENETICA VEGETALE
Universita Cattolica del S.Cuore
Piacenza, Italy

1. Opaque endosperm mutants in Italian varieties.

By self pollination of about 500 samples of Italian local populations, many phenotypically opaque endosperm mutants have been isolated.

Allelism tests have been performed by crossing the mutants to stocks bearing the $\underline{fl}_1$; $\underline{fl}_2$, $\underline{o}_1$, $\underline{o}_2$ factors in homozygous and/or heterozygous condition. Among the mutants, eight turned out to be allelic to the locus $\underline{o}_2$ on chromosome 7. No clear information has been obtained about the other mutants although they have been recognized as different from $\underline{o}_2$.

The $\underline{o}_2$ mutants were present in the varieties:

Comune di Vasto
Giallo bastardo di Stradalta di Basiliano
Giallo di Ferrandina
Giallo precoce di Valdarno
Locale di Sulmona
Nostrale di Montepulciano
Quarantino di Terracina
Quarantino di Vigo di Non

The opaque-2 isolated from the Giallo bastardo di Stradalta is the already known "Italian $\underline{o}_2$ source" detected and distributed by Bianchi and Coll. Among the examples turning out to be not allelic to $\underline{o}_2$, at least two cases appear different also from the tested factors $\underline{o}_1$, $\underline{fl}_1$, $\underline{fl}_2$.

C. Lorenzoni
M. Pozzi
F. Salamini

UNIVERSITY OF MARYLAND
College Park, Maryland
Agricultural Experiment Station
Department of Botany

1. Dual nucleoli at diakinesis.

A highly asynaptic plant occurred in a culture segregating for lower frequencies of asynapsis and normal. Failure of association was evident in all of the 113 cells scored at diakinesis. The number of disassociated pairs ranged from ten to two with an average of six per cell.

In contrast to the single nucleolus regularly present in normal material, 14 microsporocytes had two nucleoli at diakinesis. Chromosome 6 could be detected adjacent to each nucleolus in most of the cells. Although some of the cells had nucleoli of approximately equal size, one nucleolus was usually considerably larger than the other. Dual nucleoli are evident in somatic cells due to widespread separation of organizers resulting in failure of fusion. The dual nucleoli in the asynaptic microsporocytes presumably reflected greater than normal spatial separation of the homologues of chromosome 6 and organizers during nucleolar formation in the premeiotic division.

D. T. Morgan, Jr.

UNIVERSITY OF MASSACHUSETTS
Amherst, Massachusetts
Department of Plant and Soil Sciences

1. The culture of sweet corn inbreds in the greenhouse.

A greenhouse is of inestimable value to most plant breeding programs and is no exception to a corn breeding project. For years Dr. D. F. Jones (2) in Connecticut, Dr. F. D. Richey (4) at the U.S.D.A. and others involved with corn breeding projects have made considerable use of greenhouses for growing "off season" crops.

While it is not feasible to conduct yield trials or practice selection for specific characters when selfing in the greenhouse, plants grown under these conditions can be used for making limited quantities of seed of experimental hybrids or more especially as a time saver when

proceeding with backcross programs. This includes the backcross transfer of specific genotypes into male sterile cytoplasm as well as the development of appropriate restorers used in the cyto-sterile technique.

When working with early to mid-season maturities of sweet corn in northeastern U.S., it is possible to grow one generation out-of-doors in the summer followed by two successive generations in the greenhouse. The seed can be harvested and dried in time for the next out-of-door spring planting. Hence, with this schedule, 6 to 10 backcrosses can be completed in 1/3 to 1/2 the normal time required. It should be noted that in the autumn, crops are not usually as satisfactory as those grown in the spring; in general, the earlier the fall crop is planted, the better are the results. The spring crop is generally planted by January 15 and benefits from the lengthening days as it develops.

Years ago Dr. D. F. Jones (2) recommended using a fertile field soil as the proper media for growing corn in the greenhouse. This alone did not produce adequate growth or production among early sweet corn inbreds. Subsequently we used a fertile Scarborough very fine sandy loam supplemented with manure and 5-10-10 commercial fertilizer. On several occasions it was evident that we "burned" the plants and soluble salts had become a problem. More recently, we have used the following soil mixture with excellent results: 7 parts soil, 3 parts peatmoss and 2 parts sand; 60 grams of superphosphate and 60 grams of ground dolomitic limestone are well mixed with each bushel of the mixture.

For several years plants were grown directly in ground beds but in the greenhouse it affords more control to grow them in buckets. Heavy duty 10-quart pails are used and six 5/8 inch holes drilled in the bottoms to provide for drainage of excess water. The soil is compacted gently in the buckets to about two-thirds full and seeds are planted 3/4 to 1 inch deep. From 7 to 10 seeds are planted in each bucket; after germination, when the plants are about four inches tall they are thinned, leaving the two sturdiest ones to remain. The plants to be discarded are carefully cut off at the base to avoid disturbing the remaining ones. Since the young plants are often weak stemmed and their roots develop near the soil surface, soil is gradually added until it is within 2 inches of the top of the buckets.

It is imperative to keep the soil uniformly moist and not allowed to dry out. A soluble fertilizer is first applied when the plants are approximately 6 inches tall. This is comprised of 30 grams of soluble 20-20-20 in 8 quarts of water. One quart of this nutrient is applied every 10 days to each bucket. This has sustained a vigorous, healthy, sturdy type of growth. The fertilizer treatments are terminated shortly after the time of pollination.

It is advisable to give some support to the plants since the stalks are more succulent and somewhat weaker than when grown out-of-doors. Securing the plants with twine or "Twistems" to bamboo canes or a wire trellis is a satisfactory means of protection from damage by workmen in the greenhouse.

Supplementary illumination is necessary to produce normal, healthy growth. Extra light is provided from seedling emergence until pollination time. We have used 30 lamps in a 400 square foot area which accommodates 70 to 80 buckets (up to 160 plants) as a maximum. Ordinary 200 watt Mazda lamps are fitted with 16 inch diameter reflectors. The lamps are suspended from overhead pipes at a position of about 30 inches above the plants by means of the electric cords attached to and supported by a light guaged chain. A small hook attached to the top of the reflector allows the lamp to be raised or lowered by inserting the hook in the chain at the height desired. The lights are regulated by an automatic switch which is turned on about 3:30 P.M. and off at 10:30 P.M. It also seems beneficial to switch them on during the dark winter days when the sun is not shining.

Traditionally it has been said that corn grows best during hot weather. Livingston (3) showed that the rate of growth of corn increased with increasing temperature up to 89°F. We have obtained very fast growth in the greenhouse with the temperature held constantly at 80°F. Under this condition, however, the plants become tall and slender with weak stalks; ears were often barren of seed or at best the yields were disappointing. Some improvement was noted when the night temperature was lowered 10 to 15°. At the suggestion of Dr. R. G. Creech (1) we have grown many crops maintaining the temperature constantly at 68°F. with extraordinary success. At this temperature plant growth has

been excellent with very satisfactory ear production and seed yields. Although seed size, yield and quality are inferior to seed grown out-of-doors, its germination and vigor are sufficient to produce extra generations in a given time and this allows for speeding the program.

Literature Cited

1. Creech, R. G. 1963. Personal communication.

2. Jones, D. F. 1945. Personal communication.

3. Livingston, Burton Edward. 1916. Physiological temperature indices for study of plant growth in relation to climatic conditions. Physiological Researches 1:399-420.

4. Richey, Frederick D. 1927. Corn breeding. U.S.D.A. Dept. Bul. 1489:1-64.

W. H. Lachman

UNIVERSITY OF MASSACHUSETTS - BOSTON
Boston, Massachusetts
Department of Biology

1. Hybridization and parallel variation studies with the wild relatives of maize.

Genetic and cytogenetic studies of Tripsacum, teosinte, maize x teosinte hybrids, and maize from field collections made in Central Mexico (MGNL 44:188) are now under investigation. Previous studies have indicated that the maize grown in fields where teosinte was present as a weed often exhibited more variation than maize from fields some distance from a teosinte population; yet this variation was not as great as the segregation seen in experimentally produced maize x teosinte hybrids and subsequent backcross generations. A second observation, in fields where teosinte was present and did hybridize with maize, was the surprisingly low frequency of hybrids.

The frequency of maize x teosinte hybrids, which seems to be lower than what one might expect in certain fields, is under study to determine if there are in fact genetic isolating mechanisms operating between the two taxa. Previous studies have indicated the presence of the Ga_1 (Ch. 4) allele in teosinte and, since most maize is ga/ga, this gene might be operating as a barrier to gene exchange where teosinte is the maternal

parent. At present a survey of the Ga locus in all the teosinte races is underway.

The problem of detecting the effect of teosinte introgression in the maize of the region is also under study and several lines of investigation are being pursued. Seed ears and obviously introgressed ears from the study fields are being inbred to recover plants with more pronounced effects of teosinte introgression and homozygous chromosome segments from teosinte. Cytological studies of these plants have indicated a frequency of B chromosomes from 0-6. The number of B chromosomes appears to be higher (4^+) in those plants which exhibit pronounced introgressed morphology. A working hypothesis in these studies is that B chromosomes may act as sponges "absorbing or buffering" the extreme effects of homozygous teosinte introgression. Teosinte from these same fields exhibits 0-4 B chromosomes. Parallel with the investigation of the B chromosomes are cytological studies of the effects of homozygous knobs on the morphology of the inbred maize from these fields.

Further attempts to hybridize the clones of T. lanceolatum from the study area with both maize and teosinte are continuing.

H. Garrison Wilkes

UNIVERSITY OF MASSACHUSETTS
Waltham, Massachusetts
Department of Environmental Sciences

and

HARVARD UNIVERSITY
Cambridge, Massachusetts
Bussey Institution

1. On the longevity of corn seed.

A collection of the sweet corn, "Chuspillo," made by Fr. Steinbach, Cochabamba, Bolivia and placed in cold storage at the Harvard Biological Lab. by P. C. Mangelsdorf in 1941 was found to germinate 100% in May 1970, almost 30 years after its harvest. This is believed to be the oldest corn seed ever found to be viable and it is hoped that this note will help to inspire the continued maintenance of cold storage collections.

The Chuspillo seed was stored in a small screw cap bottle under constant refrigeration below 40°F. It is part of a collection of about 1900 items from Latin America collected chiefly in the period from 1940 to 1950 and stored at Harvard University until June 1968 and since then at the University of Massachusetts in Waltham.

W. C. Galinat

2. The morphological nature of "string-cob" corn.

In my previous study of the inheritance of the string cob trait in which the cob has about the same diameter as the central spike of the tassel, segregation in one background revealed control by two incompletely dominant genes (Mass. Agric. Exper. Sta. Bull. 577). Correlations of condensation in the tassel and kernel row number with cob diameter indicated that one of the dominant factors was the normal allele to a recessive gene for fasciation.

In the present studies, the effects of this dominant allele involved in the string cob trait have been identified as also including narrow cupules. In the recessive condition, wide cupules become associated with fasciation or sometimes a branching of the cob when the requirements for cupule space cramped by high row numbers (via condensation and ramosa) require an increase in surface area.

The second component of the string cob trait is now recognized as one for reduced pedicels (foot stalks). The pedicel is that portion of the spikelet axis beneath the glumes and the rachilla is above the glumes. In a thick cob, an elongate pedicel is embedded in the floor of the cupule where it is commonly identified in error as the spikelet trace.

In the tassel, only one member of the pair of spikelets is usually pedicellate. In the thick cob of Corn Belt corn usually both members of the pair are pedicellate although pedicels are fused into the cupule floor. All combinations of pedicellate and sessile spikelets in the ear have been identified in variant forms.

The reduction of pedicels appears to involve the interaction of two complementary genes, thus increasing to three the number of genes

involved in the string cob trait. When the area of pith is expanded, as in inbred C13, the inheritance is further complicated.

W. C. Galinat

3. Relationship between pedicel length and cob diameter and its inheritance.

The direct control of pedicel length over cob diameter has been proven beyond any doubt in these studies (Table 1). The pedicel has not been recognized previously as a morphological factor of importance in cob structure. Rather the length of rachilla or spikelet stalk above the glumes has been measured with little or no apparent significance such as in the series of monographs on the races of maize in Latin America. The pedicel or foot stalk below the glume has been overlooked because it was erroneously considered as a spikelet trace or a part of the vascular system of the cob rather than a stalk fused within the floor of the cupule, as is now apparent.

When cob diameter is plotted against pedicel length the shape of the curve is slightly sigmoid. At the low end of the curve, the slope is gradual because the pedicel length is small in relation to pith diameter. At the high end of the curve, the slope tapers off once again, as growth factors limit the pedicel's capacity for elongation.

The short pedicels of string-cob maize and presumably of teosinte as well are determined by the interaction of two incompletely dominant genes, as indicated by a highly significant fit of an F_2 segregation of string cob X Corn Belt dent to a 9:7 ratio (Table 2). The F_1 hybrid is intermediate in pedicel length between its parents. Recovery of the string cob phenotype depends upon the homozygous recessive condition at either one of two loci. There is a clear cut separation between the means by over three standard deviations (Table 2).

Table 1

Correlation of pedicel length to cob diameter in an F_2 population of cobs with a relatively constant pith diameter

Cob Diameter* (mm, .0 to .9)	n Diams.	Pedicel Length** (Mean)	Standard Deviation
10	4	1.15	.38
11	8	1.75	.35
12	24	1.62	.61
13	19	1.77	.42
14	31	1.60	.54
15	59	1.78	.60
16	68	1.95	.54
17	69	1.90	.53
18	85	2.10	.57
19	78	2.06	.56
20	79	2.33	.62
21	80	2.44	.60
22	79	2.79	.64
23	75	3.00	.80
24	80	3.32	.65
25	108	3.50	.56
26	103	3.60	.70
27	129	3.85	.75
28	99	4.30	.61
29	63	4.20	.58
30	54	4.17	.64
31	28	4.38	.69
32	13	4.09	.69
33	7	4.60	.31

*Measured to kernel attachment.

**Measured to divergence point of lower glume.

Correlation Coefficient (r) = .94 at 23 d.f., 5% level = .396 and 1% level = .505. Since .94 is greater the correlation is highly significant.

Table 2

Inheritance of pedicel length in an F_2 of string cob X Corn Belt dent.

Observed	Theoretical on a 9:7	Pedicel Length Mean (mm)	Standard Deviation
786	841	3.9	.6
710	654	2.0	.5

chi sq = 8.38

1 d.f. at 1% level = 6.64

Therefore the fit to a 9:7 ratio is highly significant.

W. C. Galinat
P. J. Eugene

4. On the possible assemblage of a synthetic teosinte.

The following mutant genes and variants involving the maize cob are being recombined to produce a synthetic teosinte.

1. pd: single female spikelets, a mutant discovered by Hepperly (1949) and others.
2. tr: two ranked (distichous); although most mutants are unstable, rare stable forms were discovered by Tavcar (1935), Lindstrom (in Burdick, 1951) and myself (unpub.).
3. Sg: string cob (reduced pedicels), extracted from Confite Morocho (Galinat, 1969).
4. is: cupulate interspace, extracted from Coroico (Galinat, unpub.).
5. chr 4 complex: inclination of spikelets toward cupule, induration of the outer glume and development of an abscission layer. The extraction of this complex from Maiz Amargo or Enano, both of South America, may be critical. However, its components may be discovered and isolated individually. For example, the sweet corn inbred W400 out of the variety Buttercup has weak abscission layers in the rachis.

Other teosinte-like mutations, tsb (teosinte branched), nl (narrow leaf) and id (short-day response), are not considered as essential in

synthesizing the species and, therefore, they may be omitted as unnecessarily complicating the breeding work.

If this experiment is successful in synthesizing a teosinte-like spike, it would demonstrate three important things: (1) That the above few essential features are sufficient for the transformation; (2) That the teosinte-like races of South America contain a fourth chromosome complex comparable to that extracted from teosinte; (3) That the reverse transformation from teosinte to maize is possible, if not probable. It would also illustrate the well-known plastic nature of maize which makes it improbable that wild maize would have become extinct in the first place.

W. C. Galinat

5. Relative dominance of single female spikelets.

Single female spikelets, whether derived from teosinte or by mutation within maize, have dominance relative to the maize background. It was first noted by Mangelsdorf (1947) that in a cross of Durango teosinte by Guarani maize, the spikelets are predominantly single, whereas in crosses of the same teosinte by North American maize, they are predominantly paired. More recently similar results have been obtained independently by myself and Beadle. In my own material, single spikelets from northern teosinte were dominant in crosses with Confite Morocho, a primitive popcorn from Peru. Beadle (unpub.) has found dominance of single spikelets from Nobogame teosinte in a cross of Rhee Flint from North Dakota.

In my own crosses with the mutation to single spikelets, discovered by Hepperly (1949), dominance of expression occurred in combination with Wilbur's flint and a sugary string cob inbred, but not with inbred A158.

There is a good possibility that there was a reversal of dominance of the teosinte traits, at least for single versus paired spikelets, in Central America where repeated natural outcrossing to teosinte may have led to selection of a genetic background buffered against the heterozygous expression of the teosinte traits.

W. C. Galinat

6. The lack of a teosinte-like fourth chromosome complex in tripsacum.

A segment on chromosome 4, estimated to include the whole short arm, as marked by Su_1 locus, is essential to the development of the female spike of teosinte (Mangelsdorf and Reeves, 1949; Rogers, 1950). It controls an inclination of the spikelets toward the cupule, an induration of the outer glume and the development of a rachis abscission layer.

The homozygous addition stock for the Su marked chromosome (Td7) derived from Tripsacum dactyloides has none of the above-mentioned effects of teosinte's fourth chromosome nor did the hybrid of this 20+2 stock with the TB4b tester involving the Su_1 region reveal any of these teosinte characters in a population of 36 plants.

Furthermore the Td7 chromosome marked by Su_1 does not carry 5 other loci (la, gl_3 bm_3, ra_3, j_2) borne on maize chromosome 4. Another tripsacum chromosome (Tf13) derived from T. floridanum, a species closely related to T. dactyloides, is marked by the Gl_3 locus, but does not carry any of the other fourth chromosome loci of maize. Various other unidentified tripsacum chromosomes carry dominant alleles to the la, bm_3, ra_3, and j_2 markers on the fourth chromosome of maize.

On the basis of the above facts, the hypothesis that Tripsacum dactyloides contributed this fourth chromosome segment during a creation of teosinte from maize would seem to be invalid.

In view of the 9 chromosome base for the more distant relatives of maize, i.e. Manisuris and Tripsacum, it seems possible that Zea (teosinte and maize) is 9+1 and that the fourth chromosome could represent the extra pair which was assembled during a divergence from 9 chromosome Andropogonoid stock. It is possible that another species of tripsacum carries a linkage group more similar to Zea 4 from which such an addition chromosome was derived. We have already experimentally added homozygous chromosome pairs from tripsacum to maize in true breeding stocks (20+2). One of these (Td7) carries the Su_1 locus near the centromere as on Zea 4.

W. C. Galinat

7. High trivalent formation in maize-tripsacum-teosinte heterozygotes for the Su_1 chromosome.

Two maize-tripsacum-teosinte heterozygotes [M4, Tr7 and t4] for the Su_1 marked chromosomes of these three relatives were produced by hybridizing a homozygous addition [20+2] stock for Tr7 with teosinte derivatives of A158 in which teosinte chromosome 4 from Florida teosinte and Nobogame teosinte was substituted for the corresponding maize chromosome.

The stock involving Florida teosinte chromosome 4 was studied at pachytene. The tripsacum chromosome [Tr7] was found as a univalent folding back on itself. It measured on an average of 23.7 μ which compares well with the data given by Galinat et al. [MNL: 44] for this chromosome in 2n+2 condition. This chromosome showed a feeble association at pachytene with the maize-teosinte bivalent in 6 out of 17 observations. The association involved the terminal region of the Tr7 chromosome with the maize-teosinte bivalent away from the proximal region where their common Su locus is situated [Galinat et al., MNL: 43]. Three different plants [70-375-10; 70-376-5; 70-377-1] provided the material for scoring the trivalent frequency at diakinesis and metaphase I. In all three cases a high percentage of trivalent formation was observed [Table]. The second maize-tripsacum-teosinte stock is identical with the first except that the teosinte chromosome 4 is derived from Nobogame teosinte. Here also, a high trivalent frequency was observed at diakinesis and metaphase I [Table]. In this heterozygote a bridge and a fragment was observed at anaphase I. Although an isogenic control produced by crossing the homozygous addition stock [20+2] for Tr7 by normal A158 has been produced, it has not as yet been grown for cytological comparison. Data somewhat comparable are available, however, for the original 20+1 and 20+2 Tr7 addition stocks not carrying a teosinte chromosome 4. In this background homozygous for maize chromosome 4, the frequency of trivalents and/or quadrivalents observed in either the 20+1 or 20+2 Tr7 addition stocks was only about 6 percent. The increase in trivalent frequency in the maize-tripsacum-teosinte heterozygote is probably related in some way to the presence of all three of these chromosomes from the three species within the same nucleus. The final determination awaits a study of the isogenic control.

Table showing the trivalent frequencies observed at diakinesis and metaphase I in maize-tripsacum-teosinte heterozygotes

Stock No.	Diakinesis			Metaphase I		
	Total No. Cells Observed	No. of Cells with $9_{[11]}+1_{[111]}$	Percentage	Total No. Cells Observed	No. of Cells with $9_{[11]}+1_{[111]}$	Percentage
Florida teosinte						
70-375-10	50	39	78	50	31	62
70-376-5	35	25	71	32	27	84
70-377-1	54	35	65	53	32	60
Pooled Data	139	99	71	135	90	73
Nobogame teosinte						
70-382-4	45	33	73	27	19	70

Further studies on this are also in progress to determine at pachytene if the partial homology from tripsacum [Tr7] has a greater affinity to teosinte chromosome 4 rather than to chromosome 4 or others of maize or if it is the mere presence of heterozygosity for maize and teosinte 4 which enhances the crossover potential for the tripsacum homeolog with one or the other of them.

W. C. Galinat
R. V. Tantravahi
P. Chandravadana
B. G. S. Rao*

*Present address: Andhra University, Waltair, India.

8. Comparative studies of American Maydeae and the Andropogoneae: II. Morphology of pachytene chromosomes of two collections of Elyonurus tripsacoides from Texas and Veracruz.

The morphology of the pachytene chromosomes of Elyonurus tripsacoides from Veracruz, Mexico, has been previously reported [MNL 1970, item 19]. The present study consists of observations at meiosis of Elyonurus tripsacoides from Texas. The two collections have different plant habits. Hence a detailed study of their chromosome morphology may have evolutionary significance.

Extensive studies of chromosomes at the pachytene stage of meiosis in the pollen mother cells have been made. Considerable difficulty was encountered in the identification of the chromosomes because of the poor spread in the material, as a result of which not even a single cell showed clearly all the 10 chromosomes. However, data from the individual chromosomes at pachytene from 300 observations have been analyzed and the ten chromosomes identified [Table 1].

Meiosis is regular. At pachytene the twenty chromosomes form ten bivalents. The individual chromosomes are distinguished by their relative lengths and arm ratios. There are no distinct features as knobs or chromomeres to demarcate one from the other. The adjacent regions of the centromere are more darkly stained, but this is not consistent hence cannot be taken as a criteria for distinguishing one from the other. The nucleolus organizing body is terminal with the satellited portion at the distal end and is on the short arm.

Table 1

Pachytene chromosome morphology of Elyonurus tripsacoides from Texas

Chromosome No.	Length in Microns			Arm Ratio
	Short Arm	Long Arm	Total	
1	22.7	31.0	53.7	1.5
2	11.5	32.1	45.4	3.1
3	17.3	24.1	42.8	1.4
4	10.1	22.7	34.6	2.3
5	13.3	19.5	34.6	1.5
6	13.7	18.7	33.8	1.4
7	12.2	14.8	28.4	1.2
8	9.0	16.6	27.4	1.8
9	10.1	11.9	23.4	1.2
10*	5.8	11.9	19.4	2.1

Arm Ratio - Length of Long Arm/Short Arm

*Nucleolus Organizing chromosome.

In their idiograms, the two collections of Elyonurus tripsacoides from Veracruz and Texas show similarities in the total lengths of the ten bivalents at pachytene. They are also similar in their arm ratios excepting chromosomes 2, 4, 6 and 10 [Table 2]. The nucleolus organizing chromosome is assigned the ninth position in the Veracruz material and the tenth position in the Texas material by virtue of the difference in their total lengths. The organizing body itself is terminal in both the materials and is situated in the long arm of the former and the short arm of the latter.

The chromosomes of Elyonurus tripsacoides, when compared with those of corn, are found to be shorter, but show similarities in their arm ratios with the exception of chromosomes 7 and 8. Elyonurus tripsacoides [Texas] resembles corn in having uniformly stained chromosomes, while the material from Veracruz is more similar to Tripsacum dactyloides in having differentiated chromosomes. The nucleolus organizing chromosome of the Texas material corresponds to the nucleolus organizing chromosome of corn in having the nucleolus organizing body

Table 2

Comparative pachytene chromosome morphology of maize, Elyonurus and Tripsacum

Ch. No.	Maize [Rhoades, 1955]		Elyonurus tripsacoides				Tripsacum dactyloides	
			Veracruz		Texas			
	Length μ	Arm Ratio	Length μ	Arm Ratio	Length μ	Arm Ratio	Length μ	Arm Ratio
1	82.4	1.3:1	53.5	1.1:1	53.7	1.5:1	48.6	1.7:1
2	66.5	1.2:1	44.6	1.4:1	45.4	3.1:1	45.0	6.0:1
3	62.0	2.0:1	40.0	1.8:1	42.8	1.4:1	40.3	3.1:1
4	58.8	1.6:1	35.8	1.5:1	34.6	2.3:1	35.3	2.1:1
5	59.8	1.1:1	30.9	1.4:1	34.6	1.5:1	32.4	4.6:1
6	48.7	7.1:1*	29.5	6.2:1	33.8	1.4:1	27.0	6.0:1
7	46.7	2.8:1	27.5	1.1:1	28.4	1.2:1	27.0	3.2:1
8	47.5	3.2:1	26.3	1.6:1	27.4	1.8:1	27.0	1.8:1
9	43.2	1.8:1	26.3	1.3:1	23.4	1.2:1	25.9	1.7:1
10	36.9	2.8:1	20.6	1.2:1	19.4	2.1:1	25.2	3.7:1
							23.4	2.7:1
							22.3	4.7:1
							21.6	3.5:1
							20.9	4.3:1
							20.9	2.1:1
							19.8	2.0:1
							18.0	1.9:1
							16.2	3.0:1

*The value of 7.1 for chromosome 6 of maize given in my article in Corn and Corn Improvement is incorrect. The original value reported by Longley is 3.1. M. M. Rhoades

situated in the short arm. The ten chromosomes of Elyonurus tripsacoides from both the collections are found to be more similar to the first ten out of 18 chromosomes of Tripsacum dactyloides in their relative lengths than they are to those of maize.

P. Chandravadana
W. C. Galinat

UNIVERSITA DI MILANO
Milan, Italy
Istituto Di Genetica

1. Mutational analysis of R^{ch}.

The R stocks and the treatments employed. One of the various expressions of the R phenotype, such as determined by R^{ch}, is the production of anthocyanin in the aleurone, silks, pericarp, and other plant tissues.

The experimental evidence so far obtained suggests that R^{ch} is a complex separable into four components, symbolized P, S, Si, and Ch, controlling pigment synthesis in the plant, seed, silk, and pericarp tissues respectively. This evidence was provided by analysis (Sastry, 1970) of a series of mutants with colorless endosperm that appeared on ears of testcrosses involving R^{ch}/R^{st} plants. Three independent collections of R^{ch} were employed in this study. Two of them presumably carried a heterochromatic knob (K10) situated distally on chromosome 10, because they segregated preferentially for R. The majority of these mutants had lost pigment both in aleurone and in the sporophytic tissues. Because of their heterozygous R^{ch}/R^{st} parentage, either one of their parents is accountable for their origin. However, since 21 out of 25 carried the distal marker K10 it is likely that they were R^{ch} mutants. A minority of mutants had lost the aleurone pigmenting capacity but retained some of the R^{ch} features thus suggesting that R^{ch} is a complex of genes.

To find further information on this small chromosomal region, we designed additional tests. If R^{ch} is separable into various components, it should be possible to induce deletions involving two or more genes of the complex, and from a study of their pattern of loss, information

should be obtained on the number of the R components and on their sequence.

Accordingly R^{ch}/R^{ch} plants were treated with the alkylating agent EMS, effective in inducing mutation and chromosome breakage (Malling et al., 1968), and the treated individuals were crossed reciprocally with a g R^{st} / g R^{st} tester. The stem of plants of about the same developmental stage was injected with a freshly prepared buffered solution of this agent (2.37 or 4.75 x 10^{-3}M).

Cytological analysis of the male inflorescence indicated that the majority of the germ cells reached the stage of microspores while a minority was still in meiosis at the time of the treatment.

The R^{ch} form used in this study originally was obtained from the collection of Stadler, while the g R^{st} tester (without the stippled modifier M^{st}) came from Dr. Brink's laboratory. A series of spontaneous mutants from standard R^{r} were also isolated and analyzed in order to compare the mutational spectrum of the two R forms. To test whether the EMS solution injected into the stem is taken up by the plant tissues, M-2 seeds, obtained from crosses of treated $R^{ch}R^{ch}$ plants with the g R^{st}/ g R^{st} tester, were germinated in sandbenches and studied, using appropriate controls.

A significant proportion of seedlings with abnormal growth was found only in the treated population (Table 1). These seedlings, when transferred to soil, died at the stage of 2-4 leaves. A similar phenotype associated with lethality is expected as a result of chromosomal deletions or other aberrations leading to an impairment in plant metabolism. These results suggest that EMS was incorporated into the nuclei of the germ line.

Results with R^{ch}. The exceptional kernels isolated as presumed mutants from the reciprocal crosses of R^{ch}/R^{ch} plants with the g R^{st} / g R^{st} tester have either colorless or pale aleurone (Table 2). Either one of the R parents could contribute to their production.

However, their origin from the R^{st} parent can be excluded since overlying both phenotypes is a fine stippling pattern determined by R^{st}. The EMS treatment did not alter their frequency significantly.

Table 1

Frequency of lethal seedlings with an abnormal morphology obtained from the reciprocal crosses of $\underline{G}\ \underline{R}^{ch}$ / $\underline{G}\ \underline{R}^{ch}$ plants with a $\underline{g}\ \underline{R}^{st}$ / $\underline{g}\ \underline{R}^{st}$ tester

$\underline{R}^{ch}$ contributed by	Treatment	No. seedlings scored	Abnormal seedlings	Fr x 10^{-3}
Pistillate parent	Nil(1)	4,000	0	0.00
"	EMS-1(2)	4,000	12	3.00
"	EMS-2(2)	4,000	30	7.50
Staminate parent	Nil(1)	4,000	0	0.00
"	EMS-1(2)	3,500	46	13.14
"	EMS-2(2)	4,000	40	10.00

(1) 2.37×10^{-3}M; (2) 4.75×10^{-3}M

Table 2

Number and phenotype of exceptional kernels observed on ears obtained by the reciprocal crosses of a $\underline{GR}^{ch}$ / $\underline{GR}^{ch}$ $\underline{WxWx}$ line with a $\underline{g}\ \underline{R}^{st}$ / $\underline{g}\ \underline{R}^{st}$ $\underline{wx}$ / $\underline{wx}$ tester

$\underline{R}^{ch}$ contributed by	Treatment	Number of kernels	No. and phenotype of exceptions	
			Light st.	Light st. on pale backgr.
Pistillate parent	Nil	6,000	6	1
"	EMS	22,310	22	3
Staminate parent	Nil	5,250	8	0
"	EMS	8,830	9	4
Total		42,390	45	8

Almost all (16/17) the exceptional kernels isolated from ears that derived $\underline{R}^{ch}$ from the pistillate parent were germinally transmissible while about one half (6/13) of those obtained from the reciprocal cross failed to repeat that altered phenotype in the offspring (Table 3). Upon selfing they segregated about 3/4 stippled and about 1/4 homozygous colorless or pale mutants. Since they all segregated for wx and g, we ruled out contamination with alien pollen as their possible origin. The mutant progeny has been grown for a further generation to analyze pigment distribution in the sporophytic tissues, namely in the roots, coleoptile, primary internodes, anthers, silks, and pericarp by removing the husks and exposing the ears to the sunlight from 5 to 15 days after pollination.

Table 3

Germinal transmission of the characters of the exceptional kernels

$\underline{R}^{ch}$ contributed by	Treatment	Exceptional kernels			Fr. x 10^{-3}
		Tested	Mutant	Non-mut	
A. Colorless Aleurone					
Pistillate parent	Nil	3	3	0	1.0
" "	EMS	11	11	0	.98
Staminate parent	Nil	3	2	1	1.01
" "	EMS	6	3	3	.50
B. Pale Aleurone					
Pistillate parent	Nil	1	1	0	.16
" "	EMS	2	1	1	.06
Staminate parent	Nil	0	0	0	.00
" "	EMS	4	2	2	.22

From each original mutant 5 to 10 plants were studied. Surprisingly, none of the mutants tested (23) produced visible amounts of pigment in the sporophytic tissues. The loss of aleurone pigmentation is consistently associated with loss of pigment in all the tissues of the

plant. These results are unexpected on the basis of observations of $\underline{R}^r$ mutants.

Results with $\underline{R}^r$. Homozygous $\underline{R}^r$ plants were pollinated with a $\underline{r}^g$ tester carrying wx as pollen marker. Out of 24,000 kernels produced, 24 were classified as colorless seed mutants and all the 18 progeny tested transmitted the trait germinally. All of them produced, however, pigment in the roots and in the anthers.

These results are consistent with the model first proposed by Stadler (1951), envisaging R as a complex of two genes, P and S, controlling pigment synthesis in the plant and seed tissues, respectively. The results of the mutation experiments with $\underline{R}^{ch}$ do not support such a simple model. Though the possibility of exploring the structure of the locus by means of small induced R deletions did not seem practical, as we first hoped, yet our study allows us to propose the following working hypotheses concerning the organization of the R region:

1. $\underline{R}^{ch}$ is a single gene. Its mutation leads to loss of pigment production in the whole plant. Accordingly, pale mutants could be either the result of the mutation of a closely linked color modifier or they are leaky mutants.
2. $\underline{R}^{ch}$ is a gene complex:
   a. Individuals with colorless or pale aleurone result from mutation of a regulatory component of the R complex that affects the expression of the other genes of the complex.
   b. The R complex carries duplicate segments, delimiting the R region, that favour pairing of the two homologues at meiosis. Being duplicate segments they can either pair equally or unequally (see fig. a and b).

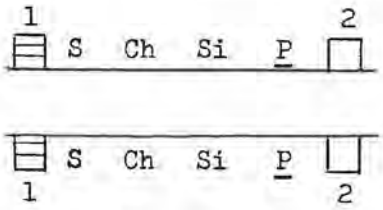


fig. a

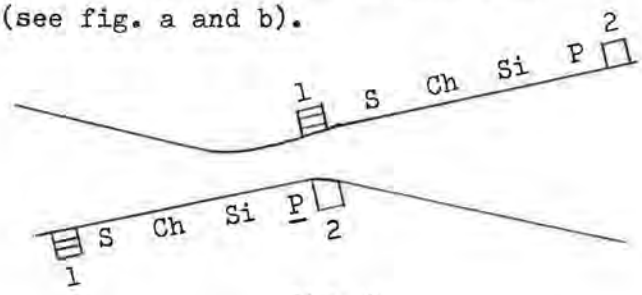


fig. b

In the first case, crossing over has no genetically detectable effect (fig. a); in the second case crossing over is limited to a region of effective pairing (fig. b). Any exchange occurring in this region gives rise to a crossover strand missing the entire R locus and to another carrying it in duplicate. Assumption (b) does not account for the pale mutants. Rather, their low frequency of production suggests that they are associated with a mutational event of a regulatory component (see 2a). Even though the data do not allow us to make a definite choice among these plausible assumptions, the working hypotheses proposed are easily amenable to experimental tests that should resolve the problems of the structural organization of R^{ch}.

Chiara Proi
G. Gavazzi

2. Effects of the "opaque-2" gene in maize: quantitative variations.

The quality of protein in the corn kernel can be genetically modified by the action of the gene opaque-2 (o_2) when homozygous. The main effect of this gene is an increase of lysine and tryptophan content (Mertz, Bates and Nelson, 1964).

In addition to this variation, some negative effects are correlated with the same genotype; the most important characters involved are kernel weight and kernel texture (Alexander et al. 1969; Salamini et al. 1969).

Some experimental results obtained during the last few years have shown that most of the effects of the o_2 gene can vary in intensity in relation to the genetical background. Therefore, it has been suggested that it should be possible to select genetical backgrounds in which the negative effects of o_2 would be minimized. Following the same reasoning, it should also be possible to select for high Tryptophan/Protein and Lysine/Protein ratios. The experiment described in this report was designed to evaluate all these possibilities, studying the genetical variability of those characters in a segregating population to which the selection could be applied.

An F_2 population segregating for o_2 was the material used for this research. The parent o_2/o_2 was an inbred line selected by Prof.

Bianchi from an Italian population and as the $+/+$ parent the line W 64 A was used. Plants from phenotypically normal F_2 kernels ($+/+$ and $+/o_2$) were crossed according to the design N.C.M.1 in order to produce progeny families from heterozygote parents ($o_2/+$). On the basis of the progeny test, all the families in which one parent was homozygous $+/+$ were discarded. After this operation, 45 full-sib families from 15 pollen parents, each mated to three seed producing plants, were left. This material was sown in an isolated field in three randomized blocks, each one including 15 full-sib families from a set of five pollen parents. Each family was replicated twice within the block. Each plot consisted of three 10-plant rows: the central one from the opaque-2 progeny (o_2/o_2) and the two lateral ones from the phenotypically normal progeny ($o_2/+$ and $+/+$). Plants of the lateral rows of each plot were detasseled and, in order to insure a sufficient amount of pollen, the field was bordered with o_2/o_2 plants of the same population. In order to obtain information about the variations of the differences between opaque-2 kernels and their normal counterparts in individual plants, observations were made on only the segregating ears of the plants in the lateral rows. On the average, 8 plants per family (four plants per plot) were considered; the family size was less than 8 only in few cases.

Opaque-2 and normal kernels from these segregating ears were separated and 40 kernels of each were weighed. Six samples of both phenotypical classes taken from six different plants of each family, making a total of 270 individuals, were analyzed for protein, tryptophan and oil content.

Total nitrogen content of each sample was determined by the "Technicon Autoanalyzer" following the method of Andress-Ferrari. The results were multiplied by 6.25 to obtain protein percent. Tryptophan content was assessed adopting the method suggested by Hernandez and Bates and using the "Technicon Autoanalyzer" for the colorimetric reading. A Soxhlet apparatus with hexane extraction was used for the oil content analysis.

The results obtained expressed as mean values for each class are given in Table 1. Clearly the weight is reduced in opaque-2 kernels. However, the degree of this effect varies according to the family

Table 1

Mean values and differences of normal and opaque-2 kernels from segregating ears

Characters		Normal ($++o_2$)	Opaque ($o_2o_2o_2$)	Differences: ($o_2o_2o_2$)-($++o_2$)	
				absolute	% of normal
40 - Kernel wt., g.	mean	9.75	8.51	-1.24**	-12.67
	range	9.15 - 10.74	7.94 - 9.08	0.92 - 1.56	-10.22 - -15.97
Protein g/100	mean	13.22	12.50	-0.72**	-5.43
	range	12.66 - 13.74	12.08 - 12.88	-0.55 - -1.01	-4.11 - 7.34
Tryptophan g/100	mean	0.099	0.166	0.067**	66.70
	range	0.089 - 0.114	0.141 - 0.183	0.051 - 0.078	56.49 - 79.23
Tryptophan % of protein	mean	0.75	1.33	0.58**	76.28
	range	0.70 - 0.87	1.17 - 1.48	0.51 - 0.67	64.68 - 90.74
Oil g/100	mean	5.57	5.83	0.26**	4.74
	range	4.91 - 6.42	5.25 - 6.65	0.09 - 0.47	1.73 - 8.72

** : indicates significant differences ($P<0.01$)

Ranges are evaluated considering half-sib family means.

considered; the range of the variation between full-sib families is wider than that shown in Table 1 and in only a few plants is the weight of opaque kernels the same as that of their normal counterparts.

The genetical control of this variation was studied by means of variance analysis of the absolute differences taking into account the family (full-sib and half-sib) classifications of the plants. As the correlation between these differences and the weight of normal kernels showed a high degree of association (r estimated within half-sib families varied around 0.75), this variation might be the result of the scaling effect. Therefore, an unbiased estimate of the genetical variance of this character can be obtained either by changing the scale or by reducing the variances of the part associated with the weight of normal kernels. Adopting this second procedure it was possible to show that there exist genes which modify the phenotypical expression of opaque-2 with mainly additive-type effects.

Protein percent in the normal and opaque phenotypes was 13.22 and 12.50, a significant difference. This difference is not very great and some plants were found in which the difference was reversed. The variability of this reduction was not correlated with protein content of normal kernels and resulted mainly from genetical effects of the non-additive type.

As expected tryptophan content of opaque-2 kernels was higher than that of their normal counterparts. Of great interest for our study was the evaluation of this character as the Tryptophan/Protein ratio. From the variability of this ratio it is possible to obtain information on the changes of the biochemical expression of opaque-2 and to determine whether this character can be genetically modified.

The results of the analysis of variance showed that the differences observed should be easily fixed in inbred lines. It is important to point out that the variation of this effect was not correlated with the value of the Tryptophan/Protein Ratio in phenotypically normal kernels.

Oil percent in normal and opaque kernels was 5.56 and 5.83 and the difference between these values is significant. This difference

can easily be explained by the increase of the germ/total weight ratio of the opaque kernels. The analysis of the data did not show that this effect was conditioned by the genetical background.

I. Cabulea
E. Ottaviano

3. Non-random transmission of chromosomes in trisomic plants.

Trisomy is an abnormal situation which would be easily eliminated in nature unless it is selected for. One of the homologous chromosomes may be lost as a univalent during meiotic divisions. Moreover, spores with eleven chromosomes sometimes abort and, in the male gametophyte, they suffer a severe competition with the normal ones. Nevertheless, we would expect these events to involve each of the three chromosomes at random unless the chromosomes differ from each other with respect to pairing, disjunction or otherwise.

Trisomic plants whose three homologous chromosomes are marked by three different alleles of the same locus offer a convenient genetic material whereby the transmission of individual chromosomes or chromosome regions can be followed. This is the case in trisomic 10 plants with the markers $\underline{R}^{st}$, $\underline{R}^{nj}$ and $\underline{r}$. Some of these plants were pollinated by an $\underline{r}$ tester and their progenies examined. Four phenotypical classes are expected ($\underline{R}^{nj}\underline{R}^{st}$, $\underline{R}^{st}$, $\underline{R}^{nj}$ and $\underline{r}$) but their relative frequencies are not readily predictable since segregation of three homologous chromosomes, as well as of the $\underline{R}$ alleles, is affected by a number of factors such as: pairing configurations, frequency and location of crossing over with respect to the centromeres, frequency of crossing over between the markers and the centromere, and frequency of loss of a chromosome as a univalent with different phenotypic consequences depending on whether a dominant or a recessive marker is lost.

However, in spite of any complication, two phenotypical classes ($\underline{R}^{st}$ and $\underline{R}^{nj}$) are expected to appear with the same frequency if the three chromosomes do not differ in any regard.

Thirteen ears were obtained from the cross: $\underline{R}^{nj}\underline{R}^{st}\underline{r}$ x $\underline{rr}$ and, after classifying their progeny, they were ordered according to the departure of the $\underline{R}^{st}$ and $\underline{R}^{nj}$ classes from the expected 1 : 1 ratio (Table 1). The series starts with a significant deficiency of $\underline{R}^{nj}$ (ear

no. 1). The following ear (no. 2) has a nonsignificant deficiency of $\underline{R}^{nj}$. Ears no. 3 and no. 4 have a nonsignificant deficiency of $\underline{R}^{st}$, and the following numbers through no. 13 have an increasingly marked deficiency of $\underline{R}^{st}$, which is significant at the .05 level in three cases and at the 0.01 level in six cases. In all significant cases, we can immediately attribute the observed departure from the 1 : 1 ratio to a loss of one of the two phenotypes rather than to an excess of the counterpart, which appears in a constant ratio to the $\underline{r}$ class; moreover, the $\underline{R}^{st}\underline{R}^{nj}$ class appears with a relatively lower frequency every time a departure from the 1 : 1 ratio is observed between the $\underline{R}^{st}$ and the $\underline{R}^{nj}$ classes.

Table 1

Progeny of the cross: $\underline{R}^{nj}\underline{R}^{st}\underline{r}$ x $\underline{rr}$, ordered according to the departure from the 1 : 1 ratio of the phenotypical classes $\underline{R}^{st}$ and $\underline{R}^{nj}$

Ear no.	$\underline{R}^{nj}\underline{R}^{st}$	$\underline{R}^{st}$	$\underline{R}^{nj}$	$\underline{r}$	χ^2 ($\underline{R}^{nj}$:$\underline{R}^{st}$)	P
1	26	39	18	34	7.7	<0.01 **
2	22	55	41	37	2.4	0.10 - 0.20
3	36	30	38	30	0.9	0.30 - 0.40
4	34	52	64	60	1.2	0.20 - 0.30
5	75	97	127	100	4.0	<0.05 *
6	15	44	69	58	5.5	<0.05 *
7	22	45	66	45	4.0	<0.05 *
8	5	6	41	28	10.9	<0.01 **
9	13	32	84	52	23.5	<0.01 **
10	31	41	117	103	36.5	<0.01 **
11	16	31	116	100	49.1	<0.01 **
12	35	20	106	72	58.7	<0.01 **
13	15	23	162	178	104.4	<0.01 **

Some of the $\underline{R}^{st}\underline{R}^{nj}\underline{r}$ putative trisomics obtained from $\underline{R}^{st}$ deficient ears (no. 8, 10, 11 and 12) were crossed to an $\underline{r}$ tester. A total of 28 such plants were crossed as female parents and gave the results reported in Table 2. The (A) series includes 21 plants showing a rather constant

Table 2

Progeny of the cross: $\underline{R}^{nj}\underline{R}^{st}\underline{r}$ x $\underline{rr}$; these trisomics were obtained from ears with a deficiency of the $\underline{R}^{st}$ phenotype, listed in Table 1

Ear no.		$\underline{R}^{nj}\underline{R}^{st}$	$\underline{R}^{st}$	$\underline{R}^{nj}$	$\underline{r}$	χ^2 ($\underline{R}^{nj}$:$\underline{R}^{st}$)	P
A	1	39	42	85	62	14.5	< 0.01 **
	2	11	14	25	19	2.8	0.05 - 0.10
	3	23	19	50	52	13.9	< 0.01 **
	4	9	9	32	31	7.3	< 0.01 **
	5	23	25	69	85	20.5	< 0.01 **
	6	39	29	113	96	88.4	< 0.01 **
	7	33	37	107	103	34.0	< 0.01 **
	8	36	39	146	87	61.9	< 0.01 **
	9	12	28	72	55	19.4	< 0.01 **
	10	45	50	154	145	53.0	< 0.01 **
	11	39	38	118	88	41.0	< 0.01 **
	12	23	20	58	56	18.5	< 0.01 **
	13	26	52	76	68	2.7	0.05 - 0.10
	14	24	32	127	104	56.8	< 0.01 **
	15	31	43	127	117	41.5	< 0.01 **
	16	24	31	120	94	52.4	< 0.01 **
	17	34	40	147	118	61.3	< 0.01 **
	18	31	30	122	82	55.7	< 0.01 **
	19	11	20	53	44	14.9	< 0.01 **
	20	49	55	139	148	36.3	< 0.01 **
	21	53	44	140	124	44.6	< 0.01 **
B	1	4	20	14	136	1.0	0.30 - 0.40
	2	93	94	150	60	12.8	< 0.01 **
						χ^2 ($\underline{R}^{nj}$:$\underline{r}$)	
C	1	0	0	233	246	0.01	0.80 - 0.90
	2	0	0	49	39	1.1	0.20 - 0.30
	3	0	0	184	191	0.14	0.70 - 0.80
						χ^2 ($\underline{R}^{st}$:$\underline{r}$)	
	4	0	70	0	218	117.7	< 0.01 **
	5	0	37	0	101	29.5	< 0.01 **

ratio of the four phenotypical classes and a consistent deficiency of the $\underline{R}^{st}$ class. The (B) series consists of two plants showing defective transmission of two markers ($\underline{R}^{st}$ and $\underline{R}^{nj}$ in one plant, $\underline{R}^{st}$ and $\underline{r}$ in the other). The (C) series includes five plants showing complete loss of either $\underline{R}^{nj}$ or $\underline{R}^{st}$. It is interesting to note that when $\underline{R}^{st}$ is lost, $\underline{R}^{nj}$ and $\underline{r}$ maintain a near 1 : 1 ratio, while when $\underline{R}^{nj}$ is lost, $\underline{R}^{st}$ is deficient compared to $\underline{r}$. Plants belonging to this series may be the result of heterofertilization, somatic loss of a chromosome or somatic breakage of a chromosome proximally to the marker lost. The different markers lost may be crossovers, but no reciprocals (normal $\underline{R}^{st}$) appeared so far.

Reciprocal crosses were also made with a few plants. Six plants of the same families were crossed as the male parents to an $\underline{r}$ tester. One of them was included in the (A) series and the other five are presumably of the same type and are referred as series (A') in Table 3. The deficiency of $\underline{R}^{st}$ is much greater than in reciprocal crosses. The $\underline{R}^{nj}$ class is reduced as compared to the $\underline{r}$ class. The $\underline{R}^{nj}\underline{R}^{st}$ shows an even greater reduction than in reciprocal crosses. These findings are explained in terms of the detrimental effect of hyperploidy on the male gametophyte.

Table 3

Reciprocal crosses involving trisomic plants as the male parent: $\underline{rr} \times \underline{R}^{nj}\underline{R}^{st}\underline{r}$

Ear no.		$\underline{R}^{nj}\underline{R}^{st}$	$\underline{R}^{st}$	$\underline{R}^{nj}$	$\underline{r}$	χ^2 ($\underline{R}^{nj}$:$\underline{R}^{st}$)	P	
A'	1	5	17	83	97	43.6	< 0.01	**
	2	14	33	265	294	180.6	< 0.01	**
	3	10	13	154	151	119.0	< 0.01	**
	4	24	43	238	286	135.3	< 0.01	**
	5	1	13	160	174	124.9	< 0.01	**
	6	15	18	122	119	77.3	< 0.01	**
	7	4	22	173	184	116.9	< 0.01	**
						χ^2 ($\underline{R}^{st}$:$\underline{r}$)		
B'	1	0	36	0	481	383.0	< 0.01	**

It is interesting to note that the $\underline{R}^{st}\underline{R}^{nj}$ class represents part of the transmission frequency of two chromosomes 10 through the pollen, which would alone be much higher than that estimated by McClintock and Hill (1931). (B') is a reciprocal cross of one of the plants listed under series (B). In this cross an even greater deficiency of the $\underline{R}^{st}$ class is observed in the absence of the $\underline{R}^{nj}$ class.

The data obtained from reciprocal crosses, namely the increased deficiency of $\underline{R}^{st}$ when transmitted through the pollen, suggest a chromosomal condition linked to $\underline{R}^{st}$ having an effect on the male gametophyte. These results seem to fit the hypothesis that a deletion (or deletions) affects the ability of a chromosome to be transmitted, although more data are needed. The trisomic stock was kindly supplied by the Coop.

Achille Ghidoni

4. Detection of chromosome aberrations involving chromosome 1 as the result of spontaneous breakage and nondisjunction.

Chromosome aberrations of any type are known to occur spontaneously. Although their frequency is relatively low, a study of such aberrations should furnish information on the underlying mechanisms causing them.

The scarcity of data on the frequency of spontaneous chromosome aberrations is mainly due to the lack of a powerful genetic method to select for a particular type of chromosome aberration.

In the spring of 1967, after crossing a chromosome 9 tester and a chromosome 5 tester as the female parents by an all dominant male parent, some exceptional F_1 progenies appeared having the female trait in the endosperm and the male trait in the embryo. These were often small seeds. After planting these exceptions, some obviously off-type plants appeared together with some apparently normal. A thorough investigation was not carried out on all of these plants. However, the same type of cross described above was extended to chromosome 1. Many $\underline{bz}_2$ plants were crossed as the female parents to BzBz plants in the summer of 1969. The F_1 progeny was estimated at 79,184 kernels. Of these, 78 or less than 1/1000 were exceptions carrying a $\underline{bz}_2$ endosperm, which made them easily detectable. Some of these exceptions were small

seeds and had colored scutellum (Bz). Chromosome counts were done on root tips after germination yielding as follows:

Chromosomes counted	19	20	21	Total
Number of plants	1	62	15	78

These findings encouraged further investigation. The plants were classified according to the chromosome number found, phenotypical traits and genetic performance:

	Chromosomes counted			19	20	21	Total
	Seedling failures			0	5	1	6
Class	Endosperm	Plant	Pollen sterility				
1	bz	bz	N	0	20	0	20
2	bz	bz	50%	1	0	0	1
3	bz	bz	50%	0	4	0	4
4	bz	Bz	N	0	17	0	17
5	bz	Bz	50%	0	16	0	16
6	bz	Bz	30-40%	0	0	14	14

Class 1 plants proved to be normal in all regards and were considered as probable self- or sib contaminants. They were true breeding in testcrosses with bz and were removed from the group of exceptional F_1 individuals (see Table 1).

Class 2 had one plant only which was regarded as a monosomic for chromosome 1. This plant gave no progeny, although semisterility was ascertained. The two sperms giving rise to this plant were both deficient. Either nondisjunction in the first pollen grain division or whole loss of chromosome 1 in the second division could be the cause of this monosomic condition.

Class 3 had four plants which, judging by their phenotype and pollen sterility, were regarded as deficient for part of chromosome 1. According to the kernel phenotype and the chromosome count, this deficiency must be the result of a chromosome breakage, proximal to Bz, taking place in one of the pollen grain divisions.

Class 4 had 17 plants with a loss of Bz limited to the endosperm, with apparently no change in the embryo. Only one of the two sperms was

Table 1

Genetic and morphological comparison of the plants described in the text

Material	Chromo-some count	Pollen steril-ity	No. plants examined	Plant pheno-type	Aver. plant height (cm)	st.err	Aver. leaf blade width (cm)	st.err
bz tester	20	N	45	Norm.	113.6	2.0	9.6	0.2
bz x Bz (Normal F_1)	20	N	25	Norm.	172.5	4.7	9.9	0.3
bz sib contaminations class 1 (normal)	20	N	20	Norm.	134.4	2.7	8.1	0.4
true exceptions: class 2 (monosomic)	19	50%	1	Def.	59.0	-	2.6	-
class 3 (prox. def.)	20	50%	4	Df.	58.7	13.4	3.5	0.6
class 4 (normal)	20	N	17	Norm.	142.4	4.5	7.7	0.4
class 5 (dist. def.)	20	50%	16	Def.	84.1	6.6	5.9	0.7
class 6 (trisomics)	21	10% - 40%	14	Abnor.	125.2	3.0	9.7	0.8

Table 2

Genetic and morphological analysis of plants with 21 chromosomes

No.	Pedigree	Plant phenotype	Pollen classification (% sterility)	Plant height (cm)	Leaf blade width (cm)	Reciprocal testcrosses			
						♀ + x ♂ bz		♂ x ♀ + bz	
						Bz	bz	Bz	bz
1	1455- 4	N Bz	10-15 %	104	5.0	43	24	95	208
2	- 7	Slow Bz	20 %	150	10.2	30	13	134	108
3	- 9	N Bz	20-30 %	152	10.7	25	9	113	76
4	- 10	Slow Bz	20 %	79	12.4	116	36	20	44
5	- 11	N Bz	30-35 %	139	9.9	111	59	64	173
6	- 12	N Bz	25 %	140	10.1	234	102	187	144
7	- 25	Def. Bz (†)	-	-	-	-	-	-	-
8	1456- 1	N Bz	30 %	153	11.4	123	125	56	54
9	- 8	Def. Bz	30-35 %	71	3.2	63	34	86	192
10	- 10	N Bz	30-35 %	131	12.7	189	103	62	171
11	- 17	Slow Bz	30-40 %	92	6.6	87	50	53	110
12	- 24	Slow Bz	30 %	99	8.1	100	56	94	200
13	- 35	Slow Bz	40 %	151	8.9	103	54	114	98
14	1460- 2	Slow Bz	20-30 %	144	13.6	141	73	79	166
15	- 3	Slow Bz	20-30 %	148	12.9	77	26	108	160

affected. No endosperm analysis could be done to discover whether loss of Bz was to be ascribed to chromosome breakage or chromosome loss.

Class 5 had 16 semisterile plants. Seed and plant phenotypes suggested deficiencies in both sperms: a proximal deficiency (with regard to Bz) was presumably in the sperm that fused with the polar nuclei, or the whole chromosome was lost; a distal deficiency in the other sperm could account for the Bz plant phenotype, plant aspect and pollen semisterility. The few genetic data obtained from these plants are not enough for a conclusive statement, but suggested abnormal segregations (they are not included in this report).

Class 6 had 15 trisomic plants one of which died at the seedling stage. Plant no. 8 gave normal disomic ratios, and could be the result of somatic loss of one of the extra chromosomes carrying Bz or, less likely, it could be trisomic for another chromosome. The remaining plants were considered to be trisomic 1 individuals. However, in testcrosses they gave quite abnormal ratios for Bz : bz which do not fit those usually found with either normal or trisomic plants (see Table 2). These data seem to suggest that the nondisjunction event, which made one of the sperms hyperploid and the other sperm deficient, was associated with (or was the cause of) chromosome breakages which could possibly account for the abnormal ratios found.

Achille Ghidoni

UNIVERSITY OF MINNESOTA
St. Paul, Minnesota

1. A chromosomal region with more crossing over in megasporogenesis than microsporogenesis.

Several chromosomal regions in maize exhibit differences in the frequency of recombination occurring in mega- versus microsporogenesis. The Rg-lg_2 and Rg-d regions in chromosome 3, the la-su region in chromosome 4, the A_2-bt, A_2-bm, bt-pr, and bm-pr regions in chromosome 5, the y-pb region in chromosome 6, the gl-In region in chromosome 7, and the sh-gl_{15} and wx^c-wx^{90} regions in chromosome 9 consistently have been

Table 1

Comparisons of recombination in mega- versus microsporogenesis

Initial cross	Number of reciprocals and (number of progeny)	Range in percent recombination***		$\bar{d}$**** (♀ - ♂)
		mega-	micro-	
Y Su_2 (inbred W23)/y su_2 $\left(\frac{64\text{-}100\text{-}3}{64\text{-}104\text{-}9}\right)$*	6(5308)	30.0 - 35.7	15.7 - 24.8	12.2**
y Su_2 (inbred Minn A188)/Y su_2 $\left(\frac{64\text{-}98\text{-}5}{64\text{-}97\text{-}10}\right)$	6(4939)	25.0 - 28.3	17.3 - 21.3	7.9**
y Su_2 (inbred Minn A188)/Y su_2 $\left(\frac{64\text{-}97\text{-}4}{64\text{-}99\text{-}1}\right)$	4(2701)	27.3 - 36.7	15.9 - 29.7	9.1**

*Maize Genetics Cooperative identification numbers in parentheses. The F_1's were backcrossed to homozygous y su_2 $\frac{61\text{-}172\text{-}1}{61\text{-}172\text{-}5}$. The various homozygous su_2 strains carry the same su_2 allele but differ somewhat in their genetic backgrounds.

**Significant at the 1% level of probability.

***The recombination data were normal in all cases in regard to allelic ratios and equality of complementary parental and recombinant classes.

****$\bar{d}$ = weighted mean difference (see Phillips. 1969. Genetics 61:117-127).

reported to have a higher recombination value in microsporogenesis, i.e. when the F_1 is used as the male parent. Greater pollen abortion than ovule abortion in the pericentric inversion 5A (5S near centromere, 5L.50) heterozygote has been attributed to the occurrence of more crossing over in the male flowers. In 1969, I reported that the $\underline{Y}$-$\underline{su_2}$ region in chromosome 6 had significantly more recombination in megasporogenesis (Genetics 61:117-127). This conclusion was based on data from seven reciprocal backcrosses (6422 progeny). More extensive tests are reported here involving two unrelated inbred lines and three additional sources of $\underline{su_2}$.

The experimental approach was simply to reciprocally backcross the F_1 to a homozygous $\underline{y}$ $\underline{su_2}$ stock. The same tester plant was used in reciprocal backcrosses with the same F_1 plant. These "exact" reciprocal backcrosses were made at the same time using only the upper ear on the main stalk. The results of these tests are given in Table 1.

Significant differences in recombination for mega- versus microsporogenesis were obtained in every reciprocal backcross. Greater recombination in megasporogenesis, therefore, appears to be an inherent quality of the $\underline{Y}$-$\underline{su_2}$ region in chromosome 6 (probably representing the physical region 6L.17-.45) which is not affected greatly by the particular genetic background. To my knowledge, this is the only chromosomal region in maize to consistently show more recombination in megasporogenesis. Additional studies are underway utilizing $\underline{Pl}$ to determine if the difference in recombination extends over the entire $\underline{Y}$-$\underline{su_2}$ region.

R. L. Phillips

2. New information on interchanges listed as T1-5.*

Symbol	Breakpoints (Longley, 1961)	Chromosomes interchanged
8347	1S.84-5L.51	1,2
8972	1S.56-5S.29	1,5
018-5	1S.53-5L.52	1,2
055-4	1S.32-5L.31	1,8
040-3	1S.17-5L.61	1,2
024-5	1S.09-5L.98	1,2
4331	1L.03-5S.02	7,10
6178	1L.04-5L.05	1,2
48-34-2	1L.19-5L.76	1,4
8388	1L.30-5S.25	1,2

*Supported by N.S.F. Grant GB8742

C. R. Burnham
Richard V. Kowles
J. T. Stout

3. Linkage data involving Tl-5 interchanges.*

The backcross data from crosses with P br f bm_2, bm_1 pr ys yg or a_2 bm_1 pr v_2 have been summarized in Tables 1 and 2 as 3-point data for use in establishing the order of the breakpoints in relation to the marker genes. A few are from F_2 and a few are from an interchange homozygote. The breakpoints are based mainly on pachytene analyses (made by John T. Stout, with a few by Dr. Wm. Weinheimer), combined with observations on intercrosses and the original observations of Longley, 1961.

An interchange designated SL has the breakpoint in the short arm of 1 and the long arm of 5, etc.

Many of the complementary recombination classes deviate greatly from 1:1. The yg class is often deficient, but not as much so as the v_2 class. For chromosome 1, based on 384 plants from backcrosses of normal plants, the recombination values were: P-br = 46.6%, br-f = 4.7%, f-bm_2 = 41.9%. For chromosome 5, based on 1086 plants from backcrosses of normal plants, the recombination values were: bm-Pr = 14.5%, pr-ys = 12.7%, ys-yg = 33.3%. Based on 180 plants in the A_2 class: a_2-bm = 3.9%, bm-pr = 20.0%, pr-v_2 = 28.9%. If the same data are used, but the Pr-pr locus omitted, the recombination values in the A_2 class (175 plants) were: A_2-bm = 4.0%, A_2-v_2 = 37.7%; and in the a_2 class (227 plants) they were: a_2-bm = 14.5%, a_2-v_2 = 48.9%. The Bm:bm ratio was a perfect 1:1 and the V_2:v_2 ratio was 231:171, a large deviation from 1:1 but only slightly greater than that for A_2:a_2. No explanation is offered.

Chromosome 5 data for the interchanges

The 3-point data for the LL-3 heterozygote are not decisive, but the test for linkage in the homozygote shows that the breakpoint was not in the pr-ys-yg region. This places it in the bm-pr region.

The data from the SL-5 homozygote (breakpoint in 5 at L.19) show that bm and pr are no longer linked. Hence pr is distal to this breakpoint. The 3-point backcrosses for the heterozygote will be grown this summer. The LS-3 data are of some interest. The break is in the short arm but the order indicated in one test is A_2-bm-T, with a recombination

*Supported by N.S.F. Grant GB8742

Table 1

Linkage data involving the Tl-5 interchanges and genetic markers in chromosome 5.
All are backcrosses unless marked otherwise.

	genotype	parental 0		recomb. in 1		recomb. in 2		recomb. in 1,2		total plants	% recomb. in 1	% recomb. in 2	% recomb. end markers	Code no.
1-5(044-10) L.05-S.83‡	no data													SS-1
1-5e S.08-S.16	$\frac{T + Pr}{+ bm\ pr}$	139	117	2	17	14	24	1	1	315	6.7	12.7	18.1	SS-2
1-5(8972) S.56-S.29‡	$\frac{T + Pr}{+ bm\ pr}$	57	46	1	5	12	20	-	1	142	4.9	23.2	26.8	SS-3
1-5(5525) S.66-S.52	$\frac{T + Pr}{+ bm\ pr}$	180	225	13	14	38	27	9	5	511	8.0	15.4	18.0	SS-4
"	$\frac{T\ A_2 +}{+\ a_2\ bm}$	37	43	2	14	1	7	-	-	103	17.1	9.7	20.5	"
1-5 i S.69-S.71	$\frac{T + Pr}{+ bm\ pr}$	119	123	23	22	11	13	8	8	327	18.7	12.2	21.1	SS-5
"	$\frac{T\ A_2 +}{+\ a_2\ bm}$	158	172	32	27	11	19	12	3	434	17.1	10.4	20.5	SS-5
1-5f L.09-L.20	$\frac{+\ T\ Pr}{bm + pr}$	149	176	1	0	6	7	1	0	340	0.6	4.1	4.1	LL-1
"	$\frac{T\ Pr + +}{T\ pr\ ys\ yg}$	15	21	7	2	4	7	8	1	65	27.7	30.8	30.8	"
"	$\frac{T\ Pr +}{T\ pr\ ys}$	17	32	10	5					64	23.4			"
1-5H L.09-L.50	no data													LL-2

Table 1. (continued)

	genotype	parental 0		recomb. in 1		recomb. in 2		recomb. in 1,2		total plants	% recomb. in 1	% recomb. in 2	% recomb. end markers	Code no.
1-5c L.44-L.34	$\frac{T + +}{+ ys yg}$	92	40	1	13	42	30	6	10	234	12.8	41.9	36.8	LL-3
"	$\frac{pr T +}{Pr + ys}$	76	82	3	8	6	16	0	2	193	6.7	12.4	17.1	"
1-5a L.58-L.45	$\frac{+ T +}{bm + ys}$	65	49	3	7	3	3	1	0	131	8.4	5.3	12.2	LL-4
1-5(7267) L.92-L.82‡	$\frac{+ T +}{ys + yg}$	95	50	33	6	5	16	10	6	221	24.9	16.7	27.1	LL-5
1-5(8782) S.02-L.01	$\frac{T + Pr}{+ bm pr}$	131	110	2	3	10	7	1	2	266	3.0	7.5	8.3	SL-1
1-5b S.09-L.05	$\frac{T + +}{+ ys yg}$	69	47	5	30	46	22	3	14	267	19.8	32.2	38.6	SL-2
1-5(7219) S.15-L.33	$\frac{T Pr +}{+ pr ys}$	122	125	1	3	3	2	1	1	258	2.3	2.7	3.5	SL-3
"	$\frac{T Pr +}{+ pr v_2}$*	62			7		33		3	105	9.5	34.3	38.1	"
"	$\frac{+ T Pr}{bm + pr}$	125	127	0	0	1	4	0	1	258	0.4	2.3	1.9	"
"	$\frac{T + +}{T ys yg}$**F_2									32	32.3			"
1-5(6899) S.37-L.11	$\frac{T Pr +}{+ pr ys}$	106	114	4	11	8	13	1	2	259	6.9	9.3	13.9	SL-4
"	$\frac{A_2 + T}{a_2 bm +}$	143	245	4	24	39	6	4	6	471	8.1	11.7	15.5	"

Table 1. (continued)

	genotype	parental 0		recomb. in 1		recomb. in 2		recomb. in 1,2		total plants	% recomb.			Code no.
											in 1	in 2	end markers	
"	$\frac{T\ Pr\ +\ +}{T\ pr\ ys\ yg}$ **F_2									92	16.7	18.0		"
1-5(4613) S.78-L.19	$\frac{T\ +\ Pr\ +}{T\ bm\ pr\ ys}$	48	44	37	52	5	5	9	11	211	51.7	14.2		SL-5
1-5(5045) S.94-L.45	$\frac{Pr\ T\ +}{pr\ +\ ys}$	238	282	4	2	6	32	4	0	568	1.8	7.5	7.7	SL-6
"	$\frac{+\ Pr\ T}{bm\ pr\ +}$	239	311	3	3	5	0	2	5	568	2.3	2.1	1.9	"
1-5(6197) L.02-S.01	$\frac{T\ +\ Pr}{+\ bm\ pr}$	164	131	10	2	11	17	1	1	337	4.1	8.9	11.9	LS-1
1-5(043-15) L.10-S.42	no data													LS-2
1-5(6401) L.16-S.19	$\frac{T\ +\ Pr}{+\ bm\ pr}$	55	82	3	-	-	2	2	-	144	3.5	2.8	3.5	LS-3
"	$\frac{A_2\ +\ T}{a_2\ bm\ +}$	129	123	4	5	4	6	0	0	271	3.3	3.7	7.0	"
1-5(070-12) L.34-S.62	no data													LS-4
1-5(7212) L.44-S.21	$\frac{T\ +\ Pr}{+\ bm\ pr}$	160	157	1	4	7	12	2	1	344	2.3	6.4	7.0	LS-5
"	$\frac{A_2\ T\ +}{a_2\ +\ bm}$	64	62	8	1	-	1	1	1	138	8.0	2.2	7.2	"
1-5(4597) L.51-S.44	$\frac{T\ +\ Pr}{+\ bm\ pr}$	114	114	4	10	12	17	-	-	271	5.2	10.7	10.7	LS-6

Table 1. (continued)

	genotype	parental 0		recomb. in 1		recomb. in 2		recomb. in 1,2		total plants	% recomb. in 1	% recomb. in 2	% recomb. end markers	Code no.
"	$\frac{T\ A_2\ +}{+\ a_2\ bm}$	132	144	4	22	3	6	12	2	325	12.3	7.1	10.8	"
1-5g L.56-S.78	$\frac{T\ +\ Pr}{+\ bm\ pr}$	115	88	45	45	25	19	8	10	355	30.4	17.5	37.7	LS-7
1-5(8041) L.80-S.10	$\frac{T\ +\ Pr}{+\ bm\ pr}$	343	432	6	5	42	28	9	3	868	2.6	9.4	9.3	LS-8

‡ - breakpoints are those reported by Longley, 1961

* - A_2 data only

** - F_2 data only

Table 2

Linkage data involving T1-5 interchanges and genetic markers in chromosome 1

	genotype	parental 0		recomb. in 1		recomb. in 2		recomb. in 1,2		total plants	% recomb.			Code no.
											in 1	in 2	end markers	
1-5e S.08-S.16	+ T + P + br	8	10	3	2	3	3	0	5	34	29.4	32.4	32.4*	SS-2
1-5(5525) S.66-S.52	T + + + P br	28	17	2	9	14	24	4	1	99	16.2	43.4	49.5	SS-4
1-5i S.69-S.71	T + + + P br	42	21	4	7	11	22	5	2	114	15.8	35.1	38.6	SS-5
1-5a L.58-L.45	+ + T br f +	86	70	4	2	4	6	0	2	174	4.6	6.9	9.2	LL-4
1-5b S.09-L.05	T + + + br bm	11	9	0	7	9	10	0	1	47	17.0	42.6	55.3	SL-2
1-5(6899) S.37-L.11	T + + + P br	30	29	1	7	18	25	2	3	115	11.3	41.7	44.3	SL-4
1-5(4613) S.78-L.19	T + + + P br	25	29	3	3	13	30	4	2	109	11.0	44.9	44.9*	SL-5
1-5(5045) S.94-L.45	T + + + P br	17	15	10	5	10	10	6	6	79	34.2	40.5	44.3	SL-6
1-5(7212) L.44-S.21	T + + + br bm	41	53	0	3	30	52	2	0	181	2.8	46.4	47.0	LS-5
1-5(4597) L.51-S.44	T + + + br bm	55	50	5	3	29	23	3	6	174	9.8	35.1	34.5	LS-6
1-5g L.56-S.78	T + + + br bm	32	33	1	5	20	22	0	2	115	7.0	38.3	41.7	LS-7
1-5(8041) L.80-S.10	+ T + br + bm	8	4	4	5	1	2	1	0	25	40.0	16.0	48.0	LS-8

value of 3.7 for bm-T. The other test is non-discriminatory. The bm marker is known to be in the short arm, very close genetically to the centromere. If it is a centromere marker, then this order shows the interchange is SL and not LS. The diakinesis observations from the intercrosses indicate this interchange is either SL or LS. The pr marker is at about 5L.3, ys is distal to SL-6 at 5L.45, yg is distal to LL-5 at 5L.82.

The data for chromosome 1 markers are based on relatively small numbers.

John T. Stout
Richard V. Kowles
Wm. Weinheimer
Chas. R. Burnham

4. A stain for pollen sterility determinations.*

A simple staining technique can be used for efficient and accurate recording of pollen sterility. Certain advantages result from the use of a gel-like mixture prepared as follows:

1 gm of agar is dissolved in 50 ml of distilled water and boiled for 3 minutes.

6 ml of strong I_2KI is added to the agar (0.3 gm I_2 and 1.0 gm KI in 100 cc H_2O).

14 ml of 1N HCl is added.

Allow to cool and mix well.

Pollen forced from the anther into the substance will stain immediately. Mixing the pollen well before placing a cover glass (one-third size) over it insures random dispersal of grains for predetermined sweeps of the slide. Differential dispersion of aborted and viable grains to the edges of the cover glass does not take place. The gel also prevents subsequent movement of grains on the slide during the counting. Three sweeps will usually constitute over 500 counted grains in a minimal amount of time. The mixture maintains its gel and staining properties for long periods of time at room temperature, even though the color of the mixture fades.

Richard V. Kowles

*Supported by N.S.F. Grant GB8742

5. Corrections and up-dating of an earlier report.

The following corrections apply to an article in MNL 42, 1968, and refer specifically to Table 1 on page 123, the "All arms" interchange tester set.

1. A double ** should be added to T4-9 (4307) and removed from T5-8a.
2. T4-6 (Conn.) is a T2-3 interchange. The stock is now homozygous.
3. T2-7c, T5-10 (6760) are now homozygous.

C. R. Burnham

6. Pollen abortion and related mutants as possible schemes for producing "all male-sterile progeny."

The pollen abortion (pa) mutants might be used for this purpose. Thirty of them, characterized by sub-normal to very small pollen, have been reported in Datura by Blakeslee (1934) and Avery et al. (1959). They show normal female transmission but none through the pollen. One pa mutant with similar behavior in maize is located between P and br (Burnham, 1941). A plant heterozygous for such a mutant with a very closely linked ms gene on the normal chromosome would transmit only ms through the pollen. The cross on ms ms $\overset{\circ}{+}$ would produce an all ms progeny.

Another possibility is an application of information published by McClintock (1944). She found that part of the deficiencies in the short arm of chromosome 9 had normal male and female transmission and also did not produce a new phenotype when homozygous. Certain normal alleles would be missing in both members of the pair. If the locus for a ms gene were one of them, the stock would breed true but when crossed on ms ms would produce all ms progeny (all hemizygous).

Such a stock might be produced by using irradiated pollen on ms plants, or on a non-lethal seedling character closely linked to ms. Various selection and test procedures could be used to establish and identify the diploid missing both alleles of ms, or the type with a usable pa mutant. (An idea developed from a proposal I made in our department in early February, 1971).

Charles R. Burnham

UNIVERSITY OF MISSOURI
Columbia, Missouri

1. Is the effect of the B-chromosomes on recombination primarily an additive one?

That the B-chromosomes can influence recombination in the A-genome chromosomes of maize is well documented. This attribute of the B-chromosome has been convincingly demonstrated by Hanson, Rhoades, and Nel. Nel has further shown that the abnormal chromosome 10 does have some sort of influence on the B-effect. Both Hanson and Rhoades observed a shift in the distribution of crossing over from the distal to the proximal region (chromosome 9) in their studies with the B-chromosomes.

In our study, where the maximum number of B's employed was five, we observed effects similar to those observed by the previously mentioned individuals. In addition, we have evidence indicative of active participation of the knobs of chromosome 9 in the B-effect. It is clear from our data that the types of knobs present do make a difference. In K^S/K^S homomorphs the decrease in recombination in the distal region (yg - sh) and the concomitant increase in the proximal region (bz - wx) are not as striking as the increases observed in both regions in K^*/K^S heteromorphs. The "total" recombination value obtained in each of the various homomorph classes is the same, approximately 42% for the yg - wx region. On the other hand, the "total" can be said to be increased in B-containing heteromorphs relative to the B-less heteromorphs. Nonetheless, the suppressive effect of the K* knob on total recombination is still visible.

Both in the proximal and the distal regions, regardless of knob composition, there is a "zig-zag" or "odd-even" effect on recombination. In the distal region an odd number of B's appears to reduce the frequency of recombination while an even number appears to reestablish the original value (0-B class value) in the homomorphs. Quite the opposite effect seems to be the case in K*-knob containing classes. In the proximal region it appears as if an odd number of B's results in an increase in recombination regardless of knob composition. The manifestation of the "odd-even" effect is more pronounced in K*-bearing plants than in K^S

Recombination values obtained from testcrosses of plants with different combinations of knobs and number of B-chromosomes

	$\frac{+\ +\ +\ +}{yg\ sh\ bz\ wx}\ \frac{o}{+}$		yg - sh	sh - bz	bz - wx	Total	Total progeny
1	0 B	K^S/K^S	23.86%	2.47%	15.72%	42.05%	7934
2		K^*/K^S	8.35	1.61	19.10	29.05	6038
3	1 B	K^S/K^S	22.08*	2.32	17.27*	41.66	5658
4		K^*/K^S	10.92**	1.69	21.39**	34.00**	9539
5	2 B	K^S/K^S	24.17	2.12	16.23	42.52	7444
6		K^*/K^S	8.12	1.43	21.05*	30.60	5121
7	3 B	K^S/K^S	21.77*	1.84	17.44*	41.06	2494
8		K^*/K^S	10.41**	2.05	23.34**	35.80**	4430
9	4 B	K^S/K^S	23.47	1.86*	16.07	41.40	5587
10		K^*/K^S	8.97	1.48	21.94**	32.39**	4202
11	5 B	K^S/K^S	23.69	1.85	18.33*	43.87	971
12		K^*/K^S	12.63**	1.86	22.77**	37.27**	966

* and ** denote deviations, significant at the 5% and 1% levels respectively, from the " 0 B " class of the same knob constitution.

homomorphs. The "zig-zag" type of effect on recombination by the supernumeraries has been observed in rye by Jones and Rees, and in Listera ovata by Vosa and Barlow. Our work on the B:Knob interaction is in need of further experimentation. It would be interesting to know whether the "zig-zag" effect observed in chromosome 9 is displayed in the other A-genome chromosomes.

It becomes increasingly clear that the effect of the B-chromosomes on recombination is not simply an additive one. It is also quite clear that the effect of the B-chromosomes on recombination is modifiable not only by the abnormal chromosome 10 but also by the knobs of chromosome 9.

C. C. Chang
Gary Y. Kikudome

2. Probable weak fusion of chromatids broken during a breakage-fusion-bridge cycle.

McClintock (Genetics 26:234) first described the chromatid type of breakage-fusion-bridge cycle in which a dicentric chromatid introduced into the initial triploid nucleus of the maize endosperm will persist throughout both the gametophyte and endosperm divisions. Variegation patterns of endosperm markers in mature kernels provide evidence of the cycle.

In the present study plants heterozygous for a ring chromosome 10 carrying the dominant R color factor allele and the dominant allele for non-striate plant (Sr_2) were crossed to chromosome 10 testers marked by the recessive alleles (figure 1). Three classes of progeny resulted. The largest class of kernels (84.4%) was colorless and yielded green, non-striate plants which were shown cytologically to contain normal chromosomes 10. These individuals arise from one of the noncrossover classes. The second class of kernels comprised 10.4% of the progeny. The kernels were variegated for R. The variegation of the endosperm tissue indicated that either the ring was present or a crossover between the ring and the rod had produced a dicentric chromosome that was undergoing the chromatid type breakage-fusion-bridge cycle. Of the 10 kernels in this class which germinated, 4 produced plants variegated for striate. These plants, designated class II-A in figure 1, represent the other noncrossover class. The presence of the ring was confirmed cytologically.

A. ORIGINAL CROSS

B. PROGENY CLASSES	PHENOTYPE	GENOTYPE*	PERCENT OF PROGENY
Class I			
Kernel color (endosperm)	Colorless	G r Sr	84.4%
Plant	Non-striate	G r Sr / g r sr	
Class II - A			
Kernel color (endosperm)	Variegated	Sr g R	
Plant	Striated	Sr g R g / sr r	10.4%
Class II - B		Sr g G R Sr r → g/G r/R Sr ← 40% striate; 60% non	
Kernel color (endosperm)	Variegated		
Plant	Non-striate	g/G r/R Sr / g r sr	
Class III		Sr g G R Sr r → g/G r/R Sr ←	
Kernel color (endosperm)	Full colored		5.2%
Plant	Non-striate	g/G r/R Sr / g r sr	

Figure 1. - Origin of progeny classes resulting from the cross k10k10 ♀ X ring-10/k10 ♂. The percent of progeny in each class is given for a total of 270 kernels from one cross. Of the 10 kernels in Class II which germinated, four were striated and six were not.

*The endosperm genotype was deduced from evidence presented in the text; only the chromosome contributed from the male parent is shown. The plant genotype was determined after cytological examination; the chromosomes contributed by both parents are shown.

The remaining six plants were not striate and contained stable rod derivatives produced when the breakage-fusion-bridge cycle ceased in the sporophytic tissues.

The third progeny class was unexpected. The kernels were full colored and yielded non-striate plants. Pachytene squashes revealed each plant had received from the male parent, a stable rod chromosome. Of the six derivatives studied from this group, five possessed the heterochromatic knob from the ring plus the three prominent chromomeres; one contained only the three chromomeres. As each included at least the three chromomeres, and since crossing over between the R locus and the knob of K10 is very rare (Rhoades, Genetics 27:395), the derivatives could not have come from a double crossover on each side of the R locus. Rather, each derivative appears to have been the result of a single crossover between the ring and the rod chromosome which led to the formation of a dicentric bridge at anaphase I. According to McClintock, the breakage-fusion-bridge cycle should have continued in the endosperm giving an endosperm variegated for R. However, in this instance it appears that the chromosomes formed by the crossover between the ring and the rod did not undergo the typical breakage-fusion-bridge cycle as described by McClintock. Two explanations could account for the non-variegated kernels. First, the broken end of the chromosome could have healed permanently in the endosperm. This contradicts all evidence accumulated by previous investigations on the healing of broken ends of chromosomes. A more plausible explanation is that the initial break of the dicentric occurred at a point in the chromosome where fusion of the sister chromatids was not complete. Because of the weak fusion, all successive breaks would occur at the same position and the original genetic constitution would be preserved. This explanation is supported by McClintock's original observation of a tendency for successive breaks to occur at positions of previous fusions, indicating that in many cases the fusion of sister chromatids is weak. In addition, Schwartz and Murray (Supplement volume of Cytologia, Proc. Intern. Gen. Symp. 1956: 277) examined cytologically the types of anaphase bridge configurations in developing endosperms containing a dicentric chromatid. Since the dicentric was introduced into the initial triploid nucleus from which

the endosperm develops, all anaphases in the young embryo should have shown single bridges as a consequence of a continuous breakage-fusion-bridge cycle. On the contrary, none of the kernels examined showed more than a scattering of single bridges. Schwartz and Murray suggested therefore, that the fusion of sister chromatids was not always complete at anaphase. An incomplete fusion would result in a weak bridge which would rupture early at the next anaphase and not be scored at middle or late anaphase.

Examination of the rod chromosomes derived from the full colored kernels provided support for the "weak fusion" hypothesis. Four of the derivatives came from breaks either through or very close to the heterochromatic K10 knob. A fifth derivative was produced after a break adjacent to one centromere, and the sixth came from a break adjacent to the other centromere. Each of the breaks was in or near heterochromatin of the knob or proximal heterochromatin surrounding the centromere. It is suggested fusion of sister chromatids may be less strong in heterochromatin.

It can be concluded that crossovers between the ring of abnormal chromosome 10 and its homologous rod produced dicentric bridges which yielded stable rods in the next sporophytic generation. Apparently, two types of chromosomes were formed. The first type (Class II-B) underwent a typical breakage-fusion-bridge cycle in the endosperm tissue as evidenced by the variegation for the R allele. The second type (Class III) presumably underwent a cryptic breakage-fusion-bridge cycle where incomplete fusion always resulted in breakage of the chromatid bridge at the point of previous fusion. Continuous breakage at the same point did not alter the genic constitution of the daughter cells, the R allele was not lost, and thus, the endosperm was not variegated.

Judith H. Miles

UNIVERSITY OF MISSOURI
and
UNITED STATES DEPARTMENT OF AGRICULTURE
Columbia, Missouri

1. Selection for resistance of pollen to ultraviolet light.

Pollen grains that survive exposure to ultraviolet light should, on average, be richer in factors that confer increased resistance to ultraviolet light. Resistance could be due to changes in repair mechanisms or in protective systems such as the UV-absorbing constituents of the cytoplasm. Experiments designed to test for selection for level of resistance were carried out with a C Sh Wx R^r line. For two successive generations plants of this line were self-pollinated with UV-treated pollen, the first treatment consisting of a 30-second exposure and the second of a 45-second exposure to two-sided radiation. Control lineages were carried forward within the same background material. In addition, lineages posited to carry increased sensitivity to UV were derived by holding the treated pollen for a 30-minute delay in aqueous medium following a short (10-second) exposure. The test generation consisted of exposing samples of pollen of the three lineages to 60 seconds of UV before pollinating c sh wx R^g ears. The resulting ears were coded at random; events were identified and verified by progeny testing where necessary, and the ears were decoded for tabulation. The event classes were (1) whole-endosperm loss of C Sh Wx; (2) whole-endosperm loss with one or more small "recovery" areas; (3) breakage fusion bridge cycles over the whole endosperm; (4) cycles over half the endosperm; (5) cycles over a quarter of the endosperm; (6) loss in one half of the endosperm; and (7) loss in one quarter of the endosperm.

The data in Table 1 show few substantial differences between the selected lineages and the control. In the control, absence of whole losses must be due to chance, since events of this type are commonly found in similar experiments. The highly significant decrease in cycles of the whole-endosperm type may be valid, but it will require further testing. If it is valid, this decrease is an interesting and unanticipated change.

Table 1

Mutational losses of C induced by ultraviolet light in pollen grains from ultraviolet-selected (UV2), unselected (Control), and delay-selected (UVD) lineages

	UV2		Control		UVD	
	Events	Freq. per 10^3	Events	Freq. per 10^3	Events	Freq. per 10^3
Total kernels obs.	2527	-	948	-	1323	-
Whole losses	14	5.54	0	0.00	3	2.27
Loss-recovery	13	5.14	5	5.27	5	3.78
Cycles whole endosp.	50	19.79**	38	40.08	42	31.75
Cycles half	20	7.91	9	9.49	6	4.54
Cycles quarter	8	3.17	1	1.05	3	2.27
Fractionals half	71	28.10	25	26.37	30	22.68
Fractionals quarter	40	15.83	18	18.99	20	15.12

**Highly significant (1% level) decrease in events relative to control.

A number of visible mutants have been identified in the selection experiments. Pollen from heterozygous plants was either treated with UV for 60 seconds or untreated, in parallel, and crossed onto c sh wx. By self-pollination of the hybrid plants, transmission data were derived for each of the mutants, with and without UV exposure. The data (Table 2) test for selective advantage of the mutant in the pollen grain. Three of the mutants showed significant increases in transmission of the mutant following UV treatment. All three are deviant to a limited extent only, but it is possible that one or more of these mutants confers on the pollen grain a degree of resistance to UV exposure.

Table 2

Transmission of mutants under ultraviolet selection[1]

Pedigree	Mutant	Treated		Control		X_h^2	X_C^2
		Nor.	Mut.	Nor.	Mut.		
7453	White seedling	23	31	38	26	2.67	6.31*
"	Pale green	18	4	29	9	0.03	0.37
7456-57	Luteus	6	6	6	8	-	0.12
"	Virescent	92	30	65	23	0.01	0.15
"	Pale Aleurone	54	75	40	56	0.03	0.00
7458-59	Virescent	24	16	46	15	2.03	5.12*
"	Viviparous	20	18	35	15	2.09	5.46*
"	Etched	30	8	37	13	0.08	0.48

*Significantly higher (5% level) transmission of mutant in the treated than in the control.

[1] X_h^2, Chi-square for heterogeneity; X_C^2, Chi-square for the control ratio.

E. H. Coe, Jr.

2. Genetic analysis of effects of an electrostatic field.

A pilot experiment was reported in the 1966 Newsletter (40:108). Fractional events (losses of A Sh_2) were more frequent in kernels from pollen that had been exposed to an intermittent field than in controls. A subsequent test by S. F. Starling (M. S. thesis, University of Missouri) indicated that fractional losses of C^I Sh Bz Wx were increased by exposure to either a steady or intermittent field; unfortunately, his analysis of the data showed very wide variations among families of tester females, and the event data were confounded with this effect. A large-scale test with a uniform tester was conducted in 1969, and progeny tests of cases have been completed. The new data do not support a substantial effect of treatment, either with steady or intermittent fields.

Treatments were carried out on tassels of C Sh Wx R^r plants ranging in development from meiosis to nearly mature pollen stages. The tassel region of the plant (wrapped and taped into a cylinder) was placed

between two foil plates separated by 6 cm of plexiglass. The plates were connected to an automatically switched DC power supply at 30 kV (i.e., intensity was 5,000 V/cm) for 18.5 hours, from mid-afternoon to early morning. The intermittent treatment was switched to ground at 40 cycles per minute. The steady treatment was continuously applied for the entire time period. Current flow was very low, averaging 5 microamperes per pair of foil plates. The plates were about 12x40 cm, and exposed to air over their entire flat surface, so that discharge flow of this magnitude is moderate. Twelve plants were given intermittent treatment, 4 steady, and 9 control. Pollinations were made onto c sh wx R^g testers. Pollen was collected each day that the tassel shed, and complete records were kept of the number of days from treatment to day of shedding, with the intent of identifying "sensitive" stages. Progeny ears were coded at random; events were identified and verified by progeny testing where necessary, and the ears were decoded for tabulation. The event classes were (1) whole-endosperm loss of C; (2) half-endosperm loss; (3) quarter-endosperm loss; (4) breakage-fusion-bridge cycles over the whole endosperm; (5) cycles over a fraction of the endosperm, down to one eighth.

The breakdown of data for two series of treated plants pollinated across a spread of 20 days (from shedding dates 4 days post-treatment to 23 days post-treatment) showed no apparent clusters of events, so only the totals for all dates are tabulated here (Table 3). The data show a significant increase in only the quarter-endosperm losses following intermittent exposure. The increase is not notable in the steady-field series. If there is any effect of the treatment it is at the sub-chromatid level and is of disappointingly small magnitude.

Table 3

Mutational losses of C following exposure of post-meiotic tassels to an intense electrostatic field

	Control	Intermittent field	Steady field	Total
Examined No.	62,175	74,867	22,190	159,232
Losses per 10^4				
Whole endosperm	0.8	0.7	1.4	0.8
Half	0.8	1.5	2.3	1.3
Quarter	12.1	17.1**	14.4	14.8
BFB cycles per 10^4				
Whole endosperm	3.1	1.6	1.4	2.1
Fractional	5.0	4.4	2.3	4.3
All events	21.7	25.2*	21.6	23.4

*,** Significantly higher than control at 5% and 1% level, respectively.

E. H. Coe, Jr.

3. Location of new mutants by A-B translocation method.

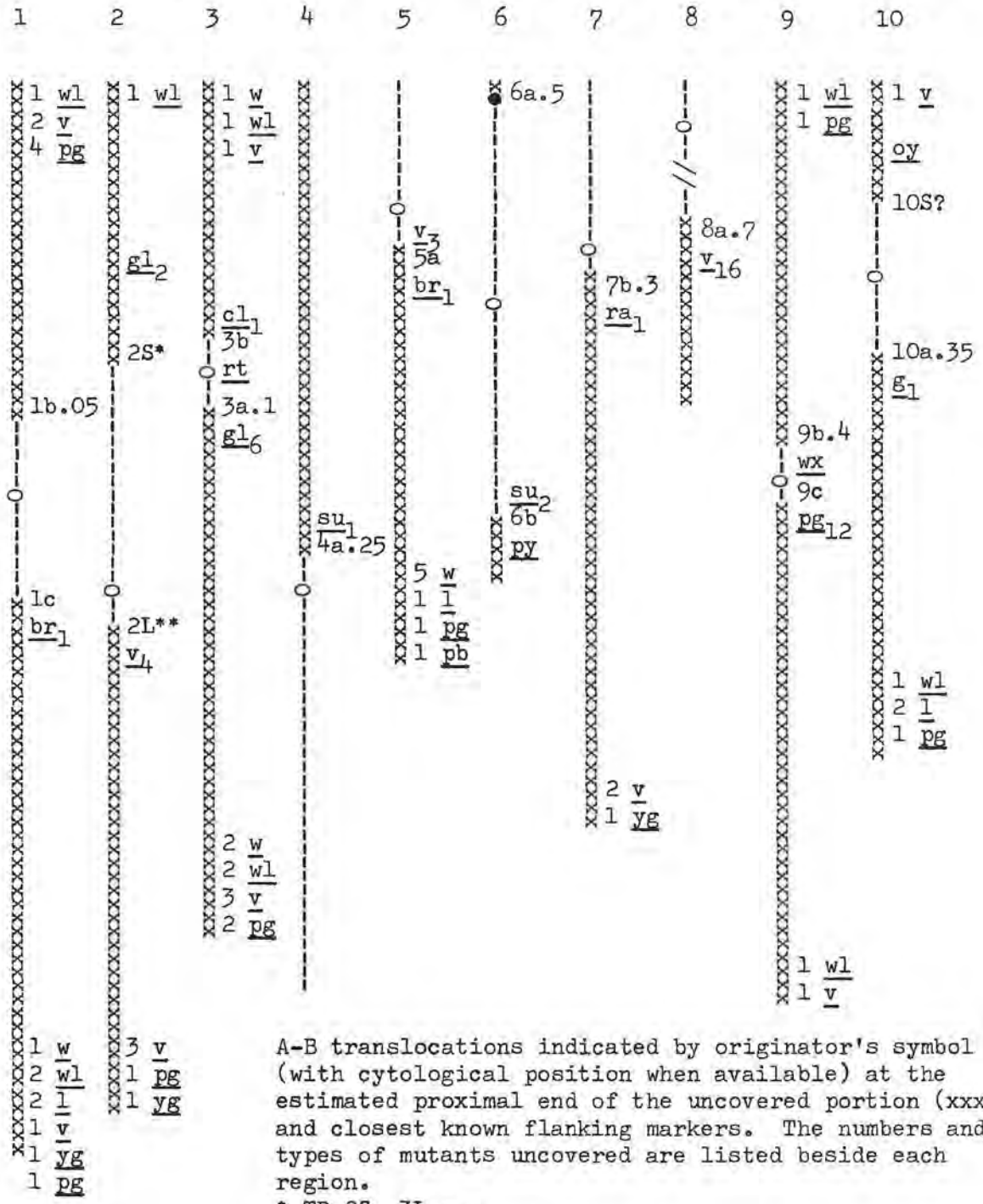
A collection of 116 chlorophyll mutants, produced either by treatment of pollen with chemical mutagens ethylmethanesulfonate or nitrosoguanidine or from experiments with the controlling elements (Ac, Dt or Spm), was prepared for linkage tests using a series of A-B translocations according to the method suggested by Roman (Genetics 32:391-409).

The collection included 75 mutants induced by EMS, 8 induced by NG and 33 derived from cultures with controlling elements. They were classified at seedling stage as w (white), wl (yellowish white), l (yellow), v (virescent), pg (pale green), yg (yellow-green) and pb (piebald). A number of unusual types, such as mutables, temperature or light sensitives, and atypical virescents, were grouped in the above categories for this report.

The procedure consisted of planting selfed seed of a known heterozygote for each mutant (since many were homozygous lethal), crossing

Figure 1

Linkage map with A-B translocations and tentatively located mutants.



A-B translocations indicated by originator's symbol (with cytological position when available) at the estimated proximal end of the uncovered portion (xxxx), and closest known flanking markers. The numbers and types of mutants uncovered are listed beside each region.

* TB 2S, $3L_{6270}$

** TB 2L, $1S_{4464}$ or TB 2L, $3L_{7285}$

three plants of each family by the A-B translocation set, planting 100 kernels from each ear in a sand bench, and noting the hypoploid seedlings that expressed the mutant phenotype.

The set of A-B translocations used covered 16 chromosome arms to some degree. They consisted of a group of 17 obtained from various sources as indicated below.

1b Roman	6a Roman
1c Beckett	6b Beckett
2S, $3L_{6270}$ Robertson	7b Roman
2L, $1S_{4464}$ Robertson	8a Roman
2L, $3L_{7285}$ Robertson	9b Roman
3b Beckett	9c Beckett
3a Roman	10S Beckett translocation not verified
4a Roman	10a Roman
5a Beckett	

The relationship of these translocations to the current linkage map and the frequency and types of mutants uncovered by each is shown in figure 1.

Fifty-two of the 116 mutants tested were tentatively located to chromosome arm. This proportion is about what one would expect, considering that the translocations covered roughly 66% (our estimate) of the known linkage map and that some of the crosses were missed, many female plants were homozygous normal and not all male parents carried the translocation.

M. G. Neuffer
J. B. Beckett

4. Chemical mutagens and in vitro germination of pollen.

An earlier report (MNL 42:124) on the use of paraffin oil as a carrier for chemical mutagens in treating corn pollen showed the method was promising. Subsequent experiments using this method have given mixed results because of difficulties in standardizing dosages. Variations occur because of purity and potency of chemicals and because of differences in mixing technics. These variations often result in complete killing as one extreme, or ineffective treatment as the other. A quick method for reducing these extremes has been developed.

Corn pollen can be germinated easily on an agar medium (Cooke and Walden, Canadian Journal of Botany 43:779-786). We found that pollen in paraffin oil, when placed on Cooke and Walden's media in a petri dish, germinated very well. The oil spread rapidly over the surface, leaving the pollen grains scattered and the whole surface covered with a film that sealed in the moisture but did not interfere with germination. This provides a convenient way to check the effect of various concentrations of chemical mutagens in paraffin oil on pollen germination and tube growth which should be correlated with seed set and mutagen effectiveness.

A stock solution of 1% ethylmethanesulfonate in paraffin oil was prepared. From this, dilutions of 1:0, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, and 1:8 with oil were used to treat pollen. The pollen was left in the oil for 16 minutes before placing on agar media. Pollen tube growth, which was prohibited by the stock solution, increased with dilution to 1:4 when growth appeared to be normal.

Pollinations were made with similar pollen (RR) held for sequential intervals of 3 to 20 minutes in 1:4 or 1:8 dilutions and crossed on Rr ears. Seed set was very poor for the 1:4 dilution while that of the 1:8 dilution was excellent. Harvested ears from the 1:8 dilution had no whole kernel and only 6 fractional losses for R among 4188 kernels in the treatments lasting 3 to 15 minutes, but had 5 whole and 15 fractional losses among 1076 kernels on the last 10 ears representing 16 to 20 minutes of treatment.

From these results we have concluded that the best concentration is a 1:7 dilution (.13% EMS) with a treatment time before pollination beginning at 15 minutes.

A solution of nitrosoguanidine (NG) was made as follows: pulverize 1 g of NG crystals to a granular consistency and mix in 100 ml of paraffin oil, stir vigorously for 2 hours, allow to sit undisturbed for 18 hours and pour off the cloudy suspension and use it as a stock solution. All these procedures must be carried out in reduced light as NG is inactivated in 3 minutes of sunlight in the greenhouse.

Using a series of dilutions as described above, it was found that a 1:19 dilution was the strongest that gave no visible effect on germination or tube growth. Pollination made from similar pollen, held 3 to 20

Table 1

Comparison of seed set and frequency of induced changes of the $\underline{\alpha}$ $\underline{\beta}$ $\underline{Sh_2}$ segment of chromosome #3 in timed treatments of $\underline{A_1^b}$-$\underline{Sh_2}$,$\underline{Dt_1}$ pollen with nitrosoguanidine in paraffin oil. The ear parent was $\underline{a^m\text{-}1}$ $\underline{sh_2}$ $\underline{dt}$.

Mean treatment time in minutes	Number of ears	Seed set in average # kernels	Induced changes per 1000 kernels*	
			Whole kernel	Fractional 1/8+ kernel
NG oil				
2	1	160	0	263
3	5	213	8.5	151
4	10	107	7.5	122
5	5	139	2.9	96
6	6	187	8.9	117
7	5	190	6.3	128
8	4	177	4.2	155
9	5	172	4.6	109
10	5	150	14.6	146
15	18	157	6.4	129
20	21	180	4.5	121
25	16	161	6.6	117
30	18	191	6.1	119
75	3	252	6.6	184
90	12	108	3.8	142
Control oil				
25	5	132	0	5
30	6	125	0	4
40	9	155	0	7
50	9	135	0	7
75	10	124	0	10
200	15	109	0	4
Control no oil				
-	9	194	0	6

*All observable types of changes of $\underline{\alpha}$ $\underline{\beta}$ $\underline{Sh_2}$

minutes in a 1:19 dilution gave fair seed set with no whole kernel and 5 fractional losses for R among 238 kernels for the 3 to 4 minute time but a rapid reduction in seed set for progressively longer treatment times.

These results contradict an earlier report (MNL 42:125) where it was stated that no reduction in seed set occurred after the first 3 minutes of treatment time. The reason for this is that in the first experiment a glass vial was used allowing the sunlight to inactivate the nitrosoguanidine, while in the second experiment a plastic vial covered with masking tape was used, thus protecting the solution which retained its activity. The effect of sunlight inactivation is demonstrated by the data in Table 1 where seed set and frequency of whole and fractional kernel changes for the A^b-Sh segment of chromosome #3 are compared. Note that there is no significant change in seed set or frequency of changes after 3 minutes.

From these tests we have concluded that the best results can be obtained with a 1:19 dilution of our stock solution which is kept in the dark but mixed with pollen in daylight and used within a 50 minute period following initiation of treatment.

M. G. Neuffer

THE NATIONAL COLONIAL FARM
Accokeek, Maryland 20607

1. The endosperm mutant described as "similar to sh_2" in News Letter 44 proved to be allelic to brittle-1. It was induced in the field corn inbred B14, and is Y Su. Aleurone constitution is AAccrrPrPr. Seed is available.

2. An induced B14 plant mutant has proved to be allelic to brachytic-2. Vigor of mutant is good. Seed is available.

3. Seed is available of most of the seedling mutants described in the Maize News Letter 44. I will not be growing any of these in 1971.

W. Ralph Singleton

UNIVERSITY OF NEBRASKA
Lincoln, Nebraska
Department of Agronomy

1. Induced "Necrotic Leaf Spot" mutation allelic to zebra necrosis (zn_1).

Irradiation of seeds of the inbred line N25 with thermal neutrons produced a mutant which developed necrotic spots on the leaves just prior to tasseling. Genetic studies (Hornbrook and Gardner, Radiation Botany 10:113-117, 1970) clearly indicated that the trait is controlled by a pair of alleles with the normal allele being completely dominant over the necrotic leaf-spot allele. In homozygous recessive plants, degree of spotting varies from leaf to leaf but is always present. In 1969, the mutant line was grown alongside zebra necrosis sources obtained from Dr. R. J. Lambert, Illinois, and Dr. Neuffer, Missouri. In the zn_1 lines the necrotic tissue is limited to areas between the veins, whereas in the necrotic leaf spot line irregular spots or blotches appear which may coalesce causing large areas of the leaf to die. The phenotypic expressions of the induced mutant and zn_1 were sufficiently different to lead us to believe that different genes were involved; however, crosses were made between the two in 1969. Both lines were used as male and female. Twelve F_1 families observed in the 1970 nursery were found to have every individual plant expressing necrotic leaf spots. In general, the F_1 plants tended to be more like the induced mutant than the zn_1 line and showed more leaf-tip damage than either parent. The mutant gene causing necrotic leaf spot appears to be allelic to the zn_1 gene or else it is the same gene interacting with other genes to produce the blotches noted. Further research is needed to determine which is the correct hypothesis.

In the 1970 nursery, the symptoms of the induced mutant appeared somewhat earlier than noted in previous years but at about the same time as the zebra necrosis symptoms. They tended to appear first on the 5th, 6th and 7th leaves as large blotched areas with very little evidence of striping. Zebra necrosis lines showed a definite striping and relatively little evidence of blotches. The first evidence of necrosis appeared when plants were about 30 to 40 cm high in 1970, whereas it was believed to have occurred just prior to tassel elongation and emergence in previous years.

C. O. Gardner

2. New evidence of heterosis in crosses between an induced mutant "Necrotic leaf spot" N25 and normal N25 inbred lines.

In an earlier report, Hornbrook and Gardner (Radiation Botany 10: 113-117, 1970) found evidence that when an induced mutant of inbred N25, which caused necrotic blotches to appear on the leaves and reduced yield, was crossed to normal N25, the F_1 hybrid outyielded the normal parent. Reviewers of our original manuscript did not believe that our data were sufficiently conclusive to justify our conclusion so more extensive tests were planned. In 1970, the mutant line, the normal parent and the F_1 hybrid were grown in 20 replications. Ten replications had a plant population density of 51,666 plants per hectare and the other 10 had 68,888 plants per hectare.

Results indicated that maximum yields were obtained at the lower rate of planting, that there is no evidence of genotype--plant population density interaction and that the F_1 hybrid does indeed show significant heterosis above the normal parent. Means are presented in Table 1.

Table 1

Mean values of agronomic traits measured on mutant and normal lines of N25 and their F_1 hybrid

Genotype	Means of traits measured							
	Grain yield	Ear length	Ear diameter	Ears/plant	Barren plants %	Plant ht.	Ear ht.	Days to flower
	(q/ha)	(cm)	(cm)			(cm)	(cm)	
Normal N25	25.6	15.7	3.70	0.80	20.2	186	46	74.9
Mutant N25	19.0	13.8	3.51	0.80	19.8	190	54	76.0
F_1 hybrid	36.2	16.5	3.84	0.95	4.9	187	50	74.6

The hybrid outyielded the better parent by over 40%, had larger ears, more ears, and fewer barren plants. The hybrid is intermediate in plant and ear height and equal to the earlier parent in earliness of flowering.

Although heterosis in yield and other traits is evident from the data, we cannot be certain whether the mutant gene causing the necrotic leaf spot is solely responsible or whether the thermal neutron seed treatment caused other mutations that contribute to heterosis.

C. O. Gardner

UNIVERSITY OF NEW HAMPSHIRE
Durham, New Hampshire 03824
Department of Plant Science

1. Role of β-glucosidase in monogenic resistance to Helminthosporium turcicum in maize.

β-glucosidase catalyzes the hydrolysis of phenolic glucosides to their corresponding aglycones. These free phenolics, which are produced after cellular disruption, are highly toxic and are believed to play a role in disease resistance. In maize, the production of the cyclic hydroxamic acid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one) from its glucoside upon cellular disruption has been recently implicated in the resistant mechanism.

We have developed a rapid fluorometric procedure for the assay of β-glucosidase. Preliminary data indicate that the susceptible genotype (htht) has a significantly higher level of β-glucosidase than the resistant genotype (HtHt). The enzyme (which has been shown to be localized primarily in the cell wall) causes the formation of the fungitoxic aglycone as a result of its mixing with DIMBOA-glucoside during cellular disruption. The toxic DIMBOA may contain the fungus until phytoalexin is produced at which point the differential genotypic reaction would begin.

It has been previously described, and observed in our study, that the infection flecks on the resistant genotype may occur as much as 15 hours earlier than in the susceptible genotype. Since apparently no phytoalexin is being produced at this early stage, it is our contention that the lower β-glucosidase levels in the resistant genotype may account for the earlier disease reaction.

R. M. Couture
D. G. Routley
G. M. Dunn
A. G. Calub

2. Effect of cyclic hydroxamates on germinating H. turcicum spores.

In maize, a single gene, Ht, conditions chlorotic lesion resistance to Northern corn leaf blight, the causal agent of which is Helminthosporium turcicum Pass. The Bx gene mediates the production of cyclic hydroxamic acids and related compounds which have been implicated recently in the resistance mechanism of maize, wheat and rye to both insects and fungi.

A bioassay test was performed to determine the fungitoxicity of cyclic hydroxamates on germinating spores of H. turcicum. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one) was isolated and purified to be used in the test which utilized solution ranging from 1-10 p.p.m.

Spore suspensions were prepared from cultures which were cut into small blocks and placed in a flask containing distilled water. The flask was shaken gently and the spore suspension filtered through glass wool. Fresh spores were added to drops of the test solution and placed on hanging drop microslides. The spores were incubated at room temperature overnight, killed and stained with IKI, and the percentage germination determined.

The results indicated that inhibition is significant at all concentrations, and is nearly complete above 6 p.p.m. Spores which germinated had significantly shorter germ tubes than the control at all concentrations. DIMBOA may therefore act in killing spores at the point of penetration and in slowing down the spread of the mycelium.

R. M. Couture
D. G. Routley
G. M. Dunn
A. G. Calub

3. Comparison of intact and detached corn leaves in bioassay tests with Helminthosporium turcicum.

Diffusates from corn leaf lesions were tested for their effects on spore germination of H. turcicum. Several genotypes were tested, including monogenic resistant (HtHt) and susceptible (htht) plus various combinations with (BxBx) and (bxbx) (MNL 1970). In the HtHt genotype, phytoalexin was apparently produced earlier in the intact than in the detached leaf. Diffusates from the intact leaf decreased both the rate

and percent of spore germination within 2-3 days, but 4-5 days were required from the detached leaf. No effects were observed with diffusates from susceptible (htht) leaves or from either resistant or susceptible control leaves (control leaves sprayed with water only).

Diffusates from HtHtBxBx leaves were more inhibitory on spore germination than diffusates from HtHtbxbx leaves. The diffusates from hthtBxBx were slightly more inhibitory than diffusates from hthtbxbx, and the former delayed the growth of germinated spores. Diffusates from homozygous resistant leaves (HtHtBxBx) inhibited spore germination much more than diffusates from the heterozygous genotype (HthtBxBx).

A. G. Calub
G. M. Dunn
R. M. Couture
D. G. Routley

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

1. An amino acid accumulating mutant from USDA P.I. 194047.

A pale-green maize mutant has been isolated from USDA P.I. 194047 and was first reported by Bell (MNL 36:73, 1962). This is a viable mutant which displays pale-green leaves for the entire life of the plant. Growth is somewhat stunted and seed set is fair to poor. Outcrosses of the mutant were made with several inbred lines, and F_2 populations produced 592 normal green and 200 pale-green plants, indicating the involvement of a single allelic pair of genes displaying simple dominance. Crosses were also made with Dr. E. G. Anderson's waxy-marked translocation series involving all chromosomes. All F_2 populations from these crosses showed normal 3:1 segregations; however, chromosomes 7, 9 and 10 cannot be eliminated as possible locations for this mutant gene. The gene has tentatively been designated pg_{13}.

A segregating population involving the mutant trait as well as a normal green line derived from the inbred Oh51A were grown in a Percival growth chamber model PGC-78 at 27$\pm$ 3°C under approximately 1000 ftc. of illumination on a 16 hour photoperiod. The plants were watered with tap

water and supplemented with Hoagland's #1 nutrient solution. Leaves were harvested after three weeks of growth. Pigment analyses, carried out according to Arnon (Plant Physiol. 24:1-15, 1949) and von Wettstein (Expt. Cell Res. 12:427-506, 1957) revealed that in the mutant segregants chlorophyll a was reduced to 37%, chlorophyll b to 63% and the carotenoids to 38% of that in the normal segregants.

Free amino acid extractions were made according to Block, Durrum and Zweig (1958) and purified on a Dowex 50-X8 ion exchange column. Total free amino acid concentrations were determined according to the method of Barrolier (Naturwissenschaften 48:554, 1961) using ninhydrin. Spectrophotometric readings were taken at 570 mμ (purple) and at 400 mμ (yellow). Separations of free amino acids were carried out on cellulose thin layer chromatograms using both one- and two-dimensional methods.

The total free amino acid concentrations of these three lines are given below in terms of absorbance at the wavelength indicated. Each value represents the mean and standard error at the 5% level, of absorbance values recorded from seven plants.

Line	440 mμ	470 mμ
Normal green segregants	0.154±0.015	0.055±0.003
Pale-green segregants	1.401±0.043	0.455±0.013
Oh51A derivative	0.153±0.007	0.055±0.004

Thin-layer chromatograms indicated that no particular amino acid had accumulated in the pale-green segregants but that the increase involved all amino acids, suggesting a block in the synthesis of one or more major proteins in the leaf.

Raymond P. Amby
David K. Shortess

2. Amino acid analyses of pale-green-11, -12 and oil yellow.

Two pale-green maize mutants, pale-green-11, pale-green-12 ($\underline{pg}_{11}\underline{pg}_{12}$) and oil yellow (oy), along with a control, a normal green derivative of Oh51A, were analyzed for pigment and free amino acid

content. Seedlings were grown for three weeks at 27±3°C under approximately 1000 ftc. of illumination on a 16 hour photoperiod. They were watered with tap water with supplements of Hoagland's #1 nutrient solution. Pigment analyses were carried out according to Arnon (Plant Phys. 24:1-15, 1949) and von Wettstein (Exptl. Cell Res. 12:427-506, 1957). Chlorophyll concentrations of $\underline{pg_{11}pg_{12}}$ and $\underline{oy}$ were 70% and 74% respectively of the normal line while the total carotenoids of the two mutant lines were 65% and 52%, respectively, of the normal line.

Free amino acids were extracted according to Block, Durrum and Zweig (1958) and purified on a Dowex 50-X8 ion exchange column. Total free amino acids were determined according to Barrolier (Naturwissenschaften, 48:554, 1961) and individual amino acids were separated on one- and two-dimensional thin-layer cellulose plates. The solvent systems used were chloroform/methanol/17% ammonium hydroxide (20/20/5,v/v) for the one-dimensional plates, and butanol/acetic acid/water (4/1/1,v/v) and the above solvent for the two-dimensional plates.

No significant differences were observed in the total free amino acid contents of either $\underline{pg_{11}pg_{12}}$ or $\underline{oy}$ when compared to the normal green plants. However, chromatographic analysis revealed that in both $\underline{pg_{11}pg_{12}}$ and $\underline{oy}$, asparagine was very much reduced or completely missing while in the normal plants it was present in significant amounts. All other amino acids appeared to be unaltered.

Raymond P. Amby
David K. Shortess

NORTH CAROLINA STATE UNIVERSITY
Raleigh, North Carolina
Department of Genetics

1. An oxidizable flavonoid difference in corn silks.

Last year (MGCNL, 1970) a polyphenol oxidase oxidizable flavonoid difference was reported in corn silks. When fresh silks were cut back prior to pollination, it was observed that the cut ends turned brown in a few minutes in certain lines while in others they did not brown but remained yellow-green (i.e. no change in color). Browning of cut ends

was due to the oxidation of polyphenols by polyphenol oxidase which resulted in a brown pigment formation. It was established that lines which did not brown after being cut back lacked the polyphenolic substrate. Both browning and non-browning types had the enzyme, polyphenol oxidase. Backcross ratios indicated a monohybrid segregation with the browning type being dominant to the non-browning. Further data involving larger numbers of progeny have confirmed the earlier genetic analysis (Table 1).

Table 1
Backcross segregation results and X^2 test for fit to a 1:1 ratio

Backcross	Brown type	Green type	Total	X^2	P
(NC236 x NC232) NC236 green brown green	316	331	647	.348	.5-.7
(Kys x T61) Kys green brown green	264	251	515	.328	.5-.7

Silks of the browning type have recently been studied to determine the type of polyphenolic compounds which are involved in the brown pigment formation. Fresh silks were ground and extracted in acidic 80% methanol. By means of PVP (polyvinyl-pyrrolidone) column chromatograph and paper chromatography in two solvent systems (BAW and 15% HOAc). We have found nine compounds present in the browning phenotype which were not present in the non-browning type. These nine compounds gave a positive reaction for ortho-dihydroxy flavones with Benedicts reagent on paper chromatograms. Although all nine compounds can be oxidized in the presence of polyphenol oxidase to form the brown pigment, three of the compounds occurred in much greater quantities than the remaining six. These three compounds were designated I, II, and III, in order of decreasing quantity.

Visible-ultraviolet spectroscopy studies have been carried out on compounds I, II, and III (Table 2). Spectra measurements were made in methanol alone and with the reagents, sodium acetate, sodium methoxide

Table 2

Diagnostic chromatographic attributes and spectra absorption maxima

Compound	Rf		Color of spot			Absorption maxima in nm					
	BAW	HOAc	UV	UV + NH_3	UV + Bened.	MeOH	MeOH-$AlCl_3$	MeOH-$AlCl_3$ +HCl	MeOH-NaOMe	MeOH NaOAc	MeOH-NaOAc+ H_3BO_3
I	.52	.67	D	Y	D	349 270 255 242*	429 332 302* 277	387 363 297* 279 267*	409 336* 278* 270	392 327* 274	377 264
II	.55	.43	D	Y	D	351 271 258 246*	429 332 302* 278	388 366 296* 280 265*	408 278	407 269	376 264
III	.40	.67	D	Y	D	350 271 255	429 331 302* 277	386 365 296* 279 267*	410	393 325* 274	377 264

*Shoulders
D = dark
Y = yellow
Bened = Benedicts solution

aluminum chloride, aluminum chloride-HCl solution and sodium acetate-boric acid solution. These results indicated the three compounds were derivatives of 5, 7, 3', 4' tetra-hydroxy flavone (luteolin). Acid hydrolysis experiments with these three compounds indicated that all had acid hydrolyzable O-glycosides. Of particular interest, acid hydrolysis of compound III yielded iso-orientin and orientin which are isomers. A milder hydrolysis of compound III gave primarily iso-orientin which indicated that compound III is an O-glycoside of iso-orientin. The isolation of iso-orientin established the presence of a C-glycosylflavone in corn.

The letters Fv have been selected to designate the dominant allele which results in browning of cut fresh silks because of the presence of at least nine oxidizable polyphenolics. Fv was chosen because the three compounds present in largest amounts have been identified as flavones. The recessive allele has been designated fv. In the homozygous recessive form, fv/fv, no browning of cut fresh silks occurs because the nine oxidizable polyphenols are absent. The dominant allele, Fv, appears to behave as a completely dominant allele since a quantitative distinction can not be demonstrated between the Fv/Fv and Fv/fv genotypes. The following commercial inbreds have been classified as Fv/Fv genotype: C121, Hy2, L317, NC7, NC232, T61, T204 and WF9, while the following inbreds are of the genotype fv/fv: Kys, NC34, NC45 and NC236. The open pollinated varieties Jarvis Golden Prolific and Indian Chief are segregating for Fv and fv alleles. Chromosomal aberration and marker stocks obtained primarily from the Coop carry both alleles, Fv and fv. It also appears that a third allele, which is intermediate in phenotype, has been isolated; however, genetic analysis is incomplete.

Several points can be made concerning the block to flavonoid synthesis imposed by the fv/fv genotype. First, several fv/fv genotypes have anthocyanin in their silk. Therefore, the fv/fv block doesn't prevent all types of flavonoid synthesis. Second, the fv/fv genotype prevents synthesis of at least nine different compounds, three of which are known to be flavones. The block may therefore occur early in flavone synthesis. Third, similar flavones appear to be produced in the leaves of plants which are of the fv/fv and Fv/Fv genotypes and some leaf

flavones appear identical to those produced in silk of the Fv/Fv genotype. These observations are based on R_f values obtained by paper chromatography in two solvent systems (BAW & 15% HOAc). Consequently, the block to flavone synthesis in the fv/fv genotype appears confined to the silks.

C. S. Levings, III
C. W. Stuber

NORTH CAROLINA STATE UNIVERSITY AT RALEIGH
Raleigh, North Carolina
Department of Statistics

1. A preliminary attempt to group Latin American races of maize.

Using a set of 30 characters including geographic characters as well as a set of ear, kernel, and cob characteristics known to be relatively slightly altered by environmental effects (see Goodman and Paterniani, 1969), principal components analysis and cluster analysis were employed to group 230 races of maize from Mexico, Venezuela, Colombia, Peru, Ecuador, Bolivia, Chile, Argentina, Uruguay, Paraguay, Brazil, the Guianas, and Cuba. The data were taken from the series of Races of Maize booklets describing these races, from Paterniani (1967), from the Ph.D. thesis of R. M. Bird (University of California, 1970), and from Goodman (1967, 1968, and unpublished). Distances between all possible pairs of races were calculated using the first 10 standardized principal components (those with eigenvalues of approximately one or greater). Unweighted cluster analysis (Sokal and Sneath, 1963) was then applied to those distances.

Thirty-six groups of races were obtained. (The following abbreviations are used: A = Argentina, Bo = Bolivia, Br = Brazil, Ch = Chile, Co = Colombia, Cu = Cuba, E = Ecuador, G = the Guianas, M = Mexico, Pg = Paraguay, Pu = Peru, U = Uruguay, and V = Venezuela). Brackets and parentheses indicate subgroupings. Races are listed within groups and subgroups in order of increasing average distance from those which precede them. Subgroups are also listed in order of increasing average distance.

Certain races described by Brieger et al. (1958) from eastern South America are assigned somewhat arbitrarily to a specific country or countries despite the fact that they are, in fact, found in other regions also. In cases where such races occur in Bolivia, they also occur in Brazil, Paraguay, or Argentina, and in all cases they have been designated here as belonging to one of the latter to avoid confusion with the races described in the Bolivian race bulletin.

The grouping presented is regarded as strictly tentative. If either the set of races or the set of characters analyzed were to be changed, a somewhat different grouping would be expected. However, a preliminary grouping such as this can be used for several purposes. It is planned to select a representative subset of races in order to further evaluate the characters already studied, as well as some additional characters considered to be potentially important. These include some internal characters of the cob which promise to be especially useful in studying archaeological maize remains.

Finally it is planned to use F_2 variability as a genetic check on the phenotypic similarities used in constructing these groupings. Such studies should be especially interesting in cases such as Oloton vs. Amagaceno and Pardo vs. the Harinoso de Ocho - Tabloncillo group, which suggest close relationships between South American and Mexican races. Similarly such studies should also be quite useful in cases of doubtful groupings such as Mishca with Rabo de Zoro and Maranon,

1. (Chapalote - M, Nal Tel - M, Reventador - M), (Yellow Pop - Cu, Arrocillo Amarillo - M)
2. Araucano - Ch
3. Chalqueno - M, Conico Norteno - M, Conico - M, Pepitilla - M, Palomero Toluqueno - M
4. (Tepecintle - M, Zapalote Grande - M, Tehua - M), (Zapalote Chico - M, Bolita - M)
5. (Tuxpeno - M, Vandeno - M, Celaya - M), (Jala - M, Comiteco - M)
6. (Harinoso de Ocho - M, Tabloncillo - M, Tabloncillo Perla - M, Pardo - Pu), (Harinoso de Ocho Occidentales - M, Olotillo - M)
7. Capia Amarillo - A, Capio Grande - Ch, Choclero - Ch

8. (Dente Rio Grandense Rugoso - Br, Dente Rio Grandense Liso - Br, Dente Paulista - Br, Semi-Dentado Rio Grandense - Br), (Cravo Paulista - Br, Cravo Rio Grandense - Br)
9. Lenha - Br, Moroti Guapi - Pg
10. Confite Puneno - Bo, Patillo - Bo
11. Rabo de Zorro - Pu, Maranon - Pu, Mishca - E
12. (Cabuya - Co, Huandango - E), (Rienda - Pu, Tusilla - E, Canilla - Cu, Pira Naranja - Co, Chimlos - Pu)
13. [(Cateto Sulino - A and U, Cateto Sulino Precoce - A, Cristalino - Ch), (Cateto Sulino Grosso - U, Cateto Sulino Escuro - U), Dentado Comercial - Ch], {[(Cateto Assis Brasil - Br, Camelia - Ch), (Canario de Ocho - U and A, Cateto Grande - Br), (Cateto - Br, Cateto - Bo)], [(Yungueno - Bo, Patillo Grande - Bo, Chutucuno Grande - Ch)]}, Cristalino Norteno - Ch
14. (Uchuquilla - Pu, Uchuquilla - Bo, Kcello - Bo, Karapampa - Bo), (Amarillo de Ocho - A, Capia Amarillo de Ocho - A), (Ninuelo - Bo, Chake Sara - Bo, Perola - Bo)
15. [(Cateto Nortista - G, Cateto Nortista Precoce - G), (Tuson - V, Cubano Dentado - Bo, Puya Grande - Co)], [(Costeno - V, Costeno - Co), (Arizona - Pu, Gallina - E), Cuba Yellow Flint - V, (Yucatan - Co, Sabanero - V, Comun - Co)], [(Comun - V, Puya Grande - V, Puya - Co, Chuncho - Pu), (Canilla - V, Chandelle - V), Puya - V], (Argentino - Cu, Perla - Pu, Criollo - Cu, Tuson - Cu)
16. [(Amagaceno - Co, Oloton - M), (Montana - Co, Uchima - E), Andaqui - Co, (Sabanero - Co, Morocho - Pu), Sabanero - E], [Patillo - E, Kcello - E, Clavito - E, (Huevito - V, Pollo, V)]
17. [(Coruca - Pu, Cuzco - Bo, Cuzco - Pu), (Huancavilicano - Pu, Cuzco Cristalino Amarillo - Pu, Cuzco-Huilcaparu - Bo), Cuzco Gigante - Pu, (Cuzco - E, Sabanero - Pu)], [Conico Dentado - E, Huachano - Pu), (Arequipeno - Pu, Chancayano - Pu)]
18. [(Marron - A, Culli - A, Checchi - Bo), (Harinoso Tarapaqueno - Ch, Achilli - A), Capio Negro - Ch, (Huilcaparu - Bo, Pisccorunto - Pu), Aysuma - Bo], {[(Kulli - Bo, Altiplano - Bo), (Shajatu - Pu, Huayleno - Pu), (Kculli - Pu, Granada - Pu, San

Geronimo Huancavilicano - Pu), (Ancashino - Pu, Marcame - Ch)], (Altiplano - A, Confite Puneno - Pu, Capio Chico - Ch)}, (Chaparreno - Pu, Oke - A, Negrito - Ch)

19. [(Negrito - Co, Cacao - V), (Negrito - V, Candela - E), (Pagaladroga - Pu, Guirua - Co)], {(Cariaco - V, Jora - Pu), [(Curugua Grande - Ch, Pojoso Chico - Bo), (Camba - Bo, Moroti - Pg)]}
20. (Cariaco - Co, Chococeno - Co, Mochero - Pu), (Pojoso Chico - E, Chococeno - E)
21. Montana - E, Clavo - Co, Morochon - E
22. Cristal - Br, Cristal Sulino - A and U, Morocho - A) (Guaribero - V, Aleman - Pu), Bola Blanca - A
23. (Dente Branco Paulista - Br, Dente Branco Rio Grandense - Br), (Tuxpeno - V, Argentino - Bo)
24. Capia Blanco - A
25. Coroico Blanco - Bo, Cristal Semi-Dentado - Pg
26. (Chirimito - V, Enano - Bo, Enano - Pu), (Araguito - V, White Pop - Cu, White Dent - Cu)
27. Pollo - Co, Confite Morocho - Pu
28. (Pororo - Bo, Avati Pichinga Ihu - Pg), (Pira - V, Pira - Co, Polulo - Ch)
29. (Pisincho - A, Pisankalla - Bo, Confite Puntiagudo - Pu), (Curagua - Ch, Avati Pichinga - Pg), Chutucuno Chico - Ch
30. Canguil - Ec, Imbricado - Co, Canguil Grueso - E
31. Paro - Pu, Paru - Bo
32. (Capio - Co, Blanco Harinoso Dentado - E, Cacahuacintle - M), (Cacao - Co, Alazon - Pu), Chillo - E
33. (Coroico Amarillo - Bo, Entrelacado - Br, Moroti Precoce - Pg), (Coroico - Bo, Piricinco - Pu, Morado - Bo)
34. Chullpi - Pu, Chulpi - Ch, Chulpi - A, Chuspillu - Bo*, Chulpi - E*
35. Maiz Dulce - M
36. Dulce Golden Bantam - Ch, Dulce Evergreen - Ch

---

*The addition of Chuspillu - Bo and/or Chulpi - E to this group makes the group distinctly more heterogeneous than the other groups.

Major M. Goodman
Robert M. Bird

2. Maize variation in relation to the natural and cultural environment.

Systematic studies of a maize collection from the Sierra of Peru and analysis of the environment were carried out at Berkeley, California and were summarized by a doctoral dissertation accepted by the University of California in June, 1970. Maize was studied with the aims of describing clusters of types, of defining character axes which reduce the variation to more comprehensive patterns, and of relating the types and character variation to the environment. The natural and cultural environments were described to help explain the maize variation, to provide a means of improving future maize collections, and to summarize those observations that the author made during the study.

Over 900 ears were collected and partially analyzed while with the "Study of Inca Provincial Life" in Huánuco, Peru, from 1964 to 1966. The project was mainly anthropological involving historical, archeological and ethnological work and was directed by Dr. John Murra. The author was responsible for ethnobotanical and ecological studies.

The systematic studies were based on 117 morphological variables and a sample of 65 ears from the collection. Factor analyses of this sample and of the published data of Grobman et al. (1961) gave similar results. Eight major factors have been described, called Ear Diameter and Kernel Length, Rachis Diameter and Thickness, Ear Length and Shape, Shank Diameter, Cupule Length and Shape, Row Number and Kernel Shape, Kernel Denting, and Kernel Hardness. There are several other minor factors. Using all the variables, numerical taxonomy of these ears, with nine additional ears from Mexico, Ecuador and two archeological sites on the South Coast of Peru, has shown that groupings of types are possible, but that many individuals remain intermediate or atypical. Factor analysis allows one to better understand the variables, but does not directly help the systematic work. Reduced sets of variables capable of classifying the material almost as well as the full set are difficult to select.

In order to explain the variation found in Andean maize races, one needs to compare their distribution and systematic descriptions with the environment and history. The thesis of Grobman et al. (1961) that Cuzco maize was spread by the Incas to many parts of their empire, from

Argentina to Ecuador, was found to be strengthened by linguistic evidence that the Cuzco dialect of Quechua shows a very parallel pattern. However, the Huánuco area, in the middle of what once was the Inca empire, has practically no Cuzco maize and a distinct set of Quechua dialects. A theory has been advanced that relates this anomaly to earlier Andean history. As a corollary it is proposed that Cuzco maize derives in part from maize of the Northern Andes, along with many other features of the Incas which were unique in Southern Peru.

Not only does maize relate to cultural history on a broad scale, but locally there are detailed parallels. Ethnic areas in the Huánuco region have been defined using many traits. Ethnographic data were gathered relating to agriculture, settlement pattern and clothing. Linguistic studies further added to the ethnic definitions and aided in comparison with other regions. The areas defined through these studies show very close similarity to tribal areas described at the Spanish Conquest.

Near the city of Huánuco, a people once called the Chupacho, living between 1900 and 3300 meters elevation, have maize more like that of Ecuador than that of people just 40 km up the same valley. On the mountain slopes nearer the continental divide, villages between 3000 and 4000 meters contain people distinct by a great many traits from those of the valley. Their maize is more like southern Peruvian maize than Chupacho maize. The boundary between these two peoples and their maize is exactly where documents of 1549 and 1562 say the tribes then had a boundary. Probably this pattern has been stable for a millenium.

Although the natural environment of Peru has been studied fairly extensively, including vegetational and climatic analyses by myself, there is little one can predict about maize using this knowledge. Even within an ethnic area there seems to be little relationship. McClintock has shown that high altitude maize has few chromosome knobs. Low altitude correlates moderately with larger ears with high glume to kernel and rachilla to kernel ratios, and it correlates poorly with thick shanks, longer cupules, and the "tripsacoid" character complex of Sehgal. However, this information is much less important than data on cultural

patterns and practices for the efficient collection of new maize races and populations and the interpretation of maize evolution.

Robert McK.Bird

3. Multivariate and quantitative studies of maize systematics and genetics.

In conjunction with Drs. S. G. Stephens and M. M. Goodman, several aspects of maize systematics are being investigated. A series of genetic studies is aimed toward understanding the genetic basis of several of the morphological traits used so frequently in determining racial interrelationships. A classification of maize races is being attempted by using different multivariate techniques. Many new morphological characters are being evaluated. Although no method seems to exist for selecting a small set of characters which would classify material almost as well as the full set, it is hoped that eventually there will be a reduction in the number of variables needed. Probably different subsets will be needed for defining maize groups and races within each geographical region and within each major subdivision of the species. A reduced set of variables accounting for most of the variation present in the maize to be studied would greatly aid research into maize evolution by allowing much larger samples to be studied.

Although several hundred maize races and subraces have been described, there has been little success in determining their interrelationships. A study of variables can be more carefully and easily made when the data are obtained from a "balanced" sample of objects, "balanced" in that all the major trends of evolution are sampled. Therefore tentative groupings of races are being described, perhaps 40 for the present species Zea mays. Many races will be unique, intermediate or too variable to be properly ascribed to a group. A more careful grouping of races should be possible when the variables have received more study.

During my doctoral work an attempt was made to use factor analysis for classifying. It was not fully satisfactory, but factor analysis helps greatly in more meaningful selection and characterization of variables.

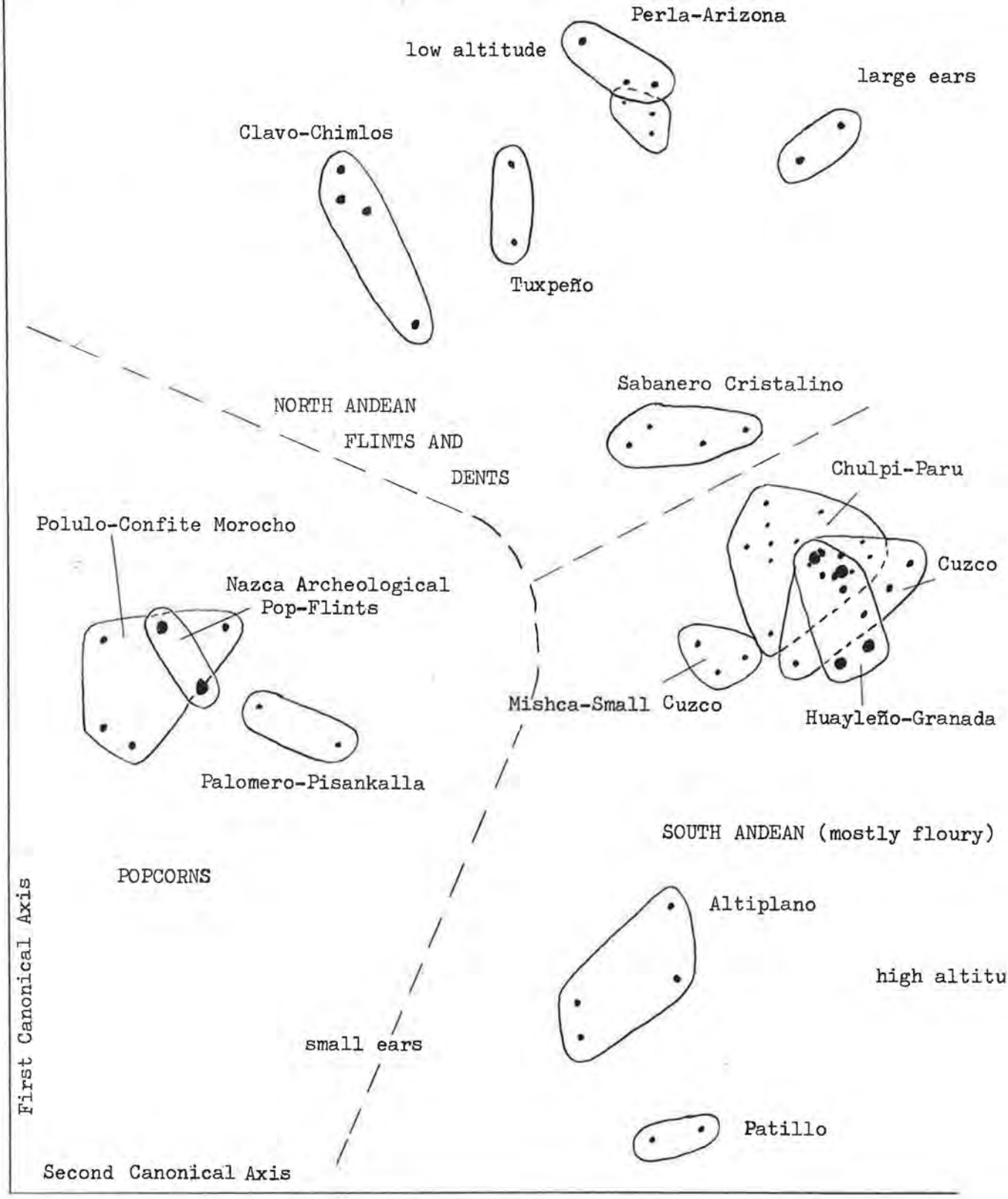
M. Goodman at this campus is continuing study of principal components of maize, the effects of the environment on variables and

classification of maize races, especially those of the Amazon area and Central America.

Numerical taxonomy provides a means of classifying that many argue holds special promise. However, the more widely available techniques do not provide a method that indicates relationship in multidimensional space, a necessity for comprehending the nearly chaotic variety of maize races.

Preliminary results using canonical analysis have convinced S. G. Stephens (for Gossypium), M. Goodman and myself that this technique provides a useful portrayal of relationships. Therefore I will briefly describe early results of a canonical analysis of 57 ears from Peru, two from Ecuador and two archeological specimens from south coastal Peru (Fig. 1). The ears are typical specimens of races already described and of new races found in Peru. These are the specimens that seem to readily fit into groups that can be defined by numerical taxonomy using 117 variables and by a prior canonical analysis using six variables. Additional material which does not easily fit into one of the 15 groups is not used in the analysis. Figure 1 results from the input of 32 variables selected to represent all the factors described in my doctoral research, these variables also having high values on a standard F test (ratio of between to within group variance).

At least three loose divisions seem possible for the sample at hand--temperate popcorns, South Andean flours and North Andean flints. The groups are tentatively labeled Polulo-Confite Morocho (South Andean small-cob popcorns), Palomero-Pisankalla (conical-eared popcorns with white, pointed kernels, probably lately introduced), Nazca Archeological Pop-Flints (multi-rowed ears with small kernels), Clavo-Chimlos (elongate flints or dents), Tuxpeño (flinty dents from the tropical Caribbean area), Amagaceño (flints of middle altitude), Perla-Arizona (large-eared flinty dents), Sabanero Cristalino (small-eared flints of middle altitude), Cuzco (large-grained, eight-rowed types not exclusively South Andean; both floury and flinty), Mishca and Small Cuzco (similar to Cuzco but smaller, floury), Huayleño-Granada (small-eared flours of high altitude), Chulpi-Paru (large-eared, multi-rowed flours of high altitude), Altiplano (very small flours of very high altitude), Patillo (very small flints of


Perla-Arizona
low altitude
large ears
Clavo-Chimlos
Tuxpeño
Sabanero Cristalino
NORTH ANDEAN
FLINTS AND
DENTS
Chulpi-Paru
Polulo-Confite Morocho
Cuzco
Nazca Archeological
Pop-Flints
Mishca-Small Cuzco
Huayleño-Granada
Palomero-Pisankalla
SOUTH ANDEAN (mostly floury)
POPCORNS
Altiplano
high altitu
small ears
Patillo
First Canonical Axis
Second Canonical Axis

very high altitude) and an unlabeled potential group of large-eared, middle altitude flours from near Huánuco at the upper right of Fig. 1.

There are several limitations to Fig. 1, a plot based on the two most important canonical variables or axes. To properly separate the several groups in close proximity in the center of Fig. 1, at least two additional axes are needed since the distances between these groups are not well demonstrated with these two axes. By using the canonical axes here presented, one does not obtain satisfactory classification of new material unless it is closely related to one or more of the 15 groups.

Canonical analyses using other sets of variables, cob characters, characters predicted to be of "low value," and the six "best" variables, also give good separation of these groups, although the relative spacing is different. Not only does this help to corroborate the grouping presented, but it means that one can classify material using whatever variables are available.

The genetic studies are only being started. Following the suggestion of M. Goodman, the variabilities found in the F_2 generations of crosses between widely separated races will be compared with the phenotypic distances. At the same time the genetic inheritance of some important traits may be studied. It should also be possible to observe introgressive effects of one race on others.

Robert McK.Bird

OSMANIA UNIVERSITY
Hyderabad 7, India
Department of Genetics

1. Chemical nature of the a_2 mutant.

About one kg. of a_2 and in a_2 seeds, after removal of pericarp, were defatted with petroleum ether and then extracted with cold methanol. The solvent was removed under reduced pressure and was treated with ether. Ether insolubles were separated by filtration and treated with distilled water. The aqueous solution was extracted repeatedly with ethyl acetate, and dried over anhydrous magnesium sulphate. The solvent is removed under cold conditions on a flash evaporator, which gave a

light brown substance (10 mg.). The leucosubstance thus obtained was converted to its corresponding flavylium chloride, repeatedly extracted with isoamyl alcohol, and was further purified by circular, ascending and descending chromatographic techniques with two different solvent systems, n-butanol: acetic acid: water (4:1:5) and Forestal (acetic acid 30: HCl 3: water 10).

The developed chromatograms of the crude methanolic solution of the leuco substance, after spraying with ferric reagent and subsequent heating for 30 minutes at 80°C in an hot air oven, showed a distinct green colour, whereas, with vanilin-toluene-p-sulphonic acid, they gave a strong violet red colour. The crude methanolic solution of the leucosubstance was spotted on a circular chromatographic paper and developed with the above mentioned solvent systems. The developed chromatograms, when sprayed with 10% sulphuric acid and heated, gave a characteristic purple colour. The detected spots on the chromatograms of converted anthocyanidins were eluted with 5% methanolic-HCl solution and subjected to Beckman DB spectrophotometer. The average Rf values and the absorption maxima of the converted anthocyanidin along with pure cyanidin chloride (control) are presented in Table 1.

Table 1

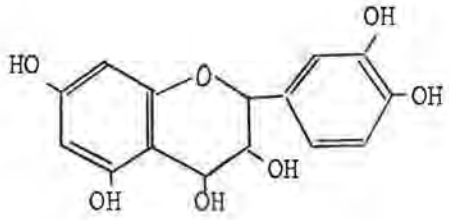
The R_f values and absorption maxima of the converted anthocyanidin and pure cyanidin chloride

Genotype	R_f values		Absorption maxima in mμ	
	BAW	FORESTAL	UV	VISIBLE
$\underline{a}_2$	0.68	0.48	279	541
$\underline{in}\ \underline{a}_2$	0.68	0.47	278	542
Cyanidin chloride	0.69	0.49	279	543

In addition to the chromatographic and spectrophotometric studies, several chemical tests have been used for further characterization and confirmation of the chemical nature of the substance. The leucosubstance

gave a negative response to the "Mollish test" for carbohydrates and also some other characteristic tests for carbohydrates, suggesting that it is an aglycone. The presence of a catechol moiety in the leucoanthocyanidin is confirmed by its characteristic green colour with ferric reagent on the developed chromatogram. Its characteristic colouration with ferric chloride suggests the phenolic nature (diol 1:2) of the substance. The presence of a phloroglucinol moiety is confirmed by the characteristic strong violet red colour on heating with vanilin-toluene-p-sulphonic acid. The tests with aqueous NaOH and concentrated sulphuric acid suggested the oxygen heterocyclic nature of the compound. A rose red colour was observed when it reacted with the cyanidin reagent (1 part of cyclohexanol and 5 parts of toluene), indicating that the converted pigment is cyanidin. The conversion of the colourless leucoanthocyanidin solution to the pink coloured anthocyanidin, i.e., cyanidin, on heating with 2N HCl in a water bath for a few minutes further suggests that the compound is leucocyanidin.

Thus the chromatographic, spectrophotometric analyses, in addition to chemical tests, clearly suggest that the accumulated substance in $\underline{a}_2$ mutant aleurones is 5, 7, 3', 4', tetrahydroxy flavan 3:4 diol (leucocyanidin).



Leucocyanidin

Preliminary studies with alcoholic extracts of various plant parts such as leafsheaths, leaves, husks, cobs and tassels of the $\underline{a}_2$ mutant gave the indication of the presence of leucoanthocyanidin.

A. R. Reddy
G. M. Reddy

2. Peroxidase isozymes in the developing endosperm of maize.

Zymograms of peroxidase isozymes from five different stages, i.e., 10, 15, 20, 25 and 30 day old developing endosperms, of opaque-2 mutant and normal Indian inbred maize (CM 201) were developed at pH 8.5 in polyacrylamide gel. The bands of zymograms were divided into three zones, a cathodal zone with slow mobility, a central zone with intermediate mobility, and an anodal zone with faster mobility. Bands within each zone were numbered in increasing order of mobility.

The opaque-2 and the normal differed widely in the number of peroxidase bands in the 10 day and 30 day old endosperms, but only by a single band in the 15 day, 20 day and 25 day old endosperms.

The 25 day old endosperm of opaque-2 exhibited the maximum number of twelve bands, five cathodal (C_1-C_5), two intermediate (I_1 and I_2), and five anodal (A_1-A_5); whereas the normal showed eleven bands with only the A_3 band of the anodal zone missing.

The 10 day old endosperm of opaque-2 showed seven bands C_2, C_3, C_4, I_1, I_2, A_1 and A_2 while the normal showed only four bands (C_2, C_4, I_1 and I_2). The 30 day old endosperm of normal showed eight bands (C_1 to C_4, I_1, I_2, A_1 and A_2) but opaque-2 showed only six, the bands I_1 and A_2 being absent.

Differences in the intensity of the bands were also observed in terms of the maturity and the type of endosperm, especially in the C-zone.

The developing opaque-2 and normal endosperms show significant qualitative differences only in the C-zone, which suggests that this zone might control the phenotypic difference and possibly the high lysine and tryptophan content in the opaque-2 mutant.

Table 1

The peroxidase isozyme pattern in normal (N) and opaque-2 ($\underline{o}_2$) endosperm

Bands	10 day		15 day		20 day		25 day		30 day	
	o_2	N	o_2	N	o_2	N	o_2	N	o_2	N
A. Cathodal zone										
C_1	-	-	-	-	-	-	+	+	+	+
C_2	+	+	+	+	+	+	+	+	+	+
C_3	+	-	+	+	+	+	+	+	+	+
C_4	+	+	+	+	+	+	+	+	+	+
C_5	-	-	+	+	+	+	+	+	-	-
B. Central zone										
I_1	+	+	+	+	+	+	+	+	+	+
I_2	+	+	+	+	+	+	+	+	-	+
C. Anodal zone										
A_1	+	-	+	+	+	+	+	+	+	+
A_2	+	-	+	+	+	+	+	+	-	+
A_3	-	-	+	-	+	-	+	-	-	-
A_4	-	-	+	+	+	+	+	+	-	-
A_5	-	-	+	+	+	+	+	+	-	-

A. Padma
G. M. Reddy

3. Induction of mutations with hydrazine.

Homozygous multiple dominant seed with $\underline{Bm}_2$, $\underline{Lg}_1$, $\underline{A}_1$, $\underline{Su}_1$, $\underline{Pr}$, $\underline{Y}_1$ $\underline{Gl}_1$, $\underline{Wx}$ and $\underline{G}_1$ markers was treated with 0.04 M and 0.08 M of hydrazine ($NH_2NH_2 \cdot H_2O$) at pH 8.5. One thousand seeds for each treatment were taken. The seeds were presoaked for 24 hours in water. The treatment duration was 24 hours.

In the case of 0.04 M hydrazine treatment, the following eleven seedling mutations were observed out of 512 plants in the M_1 generation (M_1 = the seedlings raised from treated seeds).

Table 1

Types and numbers of M_1 mutant seedlings

	Seedling mutants						Total
	Dwarf	Adherent	Old gold stripe	Golden	Pigmy leaf	Brown midrib	
Hydrazine (0.04 M)	1	5	1	1	2	1	11

The present information suggests that hydrazine may induce mutations even in the M_1 generation, most probably by altering both alleles simultaneously. Hydroxylamine, diethylsulphate or γ-rays in different doses, however, did not induce mutations in the M_1 generation. Further studies are in progress.

K. Vaidyanath
V. S. G. Chandrasekhar
G. M. Reddy

4. Chlorophyll studies in a DES-induced yellow-green mutant.

Quantitative estimations of the chlorophyll content in induced yellow-green and other known mutants were carried out and compared with the control (Table 1).

The procedure for chlorophyll determination was based on the absorption of light by aqueous acetone (80%) extracts of chlorophyll. The concentrations of chlorophyll a and b were determined by measuring the density of 80% acetone chlorophyll extracts in a Beckman DB spectrophotometer at 663 and 645 mμ.

The total chlorophyll content of the induced yellow-green, although more than that of the yellow-green-2 ($\underline{yg}_2$) and pale-green ($\underline{pg}_{11}\underline{pg}_{12}$), is about one fourth of the control.

Table 1

Quantitative estimations of chlorophyll a and b (mg/g)

Sl.No.	Phenotype	Chlorophyll a	Chlorophyll b	Total chlorophyll
1.	Yellow-green-2 ($\underline{yg_2}$)	0.1270	0.1263	0.3032
2.	Pale green ($\underline{pg_{11}}\underline{pg_{12}}$)	0.2657	0.1968	0.5027
3.	New mutant yellow-green	0.3089	0.3193	0.7394
4.	Control	1.1000	1.1270	2.9000

V. S. Bharathi
G. M. Reddy

5. Sectoring pattern in yellow opaque-2 maize.

In a yellow opaque-2 synthetic, certain seeds were observed with normal sectors. These were classified into five types, S_2, S_3, S_4, S_5 and S_6, S_1 being completely opaque. S_5 is similar to the half opaque-half normal kernels reported earlier by Paez et al. (Crop Sci. 9:1969). The sectoring pattern in these S-types extends from a few normal cells to half a kernel.

Diallele crosses were performed among these five S-types and also with the S_1 control. The preliminary studies with reciprocal crosses between S_1 and S_3 and the diallele crosses between S_6 and the other S-types suggest that the female parent may influence the pattern of sectoring. The selfed S-types segregate into one or more original S-types including some new S-types with the exception of S_2 which gives only the S_2 type. Further studies regarding their behaviour and genetic basis are in progress.

Annapurna
G. M. Reddy

PURDUE UNIVERSITY
Lafayette, Indiana
Department of Botany and Plant Pathology

1. Modification of R^{st} stability.

M^{st}, a major modifier of the R^{st} phenotype, is located about 6 crossover units distal to the R-locus. The modifier interacts with R^{st} to increase the frequency of colored spots in the aleurone. Tests by several investigators have shown that M^{st} does not alter the frequency of germinally recoverable mutations of R^{st} to self-colored aleurone (R^{sc}), despite its striking effect on aleurone phenotype.

Exceptional ears from crosses involving R^{st} M^{st} have been observed by the author and others in which it appeared that M^{st} was assorting independently of the R locus. Progeny tests from some of these ears verified the independent assortment of a modifier of the R^{st} phenotype. Two such isolates were designated transposed-$M^{st}1$ (tp-$M^{st}1$) and transposed-$M^{st}2$ (tp-$M^{st}2$), and the following two stocks were established: R^{st} +/R^{st} +, tp-$M^{st}1$/tp-$M^{st}1$; R^{st} +/R^{st} +, tp-$M^{st}2$/tp-$M^{st}2$. The phenotypic effects of tp-$M^{st}1$ and tp-$M^{st}2$ were not measured quantitatively but both gave the general impression of a darker phenotype than that produced by M^{st} in the linked position.

The above two stocks were tested for frequency of mutation to R^{sc} by pollinating them with r^{r} pollen and growing out the self-colored kernels for verification. The data from these tests are shown below together with those from other tests measuring the frequency of R^{sc} mutations in $R^{st}M^{st}$ and R^{st} + stocks.

	R^{sc} frequency	Rate X10^{-4}	Limits of expectation (P=.05) lower	upper
R^{st} +/R^{st} +, tp-$M^{st}1$/tp-$M^{st}1$	59/8,822	66.9	50.9	86.3
R^{st} +/R^{st} +, tp-$M^{st}2$/tp-$M^{st}2$	26/8,200	31.7	21.8	45.4
R^{st} M^{st}/R^{st} M^{st}	41/23,830	17.2	12.3	23.3
R^{st} +/R^{st} +	129/60,576	21.3	17.8	25.3

The two bottom lines in the above tabulation show that $\underline{M}^{st}$ in the linked position had no significant effect on the frequency of $\underline{R}^{sc}$ mutations. These two rates are lower than those in the $\underline{tp\text{-}M}^{st}$ stocks; the difference was significant for $\underline{tp\text{-}M}^{st}1$ and approached significance for $\underline{tp\text{-}M}^{st}2$. Also, the $\underline{tp\text{-}M}^{st}1$ rate was significantly higher than the $\underline{tp\text{-}M}^{st}2$ rate. If $\underline{tp\text{-}M}^{st}1$ and $\underline{tp\text{-}M}^{st}2$ are in fact $\underline{M}^{st}$ transpositions, $\underline{M}^{st}$ has been altered in such a manner that it increases the frequency of $\underline{R}^{st}$ to $\underline{R}^{sc}$ mutations, and the degree of increase was not the same in the two cases tested. The altered action of $\underline{M}^{st}$ could be the consequence of the positional change or of a transposition associated mutation (change of state).

It is possible that $\underline{tp\text{-}M}^{st}1$ and $\underline{tp\text{-}M}^{st}2$ are not in fact $\underline{M}^{st}$ transpositions but transpositions of some other element, probably from the $\underline{R}$ locus, that modifies the stippled phenotype in a manner similar to $\underline{M}^{st}$ but also increases the instability of $\underline{R}^{st}$. Kermicle (Genetics 64:247-258) has suggested that a hypothesized $\underline{R}$ locus element ($\underline{I}^{R}$) might, following transposition, have a phenotypic effect on $\underline{R}^{st}$ similar to that of $\underline{M}^{st}$.

R. B. Ashman

2. Plant color suppression by a component of the R^{st} gene.

Colorless and near-colorless aleurone mutants of several different classes have been isolated from $\underline{R}^{r}$ $\underline{R}^{st}$ plants. Mutants of one class are associated with crossing over, have near-colorless aleurone, and mutate from green to red plant color. The $\underline{R}^{st}$ allele has been resynthesized in heterozygotes between a mutant of this class, $\underline{r}^{g}$(nc)1-3, and $\underline{R}^{sc}$ (self-colored aleurone mutant from $\underline{R}^{st}$) (Ashman Genetics 64:239-245). Based on these and other data, $\underline{R}^{st}$ was postulated to be composed of an aleurone pigmenting component, $\underline{Sc}$, and a pigment inhibitor, $\underline{I}^{R}$. On this basis, the resynthesis of $\underline{R}^{st}$ in the above heterozygote resulted from a crossover that brought together on the same chromosome an $\underline{Sc}$ component from $\underline{R}^{sc}$ and an $\underline{I}^{R}$ component from $\underline{r}^{g}$(nc)1-3.

$\underline{r}^{g}$(nc)1-3 mutates from green to red plant color, and these mutants were designated $\underline{r}^{r-m}$(nc)1-3. Tests were made to determine the effect of the plant color mutation on the resynthesis of $\underline{R}^{st}$ in heterozygotes with $\underline{R}^{sc}$. The data are presented below.

Heterozygous combinations	Progeny numbers	Number of kernels	No. of $\underline{R}^{st}$ mutants
$\underline{r}^{r-m}$(nc)1-3/$\underline{R}^{sc}$	68:512-525 69:121-127	88,645	0
$\underline{r}^{g}$(nc)1-3/$\underline{R}^{sc}$	64:278-281 66:67-73 69:106-112	109,217	20

$\underline{R}^{st}$ was not resynthesized in the heterozygous combination involving the red plant mutant $\underline{r}^{r-m}$(nc)1-3. Based on the frequency of $\underline{R}^{st}$ resynthesis in the heterozygous combination involving $\underline{r}^{g}$(nc)1-3, the kernel population from the $\underline{r}^{r-m}$(nc)1-3 combination should have yielded about 16 $\underline{R}^{st}$ mutants, and their absence is clearly significant.

The data above indicate that $\underline{I}^{R}$ is lost when $\underline{r}^{g}$(nc)1-3 mutates to $\underline{r}^{r-m}$(nc)1-3. The direction of the plant color mutation, from green to red, suggests loss of a suppressor of a plant pigmenting component, and the implication is that $\underline{I}^{R}$ is the suppressor. A crossover in the parental $\underline{R}^{r}$ $\underline{R}^{st}$ plants could have brought together the plant color component (P) from $\underline{R}^{r}$ and $\underline{I}^{R}$ from $\underline{R}^{st}$.

R. B. Ashman

STATION D'AMÉLIORATION DES PLANTES
Ecole Nationale Supérieure Agronomique
Montpellier, France

1. Rapid test to screen "opaque 2" maize for approximate lysine content.

The obtaining of hybrids, from maize "opaque 2," rich in lysine with higher test weight than kernels that are entirely opaque would be interesting from an agronomic standpoint. We do not know the relations between lysine content of the entire kernel and the degree of opacity. A test would be very useful in order to screen, at the beginning of a breeding program, the young lines rich in lysine with modified opaque kernels (opaque 2 x modifier genes interactions).

Described below is a test called "double-analyzing of nitrogen" using Kjeldahl and Pro-meter, a dye-binding method.

The principle of this test is based on the following observations:

- the contents of nitrogen, determined with Pro-Meter and Kjeldahl, are identical with "normal" kernels,
- the Pro-Meter and Kjeldahl determinations give different values in tests of "opaque 2" kernels; the proteins of "opaque 2" kernels absorb more dye than those of "normal" kernels.

The importance of the difference between nitrogen content, as determined with Pro-Meter and Kjeldahl, is related with the proportions

among different protein fractions and in particular with the quantity of proteins others than zein, those having a greater biological value than zein.

The author investigated 26 different selections. He found a correlation ($r = 0.800$) between the quantity of lysine in the entire kernel and the difference of nitrogen content : Pro-Meter less Kjeldahl.

The test of "double-analyzing of nitrogen" is simple, cheap, rapid (40-50 determinations a day) and sufficiently accurate to screen corn inbred lines for approximate lysine content.

Sylvain Rautou

UNIVERSITY OF SYDNEY
Sydney, N.S.W., Australia
Department of Agricultural Botany

1. Aleurone color variegation involving the R locus.

A heritable system producing aleurone color variegation was reported in M.G.C.N.L. 43:176-178, 1969. The variegation consists of irregular colorless aleurone areas on a colored aleurone background. Further study of this system has shown the aleurone variegation to be determined by interaction of an element, designated $\underline{Ac}^R$, with alleles at the R locus.

The $\underline{Ac}^R$ element is inherited independently of the R locus, and it exhibits the following properties:

(1) $\underline{Ac}^R$ is effective in producing variegation only when present in 2 or 3 doses in the triploid endosperm.

(2) $\underline{Ac}^R$ is pollen transmitted at markedly reduced frequency. About 1/3 of the male gametes from $\underline{Ac}^R/-$ plants transmit the element.

(3) Ovule transmission of $\underline{Ac}^R$ is slightly reduced.

(4) Presence of $\underline{Ac}^R$ in a plant appears associated with some reduction in seed sets. There appears to be no reduction in pollen fertility in $\underline{Ac}^R/-$ plants.

The $\underline{Ac}^R$ element thus exhibits an unusual pattern of inheritance and spectrum of effects. The element exhibits no specificity for

Table 1

Relation of grade of variegation and number of $\underline{R}^g$ alleles

Paired matings	Number of $\underline{R}^g$ alleles	Frequency distribution of kernels by grade of variegation*							Total no. colorless squares	Diff.
		0 Not. var.	0 Var.	1-5	6-10	11-15	16-20	21-25		
1	2	16	5	9	10	7	2	1	244	231
	3	37	7	6	-	-	-	-	13	
2	2	23	7	14	3	1	2	-	204	188
	3	38	4	8	-	-	-	-	16	
3	2	26	2	9	9	4	-	-	143	87
	3	33	3	11	2	1	-	-	56	
4	2	25	1	16	6	2	-	-	127	63
	3	36	3	6	2	3	-	-	64	

*Number of colorless aleurone squares in a 5 x 5 reticule grid.

particular R alleles, since aleurone variegation was produced in interaction with all R alleles tested, including R^r, R^g, R^{sc} mutants from stippled, R^{nj} and a number of hypomorphic R^g mutants. The element produces aleurone variegation but not mutation of R alleles, since no instance of germinal mutation of R alleles has been observed in Ac^R carrying plants.

Involvement of the R locus in this aleurone variegation system was initially suggested because the phenotype appears due to loss of capacity to produce pigment, and the R locus was the only one of the complementary dominant genes involved in aleurone pigmentation that was segregating in the genetic stocks under study. This argument for association with R alleles is negated by the observation of aleurone variegation in endosperms homozygous for an R^g allele, e.g., in selfed matings on R^g R^g, Ac^R/- plants. Following these observations, aleurone variegation could involve any one, all or none of the genes involved in producing aleurone variegation.

Evidence for an integral involvement of R alleles in the variegation system was sought by examining the relationship between grade of variegation (i.e. the relative frequency of colored and colorless aleurone areas) and number of R^g alleles in the triploid endosperm. Two experiments were conducted. In the first, plants of constitution R^g R^g, Ac^R/- producing two ears were double pollinated. One ear was selfed, and the other was pollinated with r^r/r^r pollen, thus providing on the 2 ears of one plant the contrast of R^g R^g R^g and R^g R^g r^r endosperms. The resulting paired ears were scored by recording the number of colorless squares in a 5 x 5 reticule grid superimposed on the abgerminal surface of each of 50 kernels from each ear. The data so obtained are given in Table 1.

These data clearly show a relationship between frequency of variegated kernels, and grade of variegation, with number of R^g alleles in the triploid endosperm.

In the second experiment colored kernels from selfed matings on R^g/r^r, Ac^R/- plants were classified for grade of variegation and subsequently germinated for determination of seedling color. If the R locus is not associated with grade of variegation, a 2 red: 1 green seedling ratio would be expected in each phenotypic class. The data in Table 2,

Table 2

Classification of seedling color of seedlings from $\underline{R}^g/\underline{r}^r$ $\underline{Ac}^R/-$ selfed matings

Kernel phenotype	Red seedlings R^g/r^r	Green seedlings R^g/R^g	Total seedlings
Variegated-Minute	38	41	79
-Medium	86	34	120
-Extensive	74	21	95
Not variegated	370	182	552
Total	568	278	846

show that this expectation was not realized, and there was a compensated deviation from the expected ratio. In the minute variegated class of kernels there was an excess of green seedlings ($\underline{R}^g\underline{R}^g$ $\underline{R}^g$ endosperms), and in the extensive variegated class there was a deficiency of green seedlings, but the overall ratio of red to green seedlings within the variegated class of kernels is close to the 2 red : 1 green ratio expected.

Both these results are consistent with an interpretation that colored to colorless variegation is produced if $\underline{R}$ action in pigment production is lost in the aleurone, and that there is a reduced probability of loss of $\underline{R}$ action in $\underline{R}^g$ $\underline{R}^g$ $\underline{R}^g$ endosperms compared with $\underline{R}^g$ $\underline{R}^g$ $\underline{r}^r$ & $\underline{r}^r$ $\underline{r}^r$ $\underline{R}^g$ endosperm kernels.

These results implicate the $\underline{R}$ locus in production of the aleurone variegation, and they suggest that aleurone variegation is due to an interaction between the $\underline{Ac}^R$ element and a component situated at or near the $\underline{R}$ allele involved. All $\underline{R}$ alleles appear to have the latter component, and it may be a basic $\underline{R}$ gene element responsible for regulation of gene action at the $\underline{R}$ locus.

K. S. McWhirter

2. Phenotype reversal in a variegated aleurone color system.

A variegated aleurone color phenotype, produced by interaction of an element, designated Ac^{R}, with alleles at the R locus, was described in the preceding note. This phenotype consists of irregular colorless areas on a colored aleurone background. The variegated phenotype can be considered to result from a loss of pigmenting capacity in tissues normally capable of aleurone pigmentation.

A heritable alteration of one of the components of this system has given rise to phenotype reversal. A mutant which produces a "dotted" aleurone phenotype, consisting of regular, small pigmented areas on a colorless background, has been isolated. The latter phenotype can be considered to result from a gain of pigmenting capacity in aleurone cells in which this capacity is normally inhibited. The phenotype is similar to that produced by action of Dt on a_1, except that the "dots" of pigmented aleurone cells are smaller and more numerous than is characteristic of the a_1 - Dt system.

The exceptional kernels which gave rise to the "dotted" aleurone mutant stock occurred in a somatic sector of 3 "dotted" aleurone and 3 near colorless kernels on the basal portion of an ear produced by selfing a plant of R^{g}/R^{g}, Ac^{R}/-, genotype. Plants grown from the exceptional "dotted" aleurone kernels gave selfed ears segregating "dotted" aleurone, near colorless aleurone and fully pigmented kernels, whereas plants grown from parental type kernels gave ears segregating only colored, variegated and colored, nonvariegated kernel phenotypes as expected.

Testcrosses of the plants from exceptional kernels show that the R locus constitution of these plants is unchanged. All three plants were R^{g}/R^{g}.

The Ac^{R} element, present in the parental plant, could not be detected in plants grown from "dotted" kernels.

The present observations suggest that the "dotted" aleurone phenotype resulted from heritable alteration of the Ac^{R} element. The new form of this element apparently has a suppressor-mutator effect, giving a "dotted" phenotype, in contrast to the earlier form, which had an irregular suppressor effect giving a variegated aleurone phenotype.

K. S. McWhirter

3. A floury endosperm, high lysine locus on chromosome 10.

A floury endosperm mutant, which occurred spontaneously in a W22 $\underline{ACr}^g/\underline{ACr}^g$ stock, was analyzed for lysine content.* The floury mutant had 3.4 gms lysine per 100 gms protein, compared with 2.1 gms lysine/100 gms protein for the isogenic normal endosperm stock of W22. In the same analysis opaque-2 and floury-2 stocks gave values of 3.65 and 3.2 gms lysine/100 gms protein, respectively.

The floury mutant is linked with R on chromosome 10. Five separate estimates of the recombination value in $\underline{R}^r+/\underline{r}^g\underline{fl}$ plants are 23%, 26% (F_2 Data); and 22%, 24% and 26% (Backcross data). Linkage relations with other chromosome 10 markers are being determined.

*Performed by Dr. J. A. Ronalds,
C.S.I.R.O. Wheat Research Institute.

K. S. McWhirter

4. Measurement of gene effects by application of a mathematical model to triploid endosperm data.

Various models have been used by workers such as Hayman & Mather, Kempthorne & Cockerham to describe gene action and interaction for diploid genotypes. Data are available for endosperm tissue which gives sixteen triploid genotypes rather than the nine genotypes from diploids. The triploid data are used in this article for application of Cockerham's model. In the original diploid model, 9 orthogonal coefficients were used to provide each of 8 parameters which measured deviations from the mean value for the 9 genotypes. The triploid model has 16 orthogonal coefficients for each parameter, as shown in the columns of Table 1. The sixteen columns are headed by μ, the mean value of the 16 genotypes and the symbols for the 15 parameters which describe the gene action or deviation from the mean.

At the foot of each column are figures for these parameters derived from three sets of data. The sources of these data were:

1) K. S. McWhirter (1962). "A Phenotypic Comparison of Three Stippled Genes" M.G.C. N.L. 36:100-101.
2) Helm, J. L., V. L. Ferguson & M. S. Zuber (1969). "Interaction of Dosage Effects on Amylase Content of Corn at the Du and Wx loci" Hered. 60:259-260.

3) These data are derived from a maize stock in which the kernels vary in colour from yellow, through lemon, to white. This variation is due to the interaction of genes at the Y-y locus on chromosome 6 and genes at an independent, unknown locus, provisionally designated ly-iy.

The gene ly acts as a partially dominant inhibitor of the yellow colour produced by Y. The full yellow colour of Y when only iy is present is reduced to a lemon colour when ly is present. y gives a white or cream colour when only iy is present and the presence of ly changes the cream colour to pure white.

Genetic experiments were completed to prove that this system did in fact involve an interaction between genes at two independent loci. From a cob which carried yellow, lemon and white kernels in the ratio of 3Y:9L:4W the results shown in Table 2 were obtained.

A hexane extract of the maize kernels was used to give total carotenoid content in p.p.m. as a measure of yellow pigments. These data are recorded in Table 3.

The groups of figures in Table 3 are from two sources. Figures without brackets are the observed figures for the particular character, i.e., carotenoid content. Figures enclosed by brackets are obtained by summing the additive, dominance and interaction effects for a particular genotype. These additive, dominance and interaction effects are obtained from the parameters at the foot of Table 1 in the following manner.

Additive = α component for the particular gene, e.g., $\underline{ly}_\alpha$ or $\underline{Y}_\alpha$

Dominance = $\beta+\delta$ components for a particular gene, e.g., $\underline{ly}_\beta+\underline{ly}_\delta$

Interaction = $\alpha\alpha+\beta\beta+\delta\delta+\alpha\beta+\beta\alpha+\alpha\delta$ etc. or the sum of all the interacting terms for the two genes.

For each genotypic value, the expected value (Figures in brackets Table 3) can be calculated by summing the term x orthogonal coefficient for that term and that genotype.

Using genotype ly ly ly YYY as an example.

Expected Value = $1x\mu+3x1y_\alpha - 1x1y_\beta+1x1y_\delta+3x\underline{Y}_\alpha- 1x1y_\beta+1x1y_\delta \ldots-1x1y_\delta\, Y_\beta$

= 1x5.94+3x-1.72-1x-2.61+1x-0.38+3x1.42-1x0.79+1x0.04+9x-0.54-3x-0.76-3x-0.14+1x-0.23+3x0.2-1x0.0+1x-0.03+3x-0.09-1x-0.08

= 3.97

Using this set of figures, the effects of the various components can be gauged with more accuracy than a subjective analysis will allow.

For example, the additive $\underline{ly}_\alpha$ effect reduces yellow colour more than the $\underline{Y}_\alpha$ effect increases colour.

Table 1

Orthogonal coefficients used with triploid data to determine the deviation from the mean value due to the fifteen parameters shown

Genotypes	μ	l_α	l_β	l_δ	Y_α	Y_β	Y_δ
lylyly YYY	1	3	-1	1	3	-1	1
" YYy	1	3	-1	1	1	1	-3
" Yyy	1	3	-1	1	-1	1	3
" yyy	1	3	-1	1	-3	-1	-1
lylyiy YYY	1	1	1	-3	3	-1	1
" YYy	1	1	1	-3	1	1	-3
" Yyy	1	1	1	-3	-1	1	3
" yyy	1	1	1	-3	-3	-1	-1
lyiyiy YYY	1	-1	1	3	3	-1	1
" YYy	1	-1	1	3	1	1	-3
" Yyy	1	-1	1	3	-1	1	3
" yyy	1	-1	1	3	-3	-1	-1
iyiyiy YYY	1	-3	-1	-1	3	-1	1
" YYy	1	-3	-1	-1	1	1	-3
" Yyy	1	-3	-1	-1	-1	1	3
" yyy	1	-3	-1	-1	-3	-1	-1
Calculated Parameters							
ly Y	5.94	-1.72	-2.61	-0.38	1.42	0.79	0.04
	μ	Du_α	Du_β	Du_δ	Wx_α	Wx_β	Wx_δ
Du Wx	21.05	-0.98	-0.98	-0.22	4.34	6.28	1.09
	μ	M^{st}_α	M^{st}_β	M^{st}_δ	R^{st}_α	R^{st}_β	R^{st}_δ
R^{st} M^{st}	39.08	6.20	-4.27	0.11	11.90	-4.02	0.61

These figures, when multiplied by the appropriate orthogonal coefficient (i.e., in the same column and the row corresponding to the required genotype), are summed to give the expected value for that genotype.

Table 1 (Continued)

Parameters								
$I_{\alpha}^{Y}{}_{\alpha}$	$I_{\beta}^{Y}{}_{\alpha}$	$I_{\alpha}^{Y}{}_{\beta}$	$I_{\beta}^{Y}{}_{\beta}$	$I_{\alpha}^{Y}{}_{\delta}$	$I_{\beta}^{Y}{}_{\delta}$	$I_{\delta}^{Y}{}_{\delta}$	$I_{\delta}^{Y}{}_{\alpha}$	$I_{\delta}^{Y}{}_{\beta}$
9	-3	-3	1	3	-1	1	3	-1
3	-1	3	-1	-9	3	-3	1	1
-3	1	3	-1	9	-3	3	-1	1
-9	3	-3	1	-3	1	-1	-3	-1
3	3	-1	-1	1	1	-3	-9	3
1	1	1	1	-3	-3	9	-3	-3
-1	-1	1	1	3	3	-9	3	-3
-3	-3	-1	-1	-1	-1	3	9	3
-3	3	1	-1	-1	1	3	9	-3
-1	1	-1	1	3	-3	-9	3	3
1	-1	-1	1	-3	3	9	-3	3
3	-3	1	-1	1	-1	-3	09	-3
-9	-3	3	1	-3	-1	-1	-3	1
-3	-1	-3	-1	9	3	3	-1	-1
3	1	-3	-1	-9	-3	-3	1	-1
9	3	3	1	3	1	1	3	1
-0.54	-0.76	-0.14	-0.23	0.02	0.00	-0.03	-0.09	-0.08
du_{α} Wx_{α}	Du_{β} Wx_{α}	Du_{α} Wx_{β}	Du_{β} Wx_{β}	Du_{α} Wx_{δ}	Du_{β} Wx_{δ}	Du_{δ} Wx_{δ}	Du_{δ} Wx_{α}	Du_{δ} Wx_{β}
-0.18	-0.20	-0.40	-0.40	-0.10	-0.13	-0.02	-0.04	-0.15
M_{α}^{st} R_{α}^{st}	M_{β}^{st} R_{α}^{st}	M_{α}^{st} R_{β}^{st}	M_{β}^{st} R_{β}^{st}	M_{α}^{st} R_{δ}^{st}	M_{β}^{st} R_{δ}^{st}	M_{δ}^{st} R_{δ}^{st}	M_{δ}^{st} R_{α}^{st}	M_{δ}^{st} R_{β}^{st}
1.26	0.69	0.70	-2.20	-1.30	-0.25	0.49	0.21	0.44

Table 2

Genetic analysis of genotypes derived from ears segregating yellow, lemon and white kernels

Proposed Genotype	# ears	Mating	Segregation	X^2_{dev}	Pr	$X^2_{het.}$	Pr
(1) From Yellow Kernels							
iyiyiy YYY	8	x	All Yellow	-	-	-	-
iyiyiy YYy or iyiyiy Yyy	9	x	3Y:1L	0.18	0.7-0.5	6.78	0.7-0.5
(2) From Lemon Kernels							
lylyly YYY	10	x	All Lemon	-	-	-	-
lylyly YYy or Yyy	10	x	3L:1W	1.84	0.2-0.1	12.07	0.3-0.2
lylyiy or lyiyiy YYY	6	x	1Y:3L	0.006	0.95-0.9	1.83	0.9-0.8
lylyiy YYy etc.	18	x	3Y:9L:4W	0.45	0.8	37.24	0.2
(3) From White Kernels							
lylyly yyy	2	x	All pure white	-	-	-	-
lyiyiy or lylyiy yyy	2	x	3W:1C	2.7	1.0	5.3	0.02
iyiyiy yyy	-	none found					
lyiyiy yyy or lylyiy yyy	7	x iyiy YY	1Y:1L	3.8	0.5	2.33	0.9-0.8

Table 3

Yellow pigmentation of maize kernels, expressed as ppm of carotenoid in a hexane extract measured at 540 mμ and absorbancy = 250

	YYY	YYy	Yyy	yyy
lylyly	3.98(3.97)	3.79(3.79)	3.28(3.27)	1.00(1.01)
lylyiy	3.43(3.41)	3.40(3.35)	3.42(3.47)	0.76(0.77)
lyiyiy	6.12(6.14)	5.46(5.51)	3.28(3.23)	0.75(0.73)
iyiyiy	24.20(24.25)	19.50(19.47)	11.73(11.75)	0.94(0.89)

The $\underline{ly}_{\beta}$ effect, one of the dominance components has an increasing effect on yellow colour which counteracts the effect of $\underline{ly}_{\delta}$ & $\underline{Y}_{\beta}$.

Interaction effects are mainly $\alpha\alpha$ or additive x additive at -4.86, but $\alpha\beta$ & $\beta\alpha$ or additive x dominance effects of 2.28 & 0.42 help to counteract the $\alpha\alpha$ effect.

Using the data on amylase content, the obviously greater effect of Wx over Du is highlighted.

Using WxWxWx DuDuDu as an example.

Expected Value = $1x\mu+3xDu_{\alpha}-1xDu_{\beta}+1xDu_{\delta}$ ..... $-1xDu_{\delta}$ Wx_{β}

= 1x21.05+3x-0.975-1x-0.975+1x-0.225+3x4.340-1x6.275+1x1.09+ 9x-0.175-3x-0.200-3x-0.04+1x-0.400+3x-0.103-1x-0.125+1x-0.024+ 3x-0.035-1x-0.150

= 26.372

These figures show that the positive $\underline{Wx}_{\alpha}$ effect = +13.02 has an effect about 4½ times greater than the negative $\underline{Du}_{\alpha}$ effect = -2.93.

Similarly the Wx dominance effect is about 6 times greater than the Du dominance effect. The use of Cockerham's model allows a numerical comparison to be made, rather than a subjective one.

C. C. Blackley

UNIVERSITY OF TEXAS
Austin, Texas

1. Centromeric meiotic abnormalities in experimental maize plants.

For about six years maize plants have been grown in growth chambers in this laboratory for sporocyte collection in connection with recombination and other studies. Until last May cytological abnormalities in the collected sporocytes were restricted to occasional asynchronies (with probable retardation of some cells) and occasional cellular disintegration, both apparently attributable to damage of parts roughly handled during the collection of other parts. Since last May meiotic abnormalities have been sporadically, and to date unpredictably, observed in growth chamber microsporocyte material. These have included synaptic failures, irregularities of chromosome contraction, and loss of chiasmate association to produce univalents recombinant for a heterozygous knob

(reported briefly elsewhere). More recently we have repeatedly found an abnormality which (to my knowledge) has never been observed elsewhere. It appears that bivalents cooriented on the metaphase I plate may separate into pairs of dyads in which the centromeres of individual chromatids then orient on the plate. The ensuing first meiotic division then seems to be equational. Some second division anaphases then give the appearance of resolution of one or more chiasmata, and numerical distribution may be either very irregular (i.e. 15 from 5) or 10 from 10. We are currently searching for the cause of the abnormalities. Our original techniques have not been changed. Many normal anthers are usually found in samples in which some have abnormal cells. Seed from the same ear which has yielded many completely normal plants has produced plants with the abnormality. We are currently testing instruments and materials used in growing and handling the plants for a source of a causative agent.

Marjorie Maguire

2. Premeiotic mitosis in maize: Evidence for alignment of homologues.

Many acetocarmine squash preparations were studied of the last premeiotic mitosis in sporogenous tissue of anthers of KYS stock. This division is poorly synchronized and of short duration relative to the premeiotic interphase. At the early prophase of this division there was no obvious association of homologues or heterologues; at mid-prophase a configuration commonly observed seemed to involve many of the chromosomes by distal associations in chains (these distal associations extending proximally well into the chromosome arms). At prometaphase to metaphase observations of numerous cells suggested increasingly close side-by-side, parallel arrangement of matching chromosomes in pairs and absence of apparent non-homologous associations. Side-by-side alignment of matching pairs at anaphase was found more frequently than chance expectation would predict but not so frequently as at metaphase. It is tempting to speculate that parallel alignment of homologues is the rule in the premeiotic mitosis (at least by metaphase), that synapsis at meiosis is thus facilitated and that this alignment tends to be disrupted by the squashing procedure, particularly at anaphase.

Marjorie Maguire

UNIVERSITY OF WESTERN ONTARIO
London, Ontario
Department of Botany

1. Malate dehydrogenase in maize endosperm.

In a previous report (M.G.C.N.L. 44:189-90), the ontogenetic changes associated with the isozyme pattern of malate dehydrogenase during the development of the endosperm were described. Two isozymes comprised 45% and 35% of the total activity, respectively, and were the subject of further investigation. The use of differential and density gradient centrifugation established that both isozymes were of particulate origin. The organelles were identified as mitochondria and peroxisomes, and the latter were further examined both in situ and in cell homogenates. In addition to a single MDH isozyme, they also contained most of the catalase present in the tissue. This was verified by incubating pieces of endosperm in a medium containing diaminobenzidine, which cytochemically localizes catalase activity. Electron-microscopic examination of incubated tissue demonstrated that catalase was localized within these organelles alone, with some staining evident in the cytoplasm.

Homogeneous preparations of the two major isozymes, designated m-MDH and p-MDH, were obtained using anion-exchange chromatography; and these preparations were then used directly to determine several physical and kinetic parameters. Data from gel filtration experiments indicated that both m-MDH and p-MDH had Stokes (molecular) radii of about 34Å and were hence indistinguishable on the basis of molecular size. An investigation of some kinetic parameters did, however, provide evidence that the two isozymes were catalytically distinct. Among the parameters used were nucleotide analogue ratios, K m's and pH dependency curves.

Since the isozymes have different catalytic properties and are differentially compartmented within the cell, it is safe to assume that they have specific and unique physiological roles. The mitochondrial isozyme is most certainly involved in Krebs Cycle activity; and while a truly accurate assessment of the role of p-MDH must await further biochemical characterization of maize endosperm peroxisomes, it is probable that these organelles function in much the same way as peroxisomes

(glyoxysomes) in castor bean endosperm. It has been established that these organelles, containing the enzymes of the glyoxylate cycle, are involved in the mobilization of lipid reserves and their subsequent conversion to carbohydrate. The intimate association between lipid bodies and peroxisomes in maize endosperm, observed during the present study, would support the contention that maize peroxisomes function in a similar fashion.

It may be concluded then, that m-MDH and p-MDH are distinct molecular species with catalytic differences and unique metabolic functions.

D. B. Hayden

2. Data analysis and maize cytogenetics.

The application of standard statistical tests is generally well understood and the techniques have been described in many elementary texts. Less well known are a number of techniques which in the biological field are more commonly used by numerical taxonomists and ecologists. In general, these are techniques which are concerned not so much with validating hypotheses as with the manipulation of data to bring to light information not apparent in the original data. Information in this sense may well include new problems or the rephrasing of old questions in a way which allows of more productive experimental design in subsequent investigations.

Perhaps the greatest problem is the difficulty which is faced in evaluating a large body of diverse data which contains many simultaneous variables. Presented with such a mass of data, one's tendency is to examine it in parts, looking, the while, to see if the rest of the data supports impressions gained from the part. This is a notoriously unreliable procedure, and consequently the whole basis of the investigative method rests on the sequential examination of limited hypotheses. While reliable, the method suffers from the fact that very frequently one is examining the wrong hypothesis. Clearly, any technique which will extract more information from a mass of multivariate data will enable specific hypotheses to be formulated much more efficiently. It is possible, indeed, that a meaningful answer can only be obtained by taking the mass of data as a whole.

A generalised, non-mathematical description of the problem makes the available techniques more easily understood. Consider a population of objects, characterised by a number of descriptors, each of which may assume two or more values. It may be desired to ask questions concerning the objects or the descriptors, or to ask that the relationships be simplified within certain constraints. In many cases, which elements are taken to represent descriptors and which to represent objects is optional, depending on the questions which are being addressed. An example, using Principal Components Factor Analysis, will make this point clear. A detailed account of this technique will be found in Seal (1964). Very briefly, Factor Analysis of this type is a technique whereby the descriptors of a population are replaced by linear combinations of the descriptors, called factors. The technique maximizes the variance removed from the system by the linear combination of descriptors contributing to the first factor. The process is repeated for the second and subsequent factors. Complete description of the object relationships will require as many factors as there were original descriptors, even in intrinsically simple systems, due to error variance. The distribution of information, however, has been changed. It is now concentrated in the first few factors: hence the description of the objects has been simplified.

The data obtained by Douglas and Filion (see contributions below) illustrate this interchangeability of objects and description.

A number of clustering techniques are available (see Orloci, 1968) where the object is the detection of groups of similar objects, rather than the simplification of relationships. As in the case of factor analysis, a degree of flexibility exists in designating objects and descriptors. Applicability of clustering techniques varies considerably and must be considered individually. The choice of clustering technique may be influenced by prior logical deductions concerning the groups of similar objects considered in spatial terms. If, by the nature of the data, the groups must necessarily be spherical or hyperspherical, then multiple linkage (not to be confused with genetic linkage) techniques, possibly Multidimensional Group Analysis (Jancey, 1966), are most appropriate. Failing such information, a cluster technique more

appropriate to the demonstration of elongate clinal variation should be used. A single linkage approach, such as the Graph Theory Model, (Wirth, Estabrook and Rogers, 1966) would be suitable. For a fuller discussion of clustering techniques, see Sokal and Sneath (1963).

Work in the field of Information Theory has led to the development of a number of powerful analytical tools well suited to cytological data. It is possible to measure the total information content of various arrangements of data, thus providing an objective basis for discussion and an effective means of selecting the most meaningful hypothesis. Another valuable approach is the measurement of information contained in one set of data, conditional on knowing a second set. Such an approach is directional and applicable to any data that can be expressed in the form of frequencies. It is generally more appropriate than either correlation or regression techniques. A discussion of the application of some Information Theory models to biological problems can be found in Estabrook (1967) and in Orloci (1971).

Finally, a technique should be mentioned which, while it involves conventional tests of significance, is extremely useful in the development of fresh or difficult to evaluate hypotheses. It involves the combining of probabilities from a number of independent statistical tests. The probabilities may be based on different sets of data and may refer to different statistical hypotheses. They should, however, all test the same scientific hypothesis, and the probabilities should be known exactly. A full account of the method, including a worked example, is given in Sokal and Rohlf (1969).

In conclusion, it may be said that a valuable collection of analytical tools are available to geneticists. Though they are as yet little known to biologists in general, they are for the most part well proven in other fields, and exist as fully developed computer programs.

References

Estabrook, George F. 1967. An information theory model for character analysis. Taxon 16:86-97.

Jancey, R. C. 1966. Multidimensional group analysis. Aust. J. Bot. 14:127-30.

Orloci, L. 1968. A model for the analysis of structure in taxonomic collections. Can. J. Bot. 46:1093-1097.

Orloci, L. 1971. An information theory model for pattern analysis. J. Ecol. (in press).

Seal, H. 1964. Multivariate statistical analysis for biologists. Wiley. New York.

Sokal, R. R. and Rohlf, F. J. 1969. Biometry. W. H. Freeman and Co., San Francisco.

Sokal, R. R. and Sneath, P. H. A. 1963. Principles of numerical taxonomy. W. H. Freeman and Co., San Francisco.

Wirth, M., Estabrook, George F. and Rogers, David J. 1966. A graph theory model for systematic biology, with an example from the Oncidiinae (Orchidaceae), Systematic Zoology 15:59-69.

R. C. Jancey

3. Reanalysis of Longley's data: translocations in maize.

A. E. Longley (1958, 1961) has provided the most extensive cytological data on translocations available from any organism (Lindsley and Grell (1968) list approximately 800 translocations in Drosophila). Whereas the Drosophila data are based entirely on salivary analysis, Longley's data are determined entirely from pachytene analysis. Longley's data provide assignment of a 'breakage point' to a specific unit within a chromosome arm. He has discussed the limits of his data, e.g. sampling error, and some corrections have been listed in previous Newsletters (e.g. Burnham, 1969).

We have become interested in the distribution of genetic events and undertook to reanalyze Longley's data. Initially, we have attempted to describe the distribution, and in particular to establish any areas within chromosomes in which there can be detected a higher frequency of breakpoints than might be expected on a hypothesis of 'random distribution'. Our present report, which should be considered preliminary, consists largely of a summary of these distribution analyses. Next, however, we are reexamining the distribution in terms of probability statements for particular types of rearrangements. While not yet finished, it is clear that centromere-translocation probabilities far exceed that expected by chance alone. Lastly, designed but not yet processed, is a production of likelihood statements for different length-interstial and interchange-

segment aberrations. In turn, we propose to extent these analyses to the Drosophila data.

A retrospective study of the type reported below must necessarily be viewed with some reservations. While most of Longley's data are derived from induced translocations, it may be argued that the different treatments, plus differential survival, are responsible for any deviation from randomness rather than assigning such a property to the genetic material itself. Nevertheless, particularly in relation to Drosophila, we feel that Longley's data are in fact the least susceptible to the criticisms above of any data of this type which will become available in the near future.

Histograms per .05 arm length have been plotted and are summarized per arm in Table 1. In some later analyses length units have been converted to 'real lengths' by formulae derived from the data of Rhoades (1950) and Neuffer, Jones and Zuber (1968). These estimates are of mid-pachytene lengths. From our own data we assigned an estimate of 2μ as the average length of centromeres.

We set out to test two basic hypotheses concerning the data:

1. That the chromosome breaks did not depart from a random distribution.
2. That the association of chromosome segments did not depart from a random distribution.

Apart from testing these hypotheses for the chromosome complement as a whole, it was obviously desirable to see if departures from the hypothesis were characteristic of sub-sets of the whole.

A number of statistical tests of these hypotheses would have been appropriate, the most obvious being the X^2 test and the G test (Sokal and Rohlf, 1969). Because of general familiarity with the test, it was decided to employ X^2, though certain computational and theoretical advantages are attached to the use of the G test. The possibility of applying other analytical tools to the data has been mentioned above.

Clearly, in order to test the hypotheses, it was necessary to create a number of classes within the chromosome complement, to which break or association frequencies could be assigned. It would have been most desirable to divide the total chromosome lengths into small, say

2μ, segments; however, given the amount of data available, this would have resulted in very low expected frequencies per cell. Such a situation would greatly reduce the validity of the X^2 test, or indeed of any test serving the same purpose. The larger the class size, however, the less information would be yielded concerning localised deviations from randomness. It was concluded that taking chromosome arms as classes would provide the most useful compromise between class size and expected number of observations per class.

There was no logical basis for assigning centromere breaks to one arm in preference to the other. Consequently, the two hypotheses were tested first on arm data alone, then the first hypothesis was tested for centromere data, and finally the second hypothesis was tested between the two classes of data.

The computation of expected values for each cell requires some explanation:

Expected Cell Frequencies for 1st Hypothesis.

Since the hypothesis postulates a random distribution of chromosome breaks, the expected frequency, F, per chromosome arm is given by $F = [n/L] \cdot \ell$, where L = Total Complement Length, ℓ = Segment Length of Arm, and n = Total Number of observed breaks for chromosome complement.

Expected Cell Frequencies for 2nd Hypothesis.

If the number of translocations involving segment 1 is F_1, and the translocations involve random reassociation of breaks, then the number of translocation pairs involving segment 1 and segment 2 will be:

$$[F_1/L-L']\ell_2$$

where L = Total complement length

L' = Chromosome length of which segment 1 is a part (because no inversion data was included)

ℓ_2 = Chromosome length of which segment 2 is a part

Similarly, if the number of translocations involving segment 2 is F_2, then the number of translocation pairs involving segments 2 and 1 will be:

$$[F_2/L-L'']\ell_1$$

where L'' = Chromosome length of which segment 2 is a part

ℓ_1 = length of segment 1

Thus the total number of different translocation pairs involving

both segments is:

$$[(F_1/L\text{-}L')\ell_2] + [(F_2/L\text{-}L'')\ell_1]$$

Results

Table 1 shows the distribution of chromosome breaks by arm. It will be noticed that the most striking deviations from expected values occur in the arms 2S, 5L and 9L. Over all, the data depart from the hypothesis of a random distribution of breaks such that the probability of occurrence within the hypothesis is considerably less than 0.005.

Table 1

Distribution of breakage frequencies by arm

Chr. segment	Obs.	Expected*	$\frac{(f - F)^2}{F}$
1S	101	125.3	4.7
1L	141	163.1	3.0
2S	75	103.2	7.7
2L	135	129.5	0.2
3S	71	72.4	0.0
3L	127	144.5	2.1
4S	87	79.1	0.8
4L	115	126.7	1.1
5S	109	99.7	0.9
5L	145	109.9	11.2
6S	36	41.6	0.8
6L	148	129.1	2.8
7S	35	44.1	1.9
7L	111	119.7	0.6
8S	42	40.2	0.1
8L	151	126.0	5.0
9S	50	54.2	0.3
9L	133	97.3	13.1
10S	36	33.9	0.1
10L	88	95.20	0.5
	1936	1934.7	56.9
D.F. = 19		$X^2 = 56.9$ $p < 0.005$	

*See text. Length data from Rhoades (1950) and Neuffer, Jones and Zuber (1968).

The hypothesis of random distribution of breaks stands a little firmer in the case of breaks occurring within centromeres (see Table 2).

Departures from expected values are most noticeable in the centromeres of chromosomes 6, 7 and 8. The overall probability of the data occurring within the hypothesis is < 0.05.

Table 2

Distribution of breakage frequencies in centromeres

Centromere of Chr. No.	Obs. breaks	$\frac{(f - F)^2}{F}$
1	8	0.21
2	10	0.04
3	12	0.72
4	5	2.06
5	10	0.04
6	2	5.82
7	15	3.34
8	16	4.63
9	7	0.61
10	9	0.02
		17.48
Expected: F = 9.4	D.F. = 9	$p < 0.05$

On the basis of the arbitrary assignment of a length of 2μ to the centromeres, the break frequency within centromeres is somewhat higher than that found in the rest of the chromosome. Since this length (2μ) is probably an over-estimate, the frequency per unit-length within centromeres is likely to be even more different from the arm frequency.

The observed values for translocation pairs between all segments are contained in Table 3. (It should be emphasized that these pairs are, of course, evidenced cytologically as two translocations). Departures from expected values are contained in Table 4. Pairs which show striking departures from expectation are 5L-8S, 5L-9L, 5L-10S and 10S-8L. It will be noticed that the deviations are on an arm basis only; other pairwise values within the same chromosomes are unremarkable. The X^2 value for

Table 3

Observed values for breakage frequencies* ordered by pair associations.
Data from Longley (1958, 1961). Σ observed frequencies = 962.

		1		2		3		4		5		6		7		8		9		10	
		S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L
1	S			6	5	1	8	4	9	6	13	2	13	6	6	2	5	1	12	0	3
	L			6	9	5	12	8	9	11	9	2	8	2	14	4	15	4	7	2	11
2	S					3	10	2	7	4	5	3	8	0	4	1	4	1	6	0	5
	L					7	11	11	11	12	8	2	11	4	8	5	14	3	3	2	7
3	S							3	5	4	1	3	5	0	6	3	8	1	7	1	5
	L							2	4	2	13	1	13	2	8	4	13	3	14	0	6
4	S									8	6	4	7	2	4	0	15	0	5	0	3
	L									7	11	2	11	1	6	2	4	5	12	2	7
5	S											4	9	5	4	4	13	2	6	2	5
	L											2	12	1	8	8	11	5	19	8	6
6	S													0	4	1	1	0	5	1	2
	L													4	11	1	8	5	9	5	10
7	S															1	1	2	4	0	1
	L															2	10	5	6	1	2
8	S																	0	3	1	0
	L																	8	7	9	4
9	S																			1	3
	L																			1	6

*Excluding inversions and translocations involving centromeres.

Table 4

Within cell contributions to Chi Square Test for pair-wise associations of chromosome arms. The sum of the four components represents Chi Square values for pair-wise associations between chromosomes.

		1		2		3		4		5		6		7		8		9		10	
		S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L
1	S			0.2	1.7	3.1	0.4	0.4	4.9	0.1	3.9	0.2	1.9	3.1	0.6	0.2	1.5	1.9	4.4	2.3	1.9
	L			1.2	0.6	0.3	0.1	0.1	0.5	0.4	0.1	0.8	1.2	0.9	0.9	0.1	1.2	0.1	0.4	0.3	0.7
2	S					0.3	0.4	1.3	0.0	0.5	0.2	0.2	0.1	2.4	1.1	0.7	1.2	1.3	0.1	1.9	0.0
	L					0.7	0.0	5.3	0.4	3.4	0.0	0.3	0.3	0.2	0.0	1.7	3.2	0.2	2.2	0.1	0.0
3	S							0.0	0.0	0.0	2.5	1.2	0.0	1.7	0.4	1.4	1.9	0.5	2.7	0.1	0.4
	L							2.9	3.7	4.4	2.1	1.6	0.7	0.6	0.3	0.2	0.8	0.4	5.1	2.7	0.3
4	S									3.3	0.4	2.9	0.4	0.0	0.3	1.7	16.9	2.3	0.2	1.4	0.3
	L									0.0	1.5	0.2	0.4	1.3	0.7	0.2	2.6	0.4	4.1	0.0	0.0
5	S											1.4	0.5	3.0	1.0	1.6	5.5	0.3	0.1	0.0	0.0
	L											0.1	2.3	1.0	0.1	13.4	1.5	1.0	29.9	18.1	0.0
6	S													1.0	0.7	0.9	1.7	1.2	3.7	0.1	0.0
	L													0.3	0.7	1.7	0.1	0.4	0.7	3.0	1.6
7	S															0.0	1.3	0.4	1.3	0.8	0.7
	L															0.1	0.4	0.6	0.0	0.6	2.9
8	S																	1.2	0.4	0.1	2.0
	L																	2.8	0.0	19.5	1.0
9	S																			0.0	0.0
	L																			0.3	0.2

the whole table is significant at well below the 0.005 level.

Translocation pairs involving centromere breaks are shown as observed values in Table 5. Because of the very small size of the centromeres, the expected frequencies for pairwise translocations would have been too low for satisfactory use of the X^2 test. It will be noticed, however, that the majority of translocations involving a centromere also involve a second one. If the hypothesis of random reassociation of breaks is correct, such a situation is highly unlikely in view of the small size of the centromeres, even taking into account their greater propensity for breakage. This is shown quite clearly in Table 6, where the X^2 value of 978.77 would be significant at probability levels much more stringent then 0.005.

Table 5

Translocation pairs involving centromeres - observed values

	C2	2L	C3	C4	5S	C5	C6	C7	C8	8L	9S	C9	C10
C1	2	1				1			1				1
C2			1	3	1				2				
3S													1
C3				1		3		2	2			1	
4S													1
C4						1							
C5								2	1			1	1
C6										1	1		
6L												1	
C7									6			3	2
7L													1
C8												1	2

C = centromere S = short arm L = long arm

Table 6

Distribution of reassociation pairs, including centromeres, recognising the difference in break frequency between arms and centromeres*

	Obs	Expected	$\frac{(f - F)^2}{F}$
Between arms	962	928.28	1.22
Between arms and centromeres	8	82.84	67.61
Between centromeres	43	1.86	909.94
	1013	1012.98	978.77
D.F. = 2		$p < 0.005$	

*Expected values are on the basis of pachytene length (see text) and corrected for the demonstrated higher breakage frequencies in the centromeres.

It has been demonstrated that in maize the hypotheses of random breakage and random reassociation are not supported by the data. Inasmuch as these data represent the most extensive collection, further analyses may reveal new concepts concerning the composition and organisation of nuclear chromatin.

R. C. Jancey
D. B. Walden

4. Principal Components Analysis applied to addition segments in chromosome 9.

In an attempt to quantify differences within chromosomal parameters on a biometrical basis, several types of tests, both parametric and non-parametric, were performed. These types of repetitive testing lead ultimately to the acceptance or rejection of the null hypothesis of no difference. Other types of analyses are available (see Jancey, this News Letter). Of particular noteworthiness for our ongoing interest in karyotype analysis are principal components analysis (PCA) and graph theory.

The source material for our study consisted of six reciprocal interchange stocks. The meiotic post-interchange length of 9S in these

stocks ranged from 0% to 50% increments in steps of about 10% of the standard length. Two normal stocks, seneca '60' a commercial single cross ($\underline{su}_1/\underline{su}_1$) and a chromosome 9 tester stock ($\underline{yg}_2$ C $\underline{sh}_1$ $\underline{bz}_1$ $\underline{wx}$), also were included.

Crosses were performed to obtain the interchange stocks in both the T/N and T/T condition. Mitotic karyotypes were prepared from 25 cells with non-overlapping chromosomes in each of the 14 stocks. The mitotic chromosome arms were measured and values for the following parameters were calculated and included: total length; arm ratio; centromeric index; and homologue differences between all the preceding parameters. Each cell was considered an individual in the PCA yielding 140 characters (variables) per cell. Any type of mensuration data might be included, for example, labelling counts or density measurements. The only restriction on the number of variables used in any one analysis is the capacity of the computer facilities.

The print out from the analysis allows the investigator to examine the data both numerically and pictorially. The absolute values of the eigen vectors represent the contributions of the character used; that is, for any particular axis, comparatively high vector values represent primary or important contributors and comparatively low vector values represent characters which are of less importance in the separation of the individuals for a particular axis. Factor scores are printed out for each axis. The factor scores of an individual (cell) on any given component axis is the sum of the individual's (cell's) standardized value for each character multiplied by the contribution of that character (i.e., the vector value) to the axis. The analysis has, to some extent, a built in checking system. For example, if one or two particular cells of a specific population of cells are separated off by themselves, one can go back to the eigen vector values and see which characters are responsible for this separation. The original data can then be checked for possible errors.

Some of the more striking results from the P.C.A. applied to the 14 stocks include:

1) The characters 'arm-ratio' and 'centromeric-index' were identical contributors. Thus, the use of one rather than the other

appears to be unwarranted. Further, these two indices had a correlation of 1.0 in the correlation matrix. Thus, one or the other may be used but not both simultaneously.

2) The pictorial spatial relationships demonstrated that the T/T was separated more completely than the T/N. This would be expected since the T/N condition involved two altered chromosomes and the T/T involved four.

3) In the larger chromosomal alterations (40% and 50%), the altered chromosomes were the major contributors in stock separation. In other stocks, however, chromosomes not involved in an interchange were, in many cases, the important contributors to stock separation. These observations, that specific chromosomes within particular stocks were of major importance, indicates the existence of between stock differences, i.e., values of specific chromosome parameters may be unique to certain stocks. This was very apparent in the complete separation noted for the two normal stocks. These two stocks have a divergent pedigree in which we estimate there has been no interbreeding for at least 50 generations and probably many more.

4) The absolute changes in pachytene length (microns) between the interchanged chromosomes used and chromosomes of the normal maize complement are presented in Table 1. Included in Table 1 is a comparison between the size (microns) of these changes in pachytene and mitotic metaphase. The entries for mitotic metaphase are based on a 13x reduction from the pachytene values.

In maize, in chromosomes 9 and 10, which are submetacentric chromosomes, a change of 0.58 μ resulted in stock separation. Increments to 9S of less than 0.58 μ did not result in stock separation and a decrement of 0.46 μ in 10L was not detected via the P.C.A. However, the addition of 0.24 μ to 5L, a metacentric chromosome, was detected. Overall, our results suggest a fortiori that a percent change (x) may be easier to detect mitotically if it is involved in a decrement rather than an increment. Further, the ability to detect an anomaly may be dependent upon the type of chromosome altered (i.e., metacentric, sub-metacentric or acrocentric).

Table 1

Changes in Chromosome 9S		
Stock Code	Pachytene Length (microns)	Somatic Metaphase Length (microns)[a]
9-5 4871	0.5	0.04
9-7 a	1.6	0.12
9-5 a	3.1	0.24
9-2 c	4.7	0.36
9-10 8630	6.0	0.46
9-10 b	7.6	0.58

a) reduction factor = 13

The problem of somatic chromosome identification of an aberration such as an interchange can now be readdressed. One hypothesis suggests that a simple relationship exists between the ability to identify an anomaly and the size of the chromosomal alteration; that is, the greater the anomaly, the greater the chance of detection. Our observations, however, indicate that a relatively short alteration may be detected in some chromosomes. Further, the ability to detect an anomaly may be dependent upon it being an increment or a decrement to a chromosome. These two criteria may also be interdependent. In order to explain the non-linearity observed between increase and decrease, the following hypothesis is offered: the interstitial segment plus the exchanged piece does not always present a 'simple additive model' in somatic metaphase.

Many factors could account for this 'non-additivity', such as: (1) differential chromosome contraction; (2) addition or subtraction of a specific segment at meiosis may not be expressed in the same relative magnitude during mitosis (possibly interdependent upon the 13x reduction and differential contraction); (3) effects may be chromosome specific, arm specific or both (the data suggest that a metacentric chromosome may show more pronounced differences with small alterations than chromosomes of a more submetacentric or acrocentric type).

W. G. Filion

5. Expansions of descriptive chromosomal indices.

Chromosomal indices such as arm ratio, centromeric index, and morphological index (Giannelli and Howlett, 1967) have been employed in routine chromosome identification and in the determination of unknown chromosomal anomalies for some time. Conflicting opinions in our laboratory, concerning the usefulness of such indices, prompted their examination.

Consider the following example to introduce the point in question: a chromosome with a long arm of 2.0 units and a short arm of 1.0 units has an arm ratio of 2.0. If the short arm is altered by 25% of its standard length, the resulting arm ratios become 1.60 (for an increment) and 2.66 (for a decrement). Compared to the original arm ratio of 2.0, an increment results in an absolute change of 0.40, a decrement in 0.66. That is, a decrement of the same length alters the arm ratio by a proportionally greater amount.

What is the mathematical relationship between an increment (Y) and a decrement (X)? If a specific chromosome arm is decreased by a proportion (x), what proportion of the arm must be added (y) to result in an arm ratio in which the absolute change for an increase and decrease are equal? Thus we want to know the value of (y) in terms of (x), $y = f(x)$.

Functions for (y) in terms of (x) were derived for each index. Two functions, one for a long arm change and one for a short arm change, were derived for each ratio. The six derived functions are listed below:

Arm Ratio:

A.R. = q/p

Short Arm:

$$y_p = x/1-2x \qquad (1)$$

Long Arm:

$$y_q = x \qquad (2)$$

Centromeric Index:

C.I. = p/(p+q)

Short Arm:

$$y_p = x(p+q)/(p+q-2px) \qquad (3)$$

Long Arm:

$$y_q = -x(p+q)/(2qx-p-q) \qquad (4)$$

Morphological Index:

$$M.I. = (p/q)*(p+q)$$

Short Arm:

$$y_p = (-(2p+q) \pm \sqrt{4p(p+q)+q^2+4px(2p+px+q)}\ ) / 2p \qquad (5)$$

Long Arm:

$$y_q = x(p+q)/(p+q-2px-2px) \qquad (6)$$

where:

p = length of the short arm

q = length of the long arm

x = the proportion of an arm (decrement)

y = the proportion of an arm (increment)

Values of p, q and x, substituted in the positive root of equation (5) resulted in y = x; the negative root did not. Thus equations (2) and (5; '+' root) demonstrate that, for these examples, an addition and a deletion of a chromosome arm altered the arm ratio with the same absolute value; the remaining equations did not. Furthermore, values substituted for p, q and x demonstrated that function (1) was chromosome independent; the remaining functions (3, 4, 5; '-', 6) were all chromosome dependent, i.e., they are chromosome specific plots. Computer print out and plots have been obtained for each function x = 0 to 1.0, y = 1.0 to 0.

These functions demonstrate the inappropriateness of indices such as arm ratio, centromeric index and morphological index in a biometrical analysis designed to detect chromosomal differences. If, in a reciprocal translocation, both interchange segments involved long arms, arm ratio may be used; if both were in the short arms, then the morphological index may be used. Since an investigator may have no idea which type of chromosome alteration is present, the use of these indices becomes questionable. This argument applies only to a biometrical approach to anomaly detection and is based upon a mathematical approach. However, arguments for or against the use of indices should also have a statistical foundation. Thus, the actual validity of the functions may become a statistical problem. That is, the functions indicate that changes produced by segments

which add to or delete from the original length are not mathematically equivalent; however, we have not resolved their statistical equivalency to the present.

Giannelli, F. and R. M. Howlett 1967 Cytogenetics 6:420-435.

W. G. Filion

6. Somatic association: the effects of various methods of arresting spindle-fiber development.

Driscoll and Darvey (1970), Avivi, Feldman and Bushuk (1969) and Back and Zang (1969) have suggested that the presence of somatic association of homologous chromosomes at metaphase is to some extent determined by the chemical treatment used to accumulate metaphase figures for the study.

Avivi, et al. using colchicine and cold treatment, noted in common wheat, Triticum aestivum L, that somatic association of homologous chromosomes detected at metaphase after using cold treatment was not observed when the experiment was repeated using colchicine instead of cold to arrest spindle-fiber development.

We have studied all possible homologous and non-homologous associations of chromosomes in a normal single-cross hybrid Seneca-60 using cold (5°C for 24 hrs.), 8-hydroxyquinoline (method of C.C. Chen, 1970), and monobromonaphthalene (method D. Weber as modified by J. Miles) to arrest spindle formation. For procedural and statistical considerations, see Horn and Walden (1970).

Fifty circular metaphase spreads in which the chromosomes were non-overlapping, well squashed and in the same focal plane were selected for each study. The results are presented in Table 1.

Table 1

Mean distances between homologous chromosomes

Treatment	Chromosome Number									
	1	2	3	4	5	6	7	8	9	10
8-hydroxy-quinoline	.420	.331*	.406	.479	.421	.448	.481	.473	.418	.417
Cold Treatment	.491	.390*	.379*	.380*	.413	.423	.352*	.411*	.412*	.407
Monobromonaphthalene	.463	.434	.466	.460	.503	.402*	.490	.456	.424	.415

*indicates positive association $p < 0.05$

NOTE: There is no precise cutoff value for significance as the shape of the frequency distribution of distances between homologues as well as the mean distance is considered in the Kolmogorov-Smirnov statistical method used.

No non-homologous associations were detected. The study was repeated for both the long and short arms of the chromosomes. Similar results to those shown above were obtained.

Literature Cited

Avivi, L., Feldman, M., and Bushuk, W. 1969. The mechanism of somatic association in common wheat, Triticum aestivum L. I. Suppression of somatic association by colchicine. Genetics 62:745-752.

Back, E., and Zang, K. D. 1969. Quantitative studies on the arrangement of human metaphase chromosomes. II. Influence of the preparation techniques on other association pattern of the acrocentric Chromosomes. Cytogenetics 8:304-314.

Chen, C. C. 1969. The somatic chromosomes of maize. Canadian Journal of Genetics and Cytology. Vol. XI, 3:752-754.

Driscoll, C. J., and Darvey, N. L. 1970. Chromosome Pairing: Effect of Colchicine on an isochromosome. Science 169:290-291.

Horn, J. D. and Walden, D. B. 1970. Somatic association as a general phenomenon in maize. MGCNL 44.

Miles, J. Personal communication.

J. D. Horn

7. Polyploidy and nuclear cycle in Zea mays L. root-tips.

In MGCNL 44:192-195 (1970), we reported the duration of the nuclear cycle in diploid root tips of Seneca 60 and of a chromosome 9 tester at 25°C. These studies have been extended to undertake an examination of some of the factors which might influence the duration of the various components of the cycle. In the present study we report on the duration of the nuclear cycle as a function of genotype and polyploidy.

We obtained seeds of diploid and tetraploid stocks of inbred W23 from the Maize Genetics Coop., Urbana, Illinois, courtesy of Dr. R. J. Lambert. Triploid seeds were obtained after crossing diploid (W23) and tetraploid (W23) stocks. We chose 25°C for the study inasmuch as our previous work indicated that 25°C provided for good resolution of the cycle, i.e., it was neither telescoped nor elongated. Likewise, experimental error appeared to be minimal at 25°C.

The experimental procedures for the pulse labeling and autoradiography were those outlined in MGCNL 43:186-190 (1969) and MGCNL 44:192-195 (1970). All slides from each experiment were coded and scored blindly.

At 25°C, the labelled prophase frequency curves (as well as the metaphase and anaphase curves) were identical for all three stocks; therefore, it is clear that the cycle duration is similar in diploid, triploid and tetraploid material. The classification data are presented in Table 1. Employing the proportion method, the nuclear cycle duration and its components were estimated and are presented in Table 2.

The results presented indicate that the duration of the nuclear cycle and in particular the S and M periods in diploid, triploid and tetraploid stocks are similar. The slight differences are due undoubtedly to the experimental variation attributable to the plotting of the ascending and descending slopes of the curves. Inasmuch as we are dealing with percentage data, we used a probit regression analysis to obtain weighted mean values and standard deviations appropriate to these means (see Table 3).

Table 1

Frequency of labelled and unlabelled nuclei from primary root-tips following pulse labelling (^{3}H-TdR, 30 min) at 25° in W23 inbreds

Stock	Interphase		Prophase		Metaphase		Anaphase		Telophase	
	Lab	Unlab	Lab	Unlab	Lab	Unlab	Lab	Unlab	Lab	Unlab
Diploid	10409	19209	679	647	210	236	56	71	260	377
Triploid	9641	21747	563	518	242	306	48	84	153	236
Tetraploid	10639	22150	709	736	294	287	71	75	274	377
Total	30689	63106	1951	1901	746	829	175	230	687	990

Table 2

Estimates (from the data in Table 1) of the duration of the nuclear cycle in primary root-tips of inbred W23 stocks at 25°C

Phase	Mean Duration					
	Diploid		Triploid		Tetraploid	
	Hrs.	% of total	Hrs.	% of total	Hrs.	% of total
Interphase:						
G_1	2.10	18.8	2.89	25.1	2.65	23.1
S	5.50	49.1	5.00	43.5	5.20	45.2
G_2	2.31	20.6	2.50	21.7	2.31	20.1
sub-total	9.91	88.5	10.39	90.4	10.16	88.4
Mitosis:						
Prophase	0.68	6.1	0.56	4.9	0.68	5.9
Metaphase	0.23	2.1	0.28	2.4	0.28	2.4
Anaphase	0.06	0.5	0.06	0.5	0.07	0.6
Telophase	0.32	2.9	0.21	1.8	0.31	2.7
sub-total	1.29	11.5	1.11	9.7	1.34	11.7
Total	11.20		11.50		11.50	

Table 3

Standard deviations of the nuclear cycle component estimates in the primary root tips of Zea mays L. (inbred W23) at 25°C.

Phase	Hours		
	Diploid	Triploid	Tetraploid
G_1 + M	0.21	0.14	0.24
S	0.16	0.15	0.04
G_2 + P	0.18	0.18	0.12
Total Nuclear Cycle	0.26	0.18	0.28

M = Prophase + Metaphase + Anaphase + Telophase

P = Prophase

It is generally recognized that homologous chromosomes may synthesize their DNA at the same time and in a similar manner, although differences in the S period timing pattern have been reported within certain homologous pairs in mammalian tissues (e.g., X-chromosomes). From this point of view, one would predict that the addition of complete sets of chromosomes might not alter markedly the time required for DNA synthesis, provided that sufficient precursors and enzymes were present so as not to limit the rate of synthesis. As long as the genes are equally active in 2n, 3n, and 4n nuclei, then the triploid or tetraploid states should have little or no effect on the time needed for certain synthesis events. From our data, it is clear that there is not an increase in the duration of the S phase or of the entire nuclear cycle. These results agree with the conclusion of several other workers that the duration of the DNA synthesis period is independent of DNA content in diploid, triploid and tetraploid nuclei, although this study is the only report in which autotetraploids were examined.

Ram S. Verma

8. Effect of cycloheximide and chloramphenicol on the nuclear cycle in maize root meristems.

The present study reports the effect of cycloheximide (0.001%) and chloramphenicol (0.03%) on the nuclear cycle in maize root-tip meristems.

All treatments were carried out on attached 3 day old singlecross (Seneca 60) primary root-tips. Roots were exposed to ^{3}H-TdR for 30 minutes (1 μc/ml; specific activity 6.3/mM). The roots were then washed for 30 minutes and transferred to either cycloheximide (0.001%) or chloramphenicol (0.03%) for two hours. After incubation the roots were thoroughly washed again and returned to the germination chamber until sacrificed. Experiments were conducted at 25 or 30°C. Root-tips were collected at two hour intervals following treatment and fixed in alcohol-acetic acid, 3:1. Autoradiographs were prepared according to the schedule of Verma (MGCNL 43:186-190, 44:192-195). A minimum of four slides, one root-tip per slide, from each collection period, were coded and scored blindly.

The classification data are presented in Table 1. Employing the proportion method, the nuclear cycle duration and its components were estimated and are presented in Table 2. Treatment with either chloramphenicol (0.03%) or cycloheximide (0.001%) resulted in a delay in the appearance of labelled prophase, metaphase, anaphase, and telophase. In the histograms of labelled prophase from both the cycloheximide and chloramphenicol treatments, the peaks were delayed in comparison with the control. Reference to Table 2, 25°C, shows that tT, tS and tG_2 were affected by the cycloheximide and chloramphenicol treatments. At 30°C, all the stages were affected except tM. Our data confirm the high sensitivity of the tS and tG_2 phases to the treatment. Both chemicals appear to act mainly on the G_2 and S periods to lengthen the duration of the nuclear cycle. We also found that G_1 is affected at 30°C. Several workers have proposed that the primary effect of cycloheximide or chloramphenicol was the inhibition of protein synthesis, and further, that inhibition of DNA synthesis was an indirect effect. These hypotheses, if true, would lead to a delay in G_2 and S periods, such as recorded in our data.

Table 1

Frequency of nuclei scored from root tips following pulse labelling (^{3}H-TdR, 30 minutes)

	25°C		30°C	
	Cycloh. (0.001%)	Chloram. (0.03%)	Cyclo. (0.001%)	Chloram. (0.03%)
Interphase:				
Labelled	13139	13317	14896	16533
Unlabelled	29200	30785	20985	23891
Mitosis:				
Prophase				
Labelled	639	550	413	627
Unlabelled	717	686	459	616
Metaphase				
Labelled	230	184	160	209
Unlabelled	389	291	264	245
Anaphase				
Labelled	67	56	49	64
Unlabelled	98	95	73	99
Telophase				
Labelled	226	258	135	240
Unlabelled	372	369	194	292
Total	45077	46591	37228	42816

Table 2

Estimated (from the data in Table 1) duration of the nuclear cycle with or without a 2 hr incubation with cycloheximide (0.001%) and chloramphenicol (0.03%) in Zea mays L. (Seneca 60) root tips

Phase	Hours									
	25°C					30°C				
	Control	Cyclo-heximide	Percent increase over control	Chlora-mphenicol	Percent increase over control	Control	Cyclo-heximide	Percent increase over control	Chlora-mphenicol	Percent increase over control
Interphase:										
G_1	1.96	1.77	-	1.89	-	0.59	1.70	188	2.04	245
S	5.00	7.00	40	6.50	30	3.50	5.00	43	6.00	167
G_2	1.84	4.80	161	5.40	194	2.10	4.70	123	3.52	68
sub-total	8.80	13.57	54	13.79	57	6.19	11.40	84	11.56	87
Mitosis:										
Prophase	0.56	0.71	27	0.60	7	0.40	0.30	25	0.49	48
Metaphase	0.23	0.32	39	0.23	-	0.17	0.15	-	0.18	6
Anaphase	0.06	0.09	50	0.07	17	0.04	0.04	-	0.06	50
Telophase	0.24	0.31	29	0.30	25	0.20	0.11	-	0.21	48
sub-total	1.09	1.43	31	1.20	10	0.81	0.60	-	0.94	16
Total	9.90	15.00	52	15.00	52	7.00	12.00	71	12.50	79

Table 3

Standard deviation estimates for the nuclear cycle in root tips of Zea mays L. (Seneca 60) treated with cycloheximide and chloramphenicol (hours)

Phase	25°C			30°C		
	Control	Cyclo-heximide	Chloram-phenicol	Control	Cyclo-heximide	Chloram-phenicol
G_1 + M	0.31	0.37	0.15	0.20	0.50	0.52
S	0.22	0.14	0.07	0.33	0.51	0.13
G_2 + P	0.10	0.30	0.23	0.21	0.36	0.23
Total N.C.	0.40	0.49	0.18	0.36	0.71	0.59

M = Prophase + Metaphase + Anaphase + Telophase

P = Prophase

N.C. = Nuclear Cycle

Ram S. Verma

9. Changes in mitotic index induced by cycloheximide and chloramphenicol.

In addition to the effect noted in the previous note, we were able from the same experiments to record the data necessary to compute the mitotic index (MI). For each experiment, cycloheximide (0.001%), chloramphenicol (0.03%) and control, the MI was determined. The values are recorded in Table 1.

The decrease in the mitotic index to 1.5% was apparent between 1 and 4 hours (i.e., 3 to 6 hrs after pulse, accounting for the 2-hour treatment period) after treatment with cycloheximide or chloramphenicol; it remained at this level for up to 6 hours, at both temperatures and with both chemicals. Thus, the mitotic indices are affected immediately even during the incubation period. If roots were treated with chloramphenicol at 30°C, the mitotic indices reached the control level in seven hours; in the case of cycloheximide (30°C), the control level was reached

Table 1

Mean mitotic indices (with standard deviations) from treated and untreated primary root-tips of 'Seneca 60' stock at 25° or 30°C

Hrs. after pulse	Control		Cycloheximide (0.03%)		Chloramphenicol (0.001%)	
	25°C	30°C	25°C	30°C	25°C	30°C
1	6.3 ± 0.82	3.1 ± 0.54				
3		3.1 ± 0.62	5.58 ± 0.47	3.08 ± 0.27	2.46 ± 0.30	3.23 ± 0.31
5	10.8 ± 0.70	6.2 ± 0.51	5.59 ± 0.53	1.24 ± 0.25	2.24 ± 0.28	2.06 ± 0.27
7	8.2 ± 0.62	5.8 ± 0.74	3.35 ± 0.26	1.26 ± 0.20	1.61 ± 0.22	5.96 ± 0.37
9		6.2 ± 0.74	4.12 ± 0.31	4.91 ± 0.51	6.60 ± 0.35	4.71 ± 0.32
10	8.9 ± 0.64					
11			7.49 ± 0.85	4.97 ± 0.36	6.50 ± 0.44	6.20 ± 0.34
12		6.2 ± 0.89				
13			7.32 ± 0.46	4.98 ± 0.41	5.30 ± 0.35	5.81 ± 0.34
14	9.8 ± 0.74					
15			4.06 ± 0.42	6.30 ± 0.73	5.24 ± 0.34	7.14 ± 0.52
17			7.01 ± 0.43	7.57 ± 0.42	5.33 ± 0.33	3.03 ± 0.25
18	9.2 ± 1.00					
19			6.54 ± 0.40	5.67 ± 0.35	5.60 ± 0.38	6.10 ± 0.41
21			7.12 ± 0.45	5.10 ± 0.34	6.49 ± 0.36	6.20 ± 0.35
23			6.80 ± 0.44	5.24 ± 0.40	6.50 ± 0.42	5.36 ± 0.42
25			8.59 ± 0.49	4.23 ± 0.23	7.62 ± 0.40	
27			5.90 ± 0.36			
30			6.80 ± 0.37			
Temperature mean	9.1 ± 0.29	5.3 ± 0.28	5.68 ± 0.45	4.54 ± 0.38	5.12 ± 0.10	5.07 ± 0.10

in nine hours. At 25°C, mitotic indices did not reach the control level even after 30 hours in both experiments.

Ram S. Verma

10. The effect on the nuclear cycle of mitomycin C and the production of chromosomal aberrations.

The purpose of the present study was to examine the effect of mitomycin C (MC) on the nuclear cycle and to record from metaphases of similar experiments the frequency of chromosomal aberrations assignable to specific chromosomes. Seeds of "Seneca 60" were germinated and grown at 24 ± 1°C on filter paper kept moist with distilled water. Germinated kernels with roots about 2 cm. in length were used for these experiments. Intact roots were exposed to H^3-thymidine (1 μc/ml final concentration) for 30 min., washed thoroughly, and treated with 0.001% MC for 2 hrs., following which the roots were washed in water again. After the last washing, the root tips were returned to the germination chamber for further growth and fixed at 2-hour intervals up to 36 hrs. post-treatment. Liquid emulsion autoradiographs (Kodak NTB-2) were prepared from Feulgen squashes of this material. Slides were scored for the frequency of labelled mitotic figures and the mitotic index was calculated. For each collection period, 3 or 4 root tips were examined to give a population of $6 - 8 \times 10^3$ cells.

In the experiments with MC, we did not accumulate metaphases by addition of colchicine, any other chemical, or cold; consequently, normal anaphase cells were also observed. Apparently, MC does not affect spindle formation during mitosis. However, a reduction in the mitotic index was recorded after MC treatment, suggesting that there was an immediate delay in the rate at which cells entered mitosis (Table 1). The relation between mitotic index and prophase labelling at various times from the beginning of MC treatment indicated that mitotic index was affected immediately even during the incubation period. Within the 14 to 18 hour period after the beginning of MC treatment, the mitotic index had decreased to about 1% (9.53% in control, Table 1), which in turn indicated that those nuclei were in a very early S period.

Table 1

Relation between mitotic index and proportion of prophase labelled at various times from the beginning of MC (0.001%) treatment (2 hrs.)

Time after the beginning of MC (hr.)	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Mitotic index (%)	6.7	5.5	2.9	3.1	5.7	4.6	1.0	0.8	1.4	5.0	6.1	8.7	3.0	4.3	9.5	12.1	9.1	10.3
Prophase labelled (%)	4.3	16.7	33.1	51.1	68.5	90.5	93.3	92.9	62.1	40.1	33.4	32.6	27.6	25.0	39.0	21.4	22.1	16.9

*Control 9.53%

The duration of the nuclear cycle with a 2 hr. treatment with MC indicates (Table 2) that the nuclei which pass through a complete S to G_1 cycle under the influence of MC were delayed by the drug. The increase in mitotic index between 18 hrs. and 24 hrs. post-treatment is an indication of the entry into a new mitosis of cells which were in G_1 and M phases at the time of treatment. The mitotic index reached the control level after 30 hrs.

Table 2

The duration of the nuclear cycle with or without a 2 hr. treatment with MC (0.001%) in Zea mays root tips

treatment / phase	hours	
	control*	mitomycin C
G_2 + P	2.1	7.0
S	5.0	11.5
Total	9.9	22.0

*Verma's data

In a second experiment, roots were exposed for 30 min. to H^3 - thymidine and then for 2 hrs. to MC (0.0005%). Fixations were made at 2 hr. intervals up to 32 hrs. after the removal of MC. The information on both aberration yield and labelling index was obtained at all fixation times. In order to make the chromosomes spread well and to study the chromosomal aberrations, a 0.03% solution of 8-hydroxyquinoline was employed for pretreating the intact root tips for 2.5 hrs. before fixation. At each fixation, the metaphase cells on each of three or four slides were scored, both for aberration yield and presence of labelling. In some cases very few metaphase cells were obtained due to the mitotic depression resulting from MC treatment.

In Table 3, the data on aberration yields show clearly that very few aberrations were observed from the first post-treatment mitosis of those cells in G_2 at the time of MC treatment. The aberrations which

Table 3

Yields of the various aberration types obtained at various times after a 30 min. exposure of roots to H^3-thymidine followed by 2 hr. MC (0.0005%)

Time after treatment (h.)	Total metaphase scored	% labelled metaphase	% metaphase aberration	No. of metaphase nuclei		No. of chromosome breaks		No. of chromosome interchanges and rings		No. of chromatid breaks		Aberration frequencies per cell
				L*	U*	L	U	L	U	L	U	
2	202	0.99	4.45	2	200	0	8	0	0	0	1	0.04
4	212	15.56	8.49	33	179	2	14	0	1	0	3	0.09
6	188	42.55	12.77	80	108	7	11	0	0	4	3	0.13
8	203	80.30	15.76	163	40	30	2	2	0	1	1	0.18
10	221	94.57	24.43	209	12	44	2	4	0	2	0	0.24
12	54	94.44	38.88	51	3	25	0	3	0	0	0	0.57
14	45	95.56	64.44	43	2	38	0	3	0	0	0	0.91
16	59	79.66	62.71	47	12	41	9	2	2	0	1	1.00
18	55	63.64	65.45	35	20	31	24	0	1	0	0	1.03
20	124	41.94	75.00	52	72	42	76	3	6	5	6	1.11
22	151	46.36	40.40	70	81	21	39	0	10	0	7	0.56
24	201	45.27	42.79	91	110	32	77	0	8	1	4	0.64
28	222	55.40	55.85	123	99	75	60	6	4	8	12	0.78
32	148	37.84	52.70	56	92	29	67	2	4	2	6	0.78

*L = labelled; U = unlabelled.

Table 4

"Goodness of fit" test for random distribution of chromosome breaks in various chromosomes induced by MC, based on percentage of unit chromosome lengths

Chromosome number	1	2	3	4	5	6	7	8	9	10	Total
% of unit chromosome length	14.4	12.0	11.1	11.0	10.4	9.0	8.4	8.6	8.0	7.1	100
observed	66	38	39	12	42	89	22	36	18	7	369
expected	33.1	44.3	40.9	40.6	38.3	33.2	31.1	31.7	29.5	26.3	369
X^2	3.1	0.9	0.1	20.1	0.4	94.2	2.7	0.6	4.5	14.1	140.5**

**$P > 0.01$

were observed occurred in labelled cells and the results in general indicate that there is an increase in sensitivity with increasing fixation time, i.e., the cells in early S show a higher aberration yield than the cells in late S at the time of treatment. The aberration frequency at 20 hr. after MC treatment is higher than other treatments; these cells would have been in late G_1 or early S at the time of treatment.

MC-induced chromosomal aberrations among the 10 pairs of chromosomes showed an apparent non-random distribution. If the drug was acting randomly per unit of chromatin, then the distribution of breaks should be according to the length of the chromosomes. The relative lengths in percentage of the complement (long arm plus short arm using Chen's data, 1969) of metaphase chromosome are shown in Table 4. The observed versus expected values were tested by Chi-square and a highly significant value was obtained. We attempted to assign the location of each aberration not only to a specific arm, but in most cases to a segment within arms. While admittedly somewhat arbitrary, our distribution analysis suggests that the frequency of breaks in certain arm segments is higher than in other arm segments. Future work to identify the locations of heterochromatin or knobs on the chromosome may lead to the study of the relationship between breakage and heterochromatin in maize chromosomes. At this time we can report only that the greatest concentration of breaks induced by MC appears at the secondary constriction and centromeres.

M. S. Lin

11. <u>The identification of chromosome segments by their replication behavior using principal components analysis</u>.

Previously we described the DNA replication behavior of maize chromosomes using ^{3}H-TdR autoradiography in different genetic stocks and at two temperatures (MGCNL 44:195-198, 1970). Replication behavior was described by plotting the mean number of silver grains for each chromosome arm at hourly intervals of the S period (a 'replication profile'). The data have been extended to include a chromosome 3 tester stock containing one B chromosome. The experimental input now includes:

1) Chromosome 3 tester + one B chromosome (28°C)
2) Chromosome 3 tester + one B chromosome (18°C)
3) Chromosome 3 tester (18°C)
4) Heteromorphic abnormal 10 (18°C)

This study depends on two features: positive, unambiguous identification of metaphase chromosomes, and localization in the autoradiographs of the silver grains. Sampling appropriate to these studies was described in the earlier report.

The replication profiles of B chromosomes from the 18°C and 28°C experiments were not of the same configuration. At 28°C the S period was much shorter, and the B chromosome exhibited extensive ^{3}H-TdR uptake in the last half of the S period, although uptake did occur throughout the entire S period. At 18°C, the S period was longer, and the B chromosome demonstrated a constant rate of labelling throughout the S period, such that there was no peak in the profile. Its replication profile is thus, at 18°C, much like that of the distal segment on the long arm of abnormal chromosome 10 (MGCNL 44:195-198, 1970).

While it was possible to distinguish the profiles of either the heterochromatic segment on abnormal 10 or the B chromosome from the other chromosomes, it was difficult to identify most arms of the A chromosomes on the basis of replication profiles.

A principal components factor analysis (see Jancey this newsletter) was employed in a further attempt to characterize chromosome segments by their replication behavior. Within the data there exists for each chromosome arm a population of grain-count observations made at each hour (sample) of the S period. From such a population of grain counts a number of descriptive statistics or characters may be derived. Since a number of populations exist, a large number of characters may be accumulated to describe the replication behavior of each chromosome segment for the entire S period.

The analysis was carried out using grain count data standardized for the length of the chromosome arm concerned. Preliminary analyses demonstrated that the most useful characters were the mean, variance, g_1 (skewness), g_2 (kurtosis) at each hour of the S period, and the percentage increment or decrement in mean grain count between sampling intervals.

This form of analysis has facilitated the identification of some but not all of the A chromosome arms (chromosome arms were treated independently) suggesting at least limited autonomy of ^{3}H-TdR uptake within arms. The B chromosome, the distal segment (long arm) of abnormal 10 and the satellite on chromosome 6 could easily be identified as unique segments in the principal components analysis.

Some chromosome arms 'change' their replication behavior depending upon the experimental conditions. Notable in this regard were the short arm of chromosome 9 in the presence of a B chromosome, and the short arm of abnormal 10 compared to that of normal 10. A similar change was not detected in the proximal segment of the long arm of abnormal 10.

While B chromosomes in many other ways behave differently from the distal segment of the long arm of K10, it is interesting to note how one parallels the other with respect to replication of DNA. They both have similar and unique replication profiles and both respond similarly in a principal components analysis. Likewise, they both can influence the replication characteristics of other chromosome segments. In addition, they both exhibit similar effects on the synchrony of replication between homologous chromosomes (see following report).

G. R. Douglas

12. Analysis of synchrony of DNA replication between homologous chromosome segments in maize root-tips.

The question of homologue synchrony of DNA replication has not yet been resolved. Testing for this phenomenon by standard statistical methods is not feasible, since they require the restriction that one can distinguish between two homologous chromosomes. We present here a method by which synchrony may be analyzed statistically without the above constraint.

The data employed were silver grain counts described above (i.e., ^{3}H-TdR uptake over all homologous chromosome arms at hourly intervals of the S period, replicated in a number of cells).

The hypothesis of the synchrony of ^{3}H-TdR uptake between homologous chromosome arms was separated into two testable portions which could be approached by statistical methods, as follows:

1) For synchrony to exist, there must be a high degree of concordance between homologues in a population of cells. We would declare concordance in a pair of homologous chromosome segments to exist if both members are either labelled or unlabelled. Non-concordance would exist if one homologue was labelled, the other not labelled. A population exhibiting a significant degree of concordance may be detected using a X^2 test.

2) For synchrony to exist, there must also be little difference in the number of silver grains over each member of a homologous pair. The degree of asynchrony in this case may be tested using information theory. The specific test is an I-divergence test which tests the degree of pairwise divergence between members in a population of unordered paired observations.

The summary of the results of tests (1) and (2) is presented in Table 1 under four conditions of experimental input. Both tests were performed on populations represented by each of the 20 chromosome arms plus the satellite on chromosome 6 (21 chromosome segments) at hourly intervals throughout the S period.

Table 1

Summary of results of X^2 tests for concordance and I-divergence tests for disimilarity of ^{3}H-TdR uptake, between homologous chromosome segments

Genetic stock	Temp.	No. of tests performed	Percent of tests significant ($p \leq 0.05$)	
			(1) X^2-concordance	(2) I-divergence
3 tester + 1 B	18°C	315	36.8	51.4
K10/k10	18°C	252	38.1	62.3
3 tester	18°C	231	40.3	24.7
3 tester + 1 B	28°C	126	46.0	29.4

A higher proportion of tests of divergence between homologous segments was observed to be significant ($p \leq 0.05$) when the genome contained large segments of heterochromatin (B chromosome or K10). This proportion was greatly reduced by removing the extra heterochromatin, or by raising the temperature. While the proportion of significant tests of concordance was relatively low, it remained independent of the presence of extra heterochromatin.

While the tests themselves have been deemed valid, we are still assessing the objectivity of the test criteria in terms of the more general hypothesis of synchrony. We are extending our study to include specific translocation stocks as an experimental input to provide a further, more vigorous test of synchrony and autonomy of DNA replication.

G. R. Douglas

UNIVERSITY OF WISCONSIN
Madison, Wisconsin

1. Effect of $\underline{M}^{st}$ on mutation of $\underline{R}^{st-f}$, a modified form of the $\underline{R}^{st}$ (Wisconsin) allele.

The effect of $\underline{M}^{st}$ (modifier of $\underline{R}^{st}$) on $\underline{R}^{st}$ to $\underline{R}^{sc}$ (self-colored) mutation in the aleurone is to increase the frequency of late occurring mutational events. Kernels with equal dosage of $\underline{R}^{st}$ appear much darker if they also carry $\underline{M}^{st}$ in the genome than if they don't. On the other hand, the frequency of corresponding colored aleurone and colored embryo kernels (a class which includes mutations of $\underline{R}^{st}$ to $\underline{R}^{sc}$ occurring from meiosis up to and including the second megagametophyte division) does not appear to be influenced by $\underline{M}^{st}$. Likewise, the available data do not show any effect of $\underline{M}^{st}$ on the frequency of kernels having non-corresponding aleurone and embryo phenotypes (a class representing mutations of $\underline{R}^{st}$ to $\underline{R}^{sc}$ at any of the three megagametophytic divisions). Therefore, the control of mutation of $\underline{R}^{st}$ to $\underline{R}^{sc}$ exerted by $\underline{M}^{st}$ appears to be limited to a specific tissue (the aleurone) and a specific time in the development of this tissue (late divisions).

Several derivatives of $\underline{R}^{st}$ showing a heritably modified aleurone phenotype have been isolated. One of these responds to $\underline{M}^{st}$ in an

altered fashion: $\underline{R}^{st}$ to $\underline{R}^{sc}$ mutations in the aleurone tend to be suppressed until a very late developmental stage. As a consequence, kernels with the modified $\underline{R}^{st}$ and $\underline{M}^{st}$ show only small, fine spots in the aleurone. This new form of $\underline{R}^{st}$ has been designated $\underline{R}^{st-f}$ ($\underline{R}$-stippled fine). The effect of $\underline{M}^{st}$ upon $\underline{R}^{st-f}$ is no longer limited to a specific time in the development of the aleurone since it now displays an early suppressing effect in addition to its standard late-mutating effect.

With the intent of studying the effect of $\underline{M}^{st}$ on megagametophytic mutation of $\underline{R}^{st-f}$ to $\underline{R}^{sc}$ the following crosses were performed:

$$\underline{R}^{st-f}\ \underline{M}^{st}\ \underline{+}\ ♀ \times \underline{r}^{g}\ \underline{+}\ ♂$$

$$\underline{R}^{st-f}\ \underline{+}\ ♀ \times \underline{r}^{g}\ \underline{+}\ ♂$$

The results are reported in the following table.

Phenotype of selection	Origin	Embryo classification by progeny test		Effective population
		$\underline{R}^{sc}$	$\underline{R}^{st}$	
Colored aleurone	$\underline{R}^{st-f}\ \underline{M}^{st}$	57	1	33,510
Unselected embryo		(17.2)[1]	(0.3)	
	$\underline{R}^{st-f}\ \underline{+}$	78	48	23,075
		(33.8)	(20.8)	
Stippled aleurone	$\underline{R}^{st-f}\ \underline{M}^{st}$	-	-	44,200
Colored embryo		(nil)	(nil)	
	$\underline{R}^{st-f}\ \underline{+}$	8	3	19,415
		(4.1)		

[1]Numbers in parentheses represent frequencies x 10^{-4}.

Two major observations can be made from the data reported in the table:

1) Kernels of non-corresponding aleurone and embryo phenotypes are essentially not recovered in the progeny from $\underline{R}^{st-f}\ \underline{M}^{st} \times \underline{r}^{g}\ \underline{+}$ crosses, whereas they occur quite frequently in the progeny from $\underline{R}^{st-f}\ \underline{+} \times \underline{r}^{g}\ \underline{+}$ crosses.

2) The frequency of corresponding colored aleurone and embryo kernels is much higher when the female parent is $\underline{R}^{st-f}$ + than when it is $\underline{R}^{st-f}$ $\underline{M}^{st}$.

Both observations suggest that the effect of $\underline{M}^{st}$ is to eliminate mutation of $\underline{R}^{st-f}$ to $\underline{R}^{sc}$ in the female gametophyte. A comparison of the frequencies of the different kernel classes lends support to this interpretation. Mutation of $\underline{R}^{st-f}$ + to $\underline{R}^{sc}$ at the third megagametophyte division should account for all the cases of stippled aleurone, colored embryo kernels and for twice as many colored aleurone, stippled embryo kernels. Thus, of the 20.8 cases of the latter per 10,000 kernels, 8.2 would be accounted for by mutation at the third division and 12.6 by mutation at either the first or second division. Likewise, since mutation of $\underline{R}^{st-f}$ + to $\underline{R}^{sc}$ at either the first or second division should account for an equal number of the two classes of colored aleurone kernels (with corresponding embryo and non-corresponding embryo), 12.6 cases of the corresponding colored aleurone and embryo class for every 10,000 kernels would be due to mutation at either of the two early divisions. This leaves a residue of 21.2 cases of the latter class per 10,000 kernels to be explained. Since $\underline{R}^{sc}$ mutations do not occur in clusters in the ears, the most likely explanation for these remaining cases is that they arise during meiosis. The residual frequency of 21.2×10^{-4} agrees with the frequency of corresponding colored aleurone and embryo kernels obtained from $\underline{R}^{st-f}$ $\underline{M}^{st}$ (17.2×10^{-4}). This comparison suggests that all such mutants have a meiotic origin in $\underline{R}^{st-f}$ $\underline{M}^{st}$ females and lends support to the belief that few to no megagametophytic mutations occur in $\underline{R}^{st-f}$ $\underline{M}^{st}$ plants. However, if the above value represents the true frequency of $\underline{R}^{st-f}$ to $\underline{R}^{sc}$ meiotic mutations, then this frequency has been significantly increased above that normally obtained in standard $\underline{R}^{st}$ (Wisconsin) homozygotes.

The change of $\underline{R}^{st}$ to $\underline{R}^{st-f}$ appears, therefore, to have brought an increase in the frequency of $\underline{R}^{sc}$ mutations of meiotic origin in homozygotes and a new response to the action of $\underline{M}^{st}$, namely the complete suppression of post-meiotic $\underline{R}^{sc}$ mutations till a very late stage in aleurone development. Possibly, $\underline{R}^{st-f}$ represents a new relationship between the Sc and I^R components of the $\underline{R}^{st}$ allele.

Hugo K. Dooner

2. Transmission of a specific class of deficiency gametes.

The female transmission of a specific class of duplication-deficiency gametes has been detected during the course of an experiment designed to test the effect of proximity of a translocation breakpoint upon mutation of R to r. A series of six translocations involving the long arm of chromosome 10 were used in this test. The six reciprocal translocations, the position of their breakpoints, and the genetic maps of the resulting translocated tenth chromosomes are listed in the table below.

Translocation	Breakpoints	Genetic Map
T6-10a	6L 0.75; 10L 0.15	T 10.6 g 12.0 R
T1-10e (B98)	1L 0.16; 10L 0.31	T 7.6 g 15.7 R
T4-10b	4L 0.15; 10L 0.60	T 4.3 g 13.6 R
T1-10d (a84)	1L 0.50; 10L 0.68	T 2.1 g 7.9 R
T2-10 (5651)	2S 0.71; 10L 0.62	g 0.3 T 4.2 R
T5-10 (4384)	5L 0.13; 10L 0.79	g 0.0 T 2.3 R
NONE (control)		g 17.0 R

The map distances given above represent values obtained through the female side from crosses $\underline{T} + \underline{R}^r/\underline{N}\ \underline{g}\ \underline{R}^g_6 \times \underline{N}\ \underline{g}\ \underline{r}^g$. The order agrees with that established from $\underline{N}\ \underline{g}\ \underline{r}^g \times \underline{T} + \underline{R}^r/\underline{N}\ \underline{g}\ \underline{r}^g$ crosses, where the translocation heterozygote was used as male parent. The order in T5-10 (4384) is known from the latter crosses to be g T R, the recombination values obtained in that case being 2.4% for region I and 6.7% for region II. As can be seen from the above table, the breakpoint in 10L lies proximal to g in four of the six translocations. In two of them, namely in T2-10 (5651) and T5-10 (4384), the breakpoint occurs between g and R.

Colorless seeds were selected from crosses $\underline{T} + \underline{R}^r/\underline{N}\ \underline{g}\ \underline{R}^g \times \underline{N}\ \underline{g}\ \underline{r}^g$, germinated, and the resulting seedlings scored for coleoptile color. The selections were then transplanted to the field where they were grown to maturity for further testing. In testcrosses utilizing translocation heterozygotes with the breakpoint in 10L proximal to g, the frequency of colorless seeds was low, though variable among the different translocations, and in the order of magnitude expected from mutation of R to r. An approximately equal number of red and green seedlings was obtained from these seeds. In crosses involving translocation heterozygotes

having the breakpoint in 10L between g and R, an unexpectedly large number of colorless seeds was found. Upon germination, only a small minority produced the red seedling color expected from R^r to r^r mutation. A large majority gave green seedlings. Samples of 15 green seedlings from each of the two crosses were grown to maturity. These plants were well developed and normal in appearance, somewhat late in pollen shedding, and in general, smaller than the sib plants obtained from red seedlings. Some developed yellow stripes in the lower leaves. Pollen examination revealed two classes of pollen grains segregating in equal numbers: one class consisting of large, normal appearing grains, and the other class consisting of smaller, though well-filled grains. The ears in these plants were partially sterile.

Apparently, plants heterozygous for T2-10 (5651) and T5-10 (4384) produce a large number of functioning duplication-deficiency megaspores. Deficient in these female gametes is the distal end of 10L, a chromosome segment including R but not g. The behavior of these duplication-deficiency gametes in transmission closely parallels the behavior of a haplo-viable deficiency reported by Stadler (Missouri A.E.S.R.B. 204, 1933). This X-ray induced deficiency of the distal one fifth of the long arm of chromosome 10 also covered R but not g. Furthermore, the general appearance of the aneuploid plants obtained from translocation heterozygotes matches Stadler's description of his deficiency heterozygous plants.

On the other hand, plants heterozygous for any of the other four translocations do not produce functioning duplication-deficiency gametes. The breakpoint in 10L in these translocations lies proximal to g, the closest one mapping 2.1 units proximal to g. It would appear, therefore, from this evidence that a chromosomal region which extends short distances proximally and distally to g, but which definitely excludes R, contains a gene or genes which are essential for normal female gametophyte development.

Confirmatory evidence comes from the transmission behavior of 10^B in $10/10^B$ hemizygotes. 10^B is one of the two members of a reciprocal translocation between chromosome 10 and a B chromosome, in which the breakpoint in 10L is proximal to g. Gametes receiving only 10^B, and therefore deficient for g, are non-functional through the female.

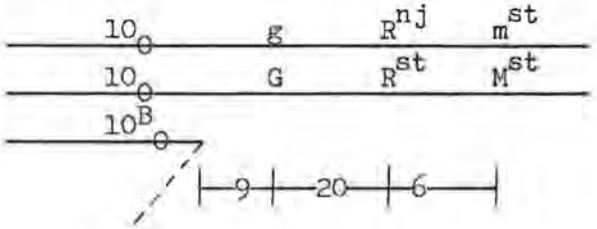
A note might be added regarding the physical location of the genes g and R in the long arm of chromosome 10, with the reservation that the variable position of the center of a translocation cross makes these placements very tentative. As can be seen from the table, a translocation breakpoint in the long arm of chromosome 10 occurring between 10L 0.60 and 10L 0.80 may be either proximal or distal to g, but is always proximal to R. Breakpoints proximal to 10L 0.60 are always proximal to g. Thus, g would be placed in the sub-terminal one-fifth and R in the terminal one-fifth of the long arm of chromosome 10. This placement of R agrees with Stadler's.

An examination of the genetic map of the long arm of chromosome 10 reveals that g lies in the proximal one-fifth of the arm and R approximately midway between the centromere and the distal end. To reconcile the genetic map with the physical map one can postulate a very high frequency of recombination in the distal two-fifths of the long arm of chromosome 10 relative to that in the remainder of the arm. The possibility of localized high recombination in the terminal segment of 10L agrees well with previous findings in maize and differs markedly from findings in Drosophila, where the frequency of recombination increases towards the center of the chromosome arm.

Hugo Dooner

3. Use of a partial trisomic in a half-tetrad analysis.

A cytogenetic system was sought for the recovery of reciprocal products of crossovers in the R region of chromosome 10. Such a system, ideally, would be disomic, of such a nature to permit recovery following a single exchange, and be efficient in terms of its yield of reciprocal products. As a preliminary to study of R intralocus crossovers, an investigation involving the g-R and R-M^{st} segments was performed utilizing the following heterozygote:



Individuals of $10/10/10^B$ constitution were synthesized initially by intercrossing primary trisomic-10 plants as female with ones heterozygous for translocation B-10a as male. Besides primary-10 trisomics and the usual B^{10} hyperploids, there occurred in progeny of this cross plants having a combination of primary trisomic-10 features complementary to those of B^{10} hyperploids. Such plants proved to have received two chromosomes 10 from the trisomic-10 parent, and the deficient 10^B chromosome from the male--hence the designation $10/10/10^B$. From successive crosses to $\underline{G}\ \underline{R}^{st}\ \underline{M}^{st}$, a $\underline{G}\ \underline{R}^{st}\ \underline{M}^{st}/\underline{G}\ \underline{R}^{st}\ \underline{M}^{st}/10^B$ stock was derived which, when mated with $\underline{g}\ \underline{R}^{nj}\ \underline{m}^{st}$, gave plants of the heterozygous constitution illustrated above.

Table 1

Chromosome 10 and golden-R classification among offspring produced in $\underline{g}\ \underline{R}^{nj}\ \underline{m}^{st}/\underline{G}\ \underline{R}^{st}\underline{M}^{st}/10^B$ ♀ x $\underline{g}\ \underline{r}^{g}\ \underline{m}^{st}$ ♂ matings

G-R constitution	Chromosome 10 class[1]		
	10 10	10 10 10^B	10 10 10
$\underline{G}\ \underline{R}^{st}$	455	175	18
$\underline{g}\ \underline{R}^{nj}$	393	136	24
$\underline{g}\ \underline{R}^{st}$	95	17	5
$\underline{G}\ \underline{R}^{nj}$	75	42	3
% of total	71.8	26.1	2.1[2]
% of recombination	16.7	15.9	16

[1]Excludes 16 morphologically abnormal or diseased plants that could not be classified according to one of the chromosome 10 phenotypic categories.

[2]Includes four cases not analyzed for marker composition.

Table 1 summarizes the classification for chromosome 10 makeup and g-R recombination of 1417 offspring obtained from 13 matings of the marked $\underline{R}^{nj}/\underline{R}^{st}/10^B$ heterozygote with $\underline{g}\ \underline{r}^{g}\ \underline{m}^{st}$ as male. 10^B was

transmitted as an extra chromosome to 26% of the progeny, whereas only two percent received an extra chromosome 10. Consideration of g-R recombination over the three chromosome 10 classes indicates independence between recombination and transmission of an extra chromosome, either 10^B or 10. Furthermore, the recombination values do not differ markedly from an average level of about 20 observed for normal diploids (inbred W22 background).

A sample of 381 R^{st} m^{st} crossovers from additional progeny of the same cross was used to examine chiasma interference. Only two of the 381 were golden, whereas some 63 simultaneous exchanges in the g-R and R-M^{st} regions would have been expected were there no interference. The resulting coincidence value of 0.03 compares with ones ranging from 0.05 to 0.14 obtained similarly in various tests involving normal 10/10 parents.

Table 2

Classification according to chromosome 10 marker relations of 10/10 spores obtained from g R^{nj} m^{st}/G R^{st} M^{st}/10^B ♀ + x g r^g m^{st} ♂ matings

Class		Number
One of each parental chromosome		13
Both chromosomes of one parental type		4
Reciprocal crossovers	G-R region	3
	R-M^{st} region	1
One parental, one crossover	G-R region	2
	R-M^{st} region	2
Identical crossovers		0
		25

Eight of the twenty-five 10/10 spores analyzed for marker composition (Table 2) carried one (four cases) or both (four cases) chromosomes 10 recombinant in the G-R-M^{st} region. In the latter event the two chromosomes were reciprocally marked and never identically so, an observation consistent with expectation based on high chiasma interference. The

four instances of one recombinant and one parental chromosome also were of one sort: markers proximal to the exchange were heterozygous whereas those distal were homozygous. Double or higher order exchanges in the chromosome arm are not necessary to account for these eight cases, provided that sister centromeres undergo normal, equational separation at the second division. Similarly, those cases (also four) where both chromosomes were of one parental type could have originated following a single exchange between the most proximal marker, golden, and the centromere.

In principle, the $10/10/10^{B}$ system therefore appears to provide for the recovery and ready identification of both products of crossovers in the chromosome 10 segment represented by B^{10}. Efficiency of the system suffers, however, from the relatively low frequency of 10/10 spores formed.

J. L. Kermicle

ADDENDUM:

HARVARD UNIVERSITY
Cambridge, Massachusetts 02138
Department of Biology

1. Scanning electron microscopy of chlamydospores of corn smut (Ustilago maydis (DC.) Corda).

In an earlier report (MGCNL 44:42, 1970) we discussed a new double-stage heat-pressure carbon replica technique. This technique was used to study chlamydospores of corn smut at the ultrastructural level using TEM (transmission electron microscope). Despite this, the conventional light microscope (LM) still has great potential, especially for measurements of size and for other comparative morphological studies of spores. In recent years a new type of electron microscope has been developed, the scanning electron microscope (SEM). The instrument is fundamentally different from the conventional TEM, in which either very thin sections or carbon replicas are used to record an image on photographic plates. In TEM an electron beam is transmitted through the objects, whereas in the SEM, entire objects (spores) are placed and a beam of electrons is used to bombard the object being studied. The electrons scattered by impact with the specimen and those emitted by the specimen during bombardment are collected to form a cathode-tube image, characterized by great depth of focus and a three-dimensional appearance. Before the objects are placed inside the SEM specimen chamber, it is important that the specimen have an even metal coating devoid of discontinuities. About 200Å to 500Å either pure gold or gold-palladium alloy coating is adequate. Stability of the image is further assured by addition of a thin coating of carbon (about 50Å to 100Å) prior to the metal coating, hence insuring greater stability of the specimens and also preventing artifacts under the electron beam. The coating process requires a vacuum-evaporator to evenly distribute the coating material which reflects the electrons. Micrographs taken with SEM further support our observations on the morphology of corn smut spores as earlier discussed. The tips of the spines are ordinarily curved, but the direction of the curvature varies from tip to tip. We postulate that this structural feature in some ways is an advantage to facilitate a mechanism for clasping the spores to the host tissues.

The chlamydospores used in this investigation were kindly supplied by Dr. J. E. Puhalla, at the Connecticut Experimental Station, New Haven, Conn.

U. C. Banerjee
E. S. Barghoorn

2. Pollen sterility in maize caused by fungus attack (Ustilago maydis (DC.) Corda).

Almost no information is available on pollen sterility caused by fungus attack on standing crops. The present investigation was carried out with respect to the influence of smut on maize pollen. A synthesized maize variety (a dent corn with the 4th chromosome from Nobogame teosinte) was chosen for this study. This maize variety exhibits an anomalous ear formation; at first the ears appear normal, but later, after the original tassel completes pollen shedding and the ears complete their normal pollination cycle, the tips of the ears elongate and produce staminate spikelets. The male spikelets show normal anther development and shed viable pollen grains which are perhaps useful for the younger developing ears at the receptive stage. Such anomalous ears are often called "laughing-ears." However, these plants also produce a normal tassel at the top. In this maize variety, the first four leaf-sheaths below the tassel are always sterile, the first ear being formed in the 5th leaf-sheath, and the second ear in the sixth leaf-sheath.

The seedlings in this study were grown in the greenhouse, then transplanted to the field. After the seedlings had reached about 6 inches in height, they were dusted with viable corn-smut chlamydospores. The chlamydospores were obtained from kernel infections. A cloudy, calm, humid afternoon was chosen for infecting the seedlings and the plants were carefully watered. The chlamydospores germinated and produced sporidia which infected the maize seedlings during the following evening. The infections appeared on the plants almost at the time when "laughing-ears" started to appear. The effect of fungus attack was appraised on the basis of the smut-gall formation on the ears bearing staminate flowers. In the control plants, staminate flowers of the "laughing-ears" developed normally and shed viable pollen grains at maturity. The male

flowers, with the infected kernels at the base, failed to show anthesis. The degree of pollen sterility was correlated with the amount of infection of the ears. A test of pollen sterility was made using the technique discussed by us earlier (MGCNL 44:45, 1970). It was also found that during severe corn smut attacks even the original tassels of the plants failed to shed pollen and exhibited a high percentage of pollen sterility. Even failure of kernel formation was observed, probably owing to inadequate development of the embryo-sacs in the kernels.

We are thankful to Dr. W. C. Galinat, University of Massachusetts, at Waltham, Mass. for the maize seeds.

U. C. Banerjee
E. S. Barghoorn

3. Formation of smut galls (Ustilago maydis) in normal and aborted kernels of maize.

It has been reported that certain smuts can produce chlamydospores on artificial culture media (Sartoris, Amer. Jour. Bot. 11:617, 1924; and Wang, Phytopath. 28:860, 1938). It was emphasized in these studies that chlamydospore development is accelerated on rich culture media over that on minimal media. This suggests that the fungus has a preference for concentration of nutrients in the culture media and matures more rapidly. But, in either case, no size or structural difference of chlamydospores was reported, when spores produced in vitro were compared with spores produced in host tissue. To demonstrate the nutrient preference of the fungus, corn smut (Ustilago maydis) was used in vivo. A few previously infected maize plants were chosen for this investigation. On each plant the second ear from the tassel was selected, because the first ear often exhibits delayed infection. At the time of pollination a limited quantity of pollen was applied to the protruding receptive stigmas of marked ears. Immediately after the ears were artificially pollinated, they were covered with paper bags to prevent further pollination. The kernels whose stigmas were not receptive at the time of pollination failed to produce a normal endosperm, due to lack of fertilization. Smut-galls appeared on the kernels of the marked ears. The ears were checked regularly to score the degree of infection. It was

found that in the host tissue corn smut has no preference for the nutrients (presence or absence of endosperm) in the normal or abortive kernels, and smut-galls are produced equally vigorously on both normal and aborted kernels.

Further, we have also observed that chlamydospores from infected kernels when transferred to a new host will produce only the kernel-galls in the next cycle of chlamydospore formation.

U. C. Banerjee
E. S. Barghoorn

4. Rootlessness, an unusual character found in some young seedlings of teosinte.

Normally, when teosinte seeds are exposed to optimum conditions of germination in the laboratory, germination begins by an orderly succession of developmental steps. The seed first imbibes water and swells and finally the coleorhiza (primary root) extends out from the root-pore (pulvinus notch) of the fruit-case and grows downward. A few hours later a coleoptile with embryonic leaves projects from the apical shoot-pore, following this the coleoptile ruptures and the young leaf expands. During this period a few secondary roots are formed which actually extend out from the shoot-pore. These secondary roots are not sensitive to gravity at first, but only gradually develop downward and become functional. When seeds are sown in the soil, the elongating embryonic leaves remain ensheathed in the coleoptile until they reach the soil surface. Occasionally, both the primary root and shoot emerge simultaneously from the seed-case and finally the seedlings become established. The germination pattern of teosinte seeds is very similar to that of maize.

In the present investigation we have studied variations in the germination pattern. The seeds of various documented teosinte races were allowed to germinate in laboratory conditions (at 24-25°C, room temp.). The seeds were first soaked in glass-distilled water for 3 hrs., then kept in paper cups with moist paper towels, covered with Saranwrap to retain moisture. The scoring for germination was done on the 9th and 11th days. Apart from the two normal germination patterns discussed above, two new types of seedlings with rootless character are recorded here. In the

first case, germination was featured by only 2 or 3 secondary roots which projected from the shoot-pore of the fruit-case; following this, the primary shoot emerged from the shoot-pore. Primary roots are never produced by these seedlings and they are comparable to the rootless maize seedlings "really rootless" as reported by Nickerson (MGCNL 40:142-144, 1966). If these seedlings are planted in soil they survive at least under greenhouse conditions. In the second rootless type no primary or secondary roots are ever produced, but the primary shoot emerges from the seeds. The seedlings remain alive as long as they can utilize the stored food in the endosperm. We consider them as "totally rootless" seedlings and they never reach maturity. Moreover, we have noticed that rootless seedlings are slightly darker green than normal seedlings. This suggests that these seedlings are metabolically more active than the normal.

The following races of teosinte from different localities were found to exhibit "rootlessness" in their seedlings: (1) Edo de Mexico, Chalco about 30%; (2) Edo de Mexico, Amecameca 20%; (3) Edo de Mexico, Los Reyes 10%; (4) Guanajuato #45121, 10%; and (5) Huehuetenango, Tzisbaj (Guatemala) 10%. The percentages reported here are based on only ten seedlings; further germination tests should be made to establish the reliability of the percentage.

No "rootless" seedlings were found in the following races: (6) Jutiapa #51186 from Guatemala; (7) Huehuetenango Huista, Guatemala; (8) Michoacan #45320; (9) Guanajuato #46452; (10) Guerrero #47259; (11) Guerrero #47269; (12) Guerrero #47335; and (13) Chihuahua, Nobogame (for the accuracy of the collection number or locality, see Wilkes, H. G., 1967, The Bussey Institution, Harvard University Publication).

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

U. C. Banerjee
E. S. Barghoorn

5. Importance of "rootlessness" in maize.

Last summer (1970), several maize varieties were tested for their germination in laboratory and greenhouse conditions. Normal seedlings with similar color, shape, and size (root and shoot length) were selected and transplanted to the field for study of their pollen grain size under various environmental conditions. The maize varieties which originated either from the arid localities of Central America (e.g. Chapalote popcorn, Papago flour corn) or from areas with a short growing season (e.g., Gaspé flint from the northeastern part of Canada) were found to exhibit a maximum percentage of rootless seedlings. Rootless seedlings in maize were previously reported by Nickerson (MGCNL 1962, 1965, and 1966) and by Lorenzoni and Salamini (MGCNL 1970). Nickerson considered that perhaps this unusual rootless character is in some way related to growth hormones which may inhibit root formation. He noticed that when "really rootless" plants survive, they flower a few days earlier than the "regular rootless" plants. He concluded that "the gene rootless forms no roots because of an excess production of IAA (indole acetic acid) rather than because of a dearth of this substance in the nodal meristems of the lower stalk of the plant as was thought heretofore." In a recent report, Dakshini and Tondon (Ann. Bot. 34:423-25, 1970) have reported a delay in radicle formation in an unusual type of germination in Oropetium thomaeum, a member of the family Gramineae, characteristic of rocky, gravelly, and semi-arid habitat in certain tropical regions of the world. Our observations also suggest that "rootlessness" in maize is possibly an adaptive device to facilitate drought resistance in seedlings growing under adverse conditions of water supply or in a short growing season. In the literature we have also found that an agronomic practice in dry regions called "presowing drought hardening" is often used. Presowing hardening is done by soaking seeds in water and then air drying. The dehydration after soaking confers a high drought resistance without interfering with germination, growth and yield, all of which are decreased when untreated seeds are subjected to soil drought during the growing period. We consider presowing hardening is the result of extensive physiological reorganization induced by the dehydration process. In our "rootless" maize seedlings, we found that "rootless" plants are metabolically quite active

and flower a few days earlier than the normal plants (with normal rooting system) of the same race. "Rootlessness" is also recorded in some teosinte races as discussed above.

U. C. Banerjee
E. S. Barghoorn

6. Identification of pollen grains of maize: importance of size.

Recently we have studied (Banerjee and Barghoorn, MGCNL 44, 1970) the differences existing among pollen grains of maize and its relatives, using scanning and transmission electron microscopy. The use of better resolution and high magnification electron micrographs further helps in precise identification using micro-morphological characters of pollen grains. However, earlier reports from this laboratory clearly suggest that pollen grain size (outer diameter at pore axis) alone could be used safely to distinguish maize pollen from that of its relatives. Maize has the largest pollen grain size so far recorded among grasses (up to 150 μ in some cultivated varieties of maize). At this stage we suggest that any pollen grains of grasses larger than 100 μ present in the sediments can be safely interpreted as maize, if the pollen shows an evenly distributed granular exine pattern with the light microscope (LM). Moreover, accurate measurements of pollen grain size are only possible with the LM. So the use of the LM still has great potential in maize palynology. If LM phase-optics or a high quality oil-immersion objective is used one can distinctly recognise the granular nature of exine (spinulate ektexine pattern) of maize and teosinte pollen from the negatively reticuloid exine pattern of Tripsacum species pollen. Problems arise when the predominantly smaller pollen grains of some cultivated maize varieties (especially of popcorn races) fall within the size range of teosinte pollen. In these cases the use of electron microscopy is essential. Recently, we have found that the pollen grains of some popcorns and teosinte among some wild populations exhibit an ektexine spinule dimorphism at the ultrastructural level which makes it very difficult to separate the two genera. The cause of such an ektexine spinule dimorphism and variation in pollen grain size, due to various environmental factors in both maize and teosinte, is under investigation.

U. C. Banerjee
E. S. Barghoorn

7. Factors controlling pollen grain size in maize.

In palynological studies various morphological characters such as pollen grain size (largest outer diameter), number of furrows and pores and their size, exine patterning and other features are often used to identify pollen grains. However, pollen size is sometimes the most useful character for separating the species of a genus. And it is generally considered that all these various characters are genetically controlled. Almost no importance has been given to variations in pollen grain size, often caused by environmental factors during their ontogeny. However, Jones and Newell (1948) have demonstrated a positive correlation of environment and pollen grain size in many grasses, including maize. In contrast, Blanco (1950) has shown a negative correlation and emphasized that no significant influence of environment was found on pollen grain size in maize. Among earlier reports on various climatic effects are those documented by Piech (1922) in Linaria, Kawecka (1926) in Oenothera, and Stow (1930) with Hyacinthus. These investigators demonstrated the effect of high temperature which favors the formation of large pollen grains. In a recent report, Kurtz and Liverman (1958) found that variation in pollen diameter is due to temperature in tomato and cocklebur. Further, they reported that pollen grain size increased with decreased day temperature, while pollen size decreased at high and low night temperatures and at high day temperatures, Michaelis (1928) found that occasional cooling of flower buds induces formation of abnormal pollen with a variable number of germ pores in Epilobium and Oenothera. Moreover, the work of Andreev (1934), of Schoch-Bodmer (1940) in Lythrum, of Mehlquist (1942) in Primula, and our work with maize indicate that the position of the flower on the plant (on the tassel in maize), the size of the anthers, and the time of anthesis and anther dehiscence all influence pollen grain size. Again Schoch-Bodmer with Lythrum and our work with maize suggest that even water deprivation causes variability in pollen grain size. Mikkelsen (1949) emphasized that nutrition has a marked influence on pollen grain size. Bell (1959) reported that pollen grain size variation in tomato, petunia and portulaca is due to mineral nutrition. In our recent report (Banerjee and Barghoorn, 1970), we found that wheat pollen grain size is greatly influenced by several environmental

factors; in addition, genetic make-up and different chromosome numbers (at different ploidy levels) directly influence the pollen grain size and density of ektexine spinules per unit area.

In our study of maize pollen, we found the various factors which influence the pollen grain size are directly or indirectly connected with the physiology of the plant. We will categorize these factors as (a) external and (b) internal. However, factors such as seed size are also important. The influence of this factor was tested using a popcorn type (chapalote). A number of the largest and smallest kernels were selected from a single cob; all kernels were considered genetically similar. After germination all seedlings were exposed to similar environmental conditions such as temperature, light, water and soil. At maturity the seedlings from the large kernels consistently produced large plants with large pollen grain size, while the seedlings from smaller kernels produced smaller plants and smaller pollen grains. It is also interesting to note that plants grown from smaller kernels flower a few days earlier than the plants grown from large kernels.

U. C. Banerjee
E. S. Barghoorn

8. Request to the readers of maize genetics cooperation News Letter.

Professor E. S. Barghoorn and U. C. Banerjee, of the Department of Biology, Harvard University, Cambridge, Mass., would like to request the readers of this News Letter to send dry mature pollen grains (at shedding stage) of pure inbred lines of sweet corn (with no gene for starchy character) and flour corn (with no gene for sugary character). They are also interested in receiving documented pollen grain samples of any primitive popcorn, pod-corn, teosinte or any other maize relatives.

They also request chlamydospores of smut from any maize relatives except corn-smut from maize.

E. S. Barghoorn
U. C. Banerjee

AGRICULTURAL RESEARCH INSTITUTE
HUNGARIAN ACADEMY OF SCIENCES
Martonvásár, Hungary

1. Correlation of earliness traits of hybrids produced from related and non related lines.

The earliness properties of 27 experimental and commercial single, three-way and double cross hybrids, produced from non related parents, were studied in four replicated trials during 1967 and 1968. Individual plants (160) from each hybrid were evaluated on the basis of the following traits:

a) grain moisture content at harvest,

b) days to 50% male flowering time,

c) total leaf number per plant,

d) number of leaves above the ear.

Highly significant positive correlations were obtained between different earliness properties. Table 1 contains the correlations of the possible pairs of traits.

Table 1

Correlation coefficients for different pairs of earliness traits for 38 maize hybrids

Martonvásár, 1967-1968 and 1969-1970

Pair of earliness traits	Correlation coefficients	
	1967-1968 27 hybrids	1969-1970 11 hybrids
Total leaf number per plant Days to 50% male flowering time	r = 0.8495***	r = 0.8398***
Total leaf number per plant Grain moisture content at harvest time	r = 0.7868***	r = 0.8118***
Total leaf number per plant Number of leaves above the ear	r = 0.8638***	r = 0.8981***
Days to 50% male flowering time Grain moisture content at harvest time	r = 0.7953***	r = 0.8944***
Days to 50% male flowering time Number of leaves above the ear	r = 0.6626**	r = 0.7706**
Number of leaves above the ear Grain moisture content at harvest time	r = 0.6291**	r = 0.7103**

***Significant at 0.1 per cent level

**Significant at 1 per cent level

*Significant at 5 per cent level

Similarly, highly significant positive correlations were observed in those 11 maize hybrids which were evaluated (except for number of leaves above the ear) during 1967-1970 (Table 2).

Table 2

Correlation coefficients of the different pairs of earliness traits for 11 maize hybrids
Martonvásár, 1967-1970

Pair of earliness traits	Correlation coefficients
Total leaf number per plant Days to 50% male flowering time	r = 0.8888***
Total leaf number per plant Grain moisture content at harvest time	r = 0.8700***
Days to 50% male flowering time Grain moisture content at harvest time	r = 0.8618***

***Significant at 0.1 per cent level.

We have also investigated the relationship of different earliness properties in hybrids which were close in type of vegetation period as well as in the building up of hybrids. The studied hybrids were special three-way cross hybrids. The maternal parent was WF 9 x N 6, the paternal parents being different selected sublines of C 5 (also called W 23) an established inbred line. The three way crosses, designated by the general formula (A x B) x $C_{1, 2, 3, C_n}$, were produced by hand pollination.

These hybrids were studied in Martonvásár during 1966-1968 and at Madison in 1966 (University of Wisconsin Department of Agronomy, Madison, Wisconsin, USA) in collaboration with Prof. N. P. Neal, to whom special thanks is due. The mean values of different earliness properties are presented in Table 3.

Table 3

Mean values of earliness properties of the three way crosses produced from WF 9 x N 6 by C 5 sublines Madison, 1966 and Martonvásár, 1966-1968

Hybrid	Grain moisture content at harvest	Days to 50% male flowering time	Total leaf number
(WF 9 x N 6) x C 5-1	27.5	82.5	19.2
(" ") x C 5-2	27.2	82.0	19.3
(" ") x C 5-5	27.4	82.0	19.0
(" ") x C 5-8	27.4	82.2	19.3
(" ") x C 5-10	27.6	82.2	19.7
(" ") x C 5-11	28.0	82.0	19.4
(" ") x C 5-12	28.1	82.2	19.1
(" ") x C 5-15	27.6	82.0	18.8
(" ") x C 5-16	27.4	82.2	19.2
(" ") x C 5-18-1	28.5	82.2	19.2
(WF 9 x N 6) x (W 23 x 014)	29.6	82.8	18.9
(WF 9 x N 6)	32.4	84.2	19.9
(WF 9 x N 6) x C 5-19	28.2	81.8	19.4
(" ") x C 5-20	27.5	81.0	19.0
(" ") x C 5-21	27.6	81.7	19.0
(" ") x C 5-22	27.2	81.7	18.9
(" ") x C 5-24	28.7	81.5	19.5
(" ") x C 5-25	27.9	81.5	19.3
(" ") x C 5-26	27.4	81.2	19.2
(" ") x C 5-28	28.0	81.2	18.9
Mean	28.1	82.0	19.2

From the data in Table 3 it can be seen that the different values of earliness are very stable and close to each other. Nevertheless, the relationships of earliness traits are also remarkable (Table 4).

Table 4

Correlation coefficients of different pairs of earliness traits of the three way crosses produced from WF 9 x N 6 by C 5 sublines

Pair of earliness traits	Correlation coefficients
Total leaf number per plant Days to 50% male flowering time	r = 0.4794*
Total leaf number per plant Grain moisture content at harvest time	r = 0.5063*
Days to 50% male flowering time Grain moisture content at harvest time	r = 0.7508**

**Significant at 1 per cent level

*Significant at 5 per cent level

The results may be summarized as follows:

1) Among the single, three way and double-cross maize hybrids produced from non selected inbred lines, the correlation between pairs of earliness traits were mostly significant at the 0.1 and 1 per cent level.

2) The correlation between earliness properties can also be observed in three way crosses produced from WF 9 x N 6 by C 5 related sublines. The correlations between pairs of earliness properties were significant at the 1 and 5 per cent levels.

István Kovács

INDIANA UNIVERSITY
Bloomington, Indiana

1. Influence of sex on crossing over in chromosome 5.

Rhoades (J. Amer. Soc. Agron. 33:603-615, 1941) found recombination in the proximal regions of chromosome 5 to be higher in male than in female meiocytes. Two explanations have been proposed to account for the lower female values, viz. (1) there is a basic difference in the rates of crossing over in mega- and microsporocytes (Rhoades 1941), or (2) selective orientation of the chromosome 5 bivalent on the meiotic spindle leads to the preferential segregation of noncrossover chromatids to the basal

megaspore (Rhoades, in Corn and Corn Improvement, pp. 123-219, 1955). If the metaphase I orientation is maintained at metaphase II in megasporogenesis, preferential recovery of noncrossover strands in the basal megaspore would result. Since all four spores arising from a microsporocyte produce viable gametes while the egg develops from the basal megaspore only, the observed amount of recombination would be less in the female gametes, even if the frequencies of crossing over in male and female flowers were identical.

These alternatives were tested by making use of one of the properties of the elongate mutant, namely the production of unreduced eggs. These diploid eggs originate by omission of one of the meiotic divisions (Rhoades and Dempsey, Genetics 54: 502-522, 1966), so half the products of meiosis can be recovered within individual cells. El el and el el sibs heterozygous for A_2 and Bt were backcrossed as males and as females to a_2 bt testers. The silks of the elongate plants were pollinated with diploid pollen so as to obtain plump, viable 4n kernels from unreduced eggs. With the exception of the double recessive individuals, the tetraploid offspring from the first testcross were grown and pollinated again by tetraploid a_2 bt testers. The genotypes of the original diploid eggs were deduced from the progeny phenotypes. A second cross was unnecessary for backcross progeny showing only recessive phenotypes. Corrections were made to the diploid egg frequencies to allow for incomplete germination of certain classes of kernels among the backcross progeny. Crossover values calculated from both the uncorrected and corrected genotypic analyses of 425 diploid eggs appear in the table below, together with data from haploid el el sperm and from haploid eggs and sperm of El el sibs.

El el			el el			
Plant No.	No. of progeny strands	Percent recombination	Plant No.	No. of progeny strands	Percent recombination: A-Bt	
					Uncorrected	Corrected
F_1 used as female parents						
453-91	387	13.2	453-77	180	7.8	9.6
453-113	185	7.0	453-96	250	10.4	10.7
453-123	315	12.7	453-103	208	11.1	12.8
453-132	581	5.9	453-138	212	20.8	21.4
453-140	284	8.1				
453-142	389	14.1				
Pooled value:	2141	10.1**		850	12.6**	13.4**
F_1 used as male parents						
453-91	109	15.6	453-77	71	18.3	
453-113	361	15.2	453-88	484	32.0	
453-123	352	24.4	453-96	267	28.5	
453-132	531	17.3	453-121	270	35.9	
453-140	434	23.5				
453-142	334	22.2				
Pooled value:	2121	20.1		1092	31.2	

**Significantly lower at the 1% level, using a one-tailed test, than the corresponding male value.

The reciprocal testcrosses of El el plants show a significant difference in A_2-Bt recombination in the haploid gametes from female and male inflorescences. According to the second hypothesis, the percent of recombinant strands found in the diploid eggs of el el plants should be similar to that in the haploid male gametes of El el sibs, because in both cases a representative sample of the meiotic products is recovered and these should include the crossover strands presumed to be preferentially lost in the El el megaspores.

Another consequence of the "orientation hypothesis" is that no difference should be found in crossing over in the male and female gametes of elongate plants. The female values are based on diploid eggs while the male values come from haploid gametes. It might be argued that comparisons should not be made between two populations originating by different meiotic mechanisms. However, Rhoades and Dempsey (1966) found no difference in Lg-A_1 or Sh-Wx crossover frequencies in the haploid and diploid eggs from the same ear. Thus, a comparison of recombination in diploid eggs and haploid sperm of the same plant should also be legitimate.

The data show that neither of the above expectations is fulfilled. Recombination in elongate megasporocytes is not significantly higher than that found in haploid eggs from El el megasporocytes and is markedly lower than the value observed in El el microsporocytes. Moreover, the amount of recombination in diploid eggs from el el plants is much less than the value obtained from haploid male elongate gametes.

The significant differences between male and female recombination values in both El el and el el plants eliminate the hypothesis involving selective orientation. It may be concluded that the amount of recombination in the A_2-Bt region of chromosome 5 is intrinsically higher in male than in female meiocytes.

It may be noted that recombination in the haploid gametes of elongate microsporocytes is higher than in those of El el sibs. This is in agreement with the report last year (MNL 44:61-65) that crossing over is increased in elongate homozygotes.

P. M. Nel

2. Mechanism of diploid egg formation in elongate homozygotes.

Rhoades and Dempsey (Genetics 54: 502-522, 1966) were able to rule out somatic doubling of the genome in sporogenous cells and doubling in the gametophytic generation as mechanisms by which the unreduced eggs of elongate plants could arise. The remaining alternatives were: (1) suppression of the first meiotic division followed by a normal second division, (2) a normal first division with omission of the second, and (3) a normal first division with chromosomal replication occurring

during interphase, followed by the second meiotic division. On the basis of genetic studies with a number of loci on chromosomes 2, 3 and 9, they concluded that their data were best explained by hypothesis 2 or 3. Both hypotheses have similar expectations in progeny tests, but second division omission was preferred since it is the simpler of the two.

The chromosome 5 backcrosses described in the preceding report permitted a more precise determination of the origin of diploid eggs because Bt is more proximally situated than the markers previously employed. The genotypic constitution of unreduced eggs produced by four F_1 plants is given below.

	Diploid egg genotype								Total
	A Bt / A Bt	a bt / a bt	A Bt / a bt	A Bt / A bt	a Bt / a bt	A Bt / a Bt	A bt / a bt	A bt / a Bt	
Uncorrected frequencies									
No. of eggs	112	136	72	7	6	48	42	2	425
Percentage	26.4	32.0	16.9	1.6	1.4	11.3	9.9	0.5	100.0
Corrected frequencies									
No. of eggs	159.3	136.3	103.7	9.1	12.0	62.8	56.7	2.6	542.5
Percentage	29.4	25.1	19.1	1.7	2.2	11.6	10.4	0.5	100.0

When no exchanges occur between a locus and its centromere, the diploid eggs from a heterozygous plant would be expected to show 0% or 50% homozygosity for the recessive allele depending on whether the first or second meiotic divisions, respectively, are omitted. With 100% single exchanges, the corresponding values are 25% and 0% (Rhoades and Dempsey 1966).

The corrected data in the table give 41% Bt Bt, 35.5% bt bt, and 23.5% Bt bt eggs. The high frequencies of the homozygous Bt Bt and bt bt classes are at variance with the hypothesis postulating suppression of the first meiotic division. However, difficulties also arise if the results

are interpreted on the basis of second division failure. On this hypothesis, the 23.5% Bt bt eggs would be ascribed to exchanges between Bt and the centromere. For short regions the frequency of recombination between a locus and the centromere is 50 minus the percentage of the homozygous recessive class (Rhoades and Dempsey 1966). Thus, a map distance of 50.0 - 35.5 = 14.5 units (or 8.1 for the uncorrected data) would be obtained between Bt in the long arm and the centromere. The close linkage (1 map unit) of Bt with Bm in the short arm makes this highly improbable. Moreover, most or all of the exchanges between A_2 and Bt (cf. the preceding report) would have occurred between the Bt locus and the centromere, which is very unlikely.

Secondly, with omission of the second division, the genotypes A_2 Bt / a_2 bt and A_2 bt / a_2 Bt must involve double exchanges in the A_2-centromere and centromere-Bt regions. In view of the small amount of recombination between A_2 and Bt (12.6%-13.4%), double exchanges should be rare, and yet nearly one-fifth (19.1%) of the diploid eggs are A_2 Bt / a_2 bt. This genotype could be derived from two-strand and one-half of the three-strand double crossovers, while the A_2 bt / a_2 Bt eggs would come from four-strand and one-half of the three-strand doubles in the same regions. Since the A_2 bt / a_2 Bt class represents only 0.5% of the diploid eggs, there would have to be a very great excess of two-strand double exchanges. The high frequency of eggs heterozygous for Bt cannot be accounted for by omission of the second meiotic division.

If eggs classified as A_2 Bt / a_2 bt were in fact A_2 Bt / -- monosomics in which an A_2 Bt chromatid had been lost during meiosis, the resulting A_2 Bt / a_2 bt / a_2 bt plants would have been aneuploid instead of full tetraploids and these would have given the 1:1 segregation ratios for both pairs of alleles on which the genotype determinations were based. However, it is unlikely that this was the case. Among 12 progeny of an F_1 plant which were examined cytologically and used in the second backcross, only one had less than 40 chromosomes and the deficient chromosome could have been any one of the 10 chromosomes of the complement.

It is therefore proposed that diploid eggs are produced by the suppression of the first meiotic division in some cells and by omission of the second division in other meiocytes of the same ear. This

hypothesis is supported by the close correlation between the observed distribution of $\underline{A}_2$ genotypes within the Bt Bt, bt bt, and Bt bt classes and that expected on the basis of the known amount of recombination between $\underline{A}_2$ and Bt. For example, if there is no crossing over between Bt and the centromere and if the Bt bt eggs come from first division elimination, the proportion expected to be homozygous at the $\underline{A}_2$ locus is 13.4% on the basis of recombination between $\underline{A}_2$ and Bt (corrected data) and 12.6% on the basis of the uncorrected crossover value. These percentages are in fair agreement with the frequencies of 16.6% and 14.9% derived from the corrected and uncorrected arrays of diploid eggs, respectively.

Similarly, if the Bt Bt and bt bt eggs arise through the omission of the second meiotic division, the expected proportions of homozygotes ($\underline{A}_2\ \underline{A}_2 + \underline{a}_2\ \underline{a}_2$) are 73.2% and 74.8%, depending on whether the corrected or uncorrected recombination values are used. The actual frequencies are in close agreement, namely 71.2% for the corrected and 73.4% for the uncorrected data. The small discrepancies can be attributed to a low frequency of crossing over between Bt and the centromere, and to sampling errors.

P. M. Nel*

*Present address: Dept. of Botany,
University of the Witwatersrand
Johannesburg, South Africa

RESEARCH INSTITUTE OF AGRICULTURE
Krasnodar, USSR

1. Studies of inbreeding in autotetraploid maize.

The inbreeding influence on depression characteristics was studied for the five characters: yield (in centner per hectare), plant height, number of tassel branches, leaf area of an ear and ear length in progenies from successive selfing based on the diploid synthetic population Krasnodarskaya 1/49 and the tetraploid population Synthetic B developed by D. Alexander.

The seeds from each selfed ear were sown in 5 m^2 one-row plots in four replications: P; I_1; I_2; I_3; I_4; I_4; I_3; I_2; I_1; P and so on.

For every inbred population 18-20 progenies were present. Measurements were made on 20 plants in every progeny.

The complete correlation analysis showed that there was a directilinear correlation between the level of heterozygosity and the characteristics of the depression curve for all five factors. But for tetraploids, there was a curvilinear correlation for plant height and yield.

In calculating the theoretically expected decrease in the heterozygosity level, it was assumed that chromosome segregation occurred and that every locus had two alleles (O. Kempthorne, 1957, An Introduction to Genetic Statistics, New York).

A comparison of depression characteristics for the populations permits certain conclusions: autotetraploidy affects the slope of the curve making it smoother; the depression characteristics depend on the nature of the character. For example, such complex characters as yield and plant height conditioned by many genes have a steeper depression slope which differs a little from that in diploids.

In the report of Dudley and Alexander (Crop Science 9 (5) 1969) it was assumed that in the process of autotetraploid inbreeding a relatively great number of aneuploids of little value would appear. These aneuploids have a low yield.

Table 1 gives the results of studies proving these suppositions.

Table 1

Correlation of aneuploids in populations at various levels of inbreeding in Synthetic B

Inbred population		Number of examined plants		
		Total	Aneuploids	$P \pm m_p$
Synthetic B	P	169	61	0.36 ± 0.037
	I_1	100	37	0.37 ± 0.048
	I_2	100	39	0.39 ± 0.049
	I_3	100	42	0.42 ± 0.05
	I_4	100	49	0.49 ± 0.05

Thus, the increase in number of aneuploids in the population on inbreeding probably has a somewhat negative effect, but its influence is not great enough to be responsible for a yield decrease of 45% in a selfed generation.

We may suppose that the main reasons conditioning a considerable reduction of yield and plant height in inbreeding of autotetraploid maize are likely genes with a series of multiple alleles involved in the expression of these characters and genes with a similar additive effect.

Probably various types of heterozygotes (simplex Aaaa, duplex - AAaa, triplex - AAAa) are not of the same value from the point of view of the maximum expression of heterosis. All these suppositions are to be checked.

M. I. Hadjinov
V. S. Shcherbak

2. Meiosis in amphidiploid maize and teosinte.

Diploid hybrids of maize and teosinte developed by Emerson and Beadle usually showed a normal process of crossing over for marker genes (Emerson, R. A. and Beadle, G. W. Zeitschrift für Inductive Abstammungs- und Vererbungslehre 62:291-304, 1932).

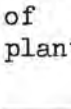
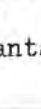
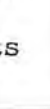
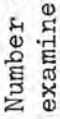
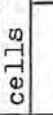
Cytological studies showed normal chromosome pairing in these hybrids and only negligible variations in their length were determined (Longley, A. E., Bot. Rev. 7:263-289, 1941). The minor deviations in the process of meiosis manifested in some diploid hybrids are explained by the presence of small inverted segments of chromosomes in some teosinte forms.

The next logically based step in determination of the level of chromosome relationship and chromosome interaction is a hybrid tetraploid test.

To determine the level of homology of maize and teosinte chromosomes we crossed maize with the annual teosinte, E. Mexicana, from Chalco.

The 2-3 leaf seedlings were treated with colchicine by the method of 0.2% colchicine solution and 0.5% water soluble methylcellulose injection into the hollow formed by the leaves above the shoot apex.

Table 2

Number of plants	Number of examined cells	Occurrence of individual quadrivalent configurations										Number of bivalents	Trivalent + univalent number
													
tetraploid maize													
6	300	0.022	0.016	0.100	0.009	0.114	0.046	0.245	0.238	0.029	0.057	0.120	0.004
Zea mays x *E. mexicana* amphidiploid													
1	50	0.062	0.032	0.042	0.022	0.100	0.068	0.232	0.170	0.044	0.056	0.168	0.004
Z. mays x *E. perennis* amphidiploid													
3	150	0.033	0.013	0.024	0.005	0.042	0.040	0.104	0.037	0.007	0.007	0.689	0.001

Young tassels of chimeric plants were fixed during the process of meiosis.

Simultaneously, we have crossed autotetraploid maize with the perrenial teosinte, E. perennis.

A study of meiosis in autotetraploid maize and in both amphidiploids has been made in pollen mother cells prepared by the aceto-carmine squash technique. Individual chromosome configurations were scored according to Darlington (Darlington, C. D., J. Genet. 24:65-96, 1931).

As chromosome pairing in the maize amphidiploids and autotetraploids is mainly observed as quadrivalents and bivalents, we have classified only individual configurations of quadrivalents and the total number of bivalents neglecting their type.

The frequencies of the various types of association are given in table 2.

In the Z. mays x E. mexicana amphidiploid, the frequency of bivalent chromosome pairing rises from 0.120 (for a tetraploid maize) to 0.168. In addition, an increased frequency of quadrivalent associations with fewer chiasmata is also observed. This tendency is evidently expressed also in the Z. mays x E. perennis amphidiploid, where the frequency of bivalent pairing is 0.689. This is explained by extension of chiasma interference across the centromere (Shaver, D. L., Caryologia 15 (1), 1962).

As a result of involving a perrenial teosinte in crosses with an autotetraploid maize a decrease of aneuploids in the population is expected.

V. S. Shcherbak

IV. REPORT ON MAIZE COOPERATIVE

Seed requests to the Maize Cooperative in 1970 numbered 154. A total of 114 requests (74%) were from 27 states in the United States and 40 (26%) requests were from 18 foreign countries. In addition, 55% of the requests were from Geneticists, 19% from Plant Breeders, 19% from Physiologists and 7% for educational purposes. The number of stocks per request ranged from 1 to 108, with the average number per request being for 8 stocks. The total number of packets sent to supply the requests were 1340.

The number of requests per year has leveled off some in the last 3 to 4 years. However, the number of requests has increased about 4 times compared to 1955. The following summary gives the total yearly requests at 5 year intervals for 1955 through 1970.

	Number of Requests		
Year	U.S.	Foreign	Total
1955	34	6	40
1960	79	5	84
1965	88	22	110
1970	114	40	154

During 1970, 89 different lethal seedling traits were increased and 1668 seedling tests conducted to determine pedigrees. In addition, certain single genes and chromosome tester combinations were increased for chromosomes 4, 5, 6, and 9. Also allele tests were made on 50 unknown genes with ones placed to chromosomes. All the endosperm genes in the collection were increased in 1970.

Considerable effort is being spent in arranging stocks in our new cold seed storage unit. 960 new all steel storage drawers have been assembled in this room. All stocks are being ordered by chromosome and tester combinations within the chromosome. Also all chromosome aberrations stocks have been assembled in an orderly manner. This should allow for a continuing inventory of all stocks and also save considerable time and effort in filling seed requests.

The attached catalogue of stocks represents a listing of currently available genetic stocks. A complete listing of all translocation stocks

in the collection has been published in Volume 43 of the Maize News Letter. Copies of these stock lists are available upon request.

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

Catalogue of Stocks

Chromosome 1

$ad_1\ an_1\ bm_2$

$ad_1\ bm_2$

$an_1\ bm_2$

as

$br_1\ Vg$

br_2

bz_2^m; M

bz_2^m; m

Kn

$Kn\ Ts_6$

lw_1

P^{CR}

P^{CW}

P^{MO}

P^{RR}

P^{RW}

P^{VV}

$P^{RR}\ ad_1\ an_1$

$P^{RR}\ ad_1\ bm_2$

$P^{RR}\ an_1\ gs_1\ bm_2$

$P^{RR}\ br_1\ f_1\ an_1\ gs_1\ bm_2$

$P^{WR}\ bm_2$

$P^{WR}\ an_1\ bm_2$

$P^{WR}\ an_1\ Kn\ bm_2$

Chromosome 1 (Continued)

$P^{WR}\ gs_1\ bm_2$

$P^{WW}\ br_1\ f_1\ bm_2$

$P^{WW}\ br_1\ f_1\ ad_1\ bm_2$

$P^{WW}\ br_1\ f_1\ an_1\ gs_1\ bm_2$

$P^{WW}\ hm\ br_1\ f_1$

rs_2

sr_1

$sr_1\ P^{WR}\ an_1\ bm_2$

$sr_1\ P^{WR}\ bm_2$

$sr_1\ P^{WR}\ an_1\ gs_1\ bm_2$

$sr_1\ zb_4\ P^{WW}$

ts_2

$ts_2\ P^{WW}\ br_1\ bm_2$

Ts_6

Vg

$Vg\ an_1\ bm_2$

vp_5

vp_8

$zb_4\ ms_{17}\ P^{WW}$

$zb_4\ P^{WW}\ bm_2$

$zb_4\ P^{WW}\ br_1$

$zb_4\ P^{WW}\ br_1\ f_1\ bm_2$

$zb_4\ ts_2\ P^{WW}$

$zb_4\ ts_2\ P^{WW}\ bm_2$

Chromosome 1 (Continued)

an_{6923}-bz_2 (apparent deficiency including an_1 and bz_2)

bm_2

bm_2 br_2

Necrotic 8147-31

tb

id

ms_9

ms_{14}

rd

Chromosome 2

al lg_1

al lg_1 gl_2 B sk

al lg_1 gl_2 b sk v_4

ba_2

d_5

fl_1

ts_1

gl_{11}

Ht

lg_1

lg_1 gl_2 wt

lg_1 gl_2 B

lg_1 gl_2 b

lg_1 gl_2 b Ch

lg_1 gl_2 b fl_1 v_4

Chromosome 2 (Continued)

lg_1 gl_2 b fl_1 v_4 Ch

lg_1 gl_2 B gs_2

lg_1 gl_2 b gs_2

lg_1 gl_2 b gs_2 sk

lg_1 gl_2 B gs_2 v_4

lg_1 gl_2 b gs_2 v_4

lg_1 gl_2 b gs_2 v_4 Ch

lg_1 gl_2 B sk v_4

lg_1 gl_2 b sk v_4

lg_1 gl_2 b sk fl_1 v_4

lg_1 gl_2 B v_4

lg_1 gl_2 b v_4

lg_1 gl_2 b v_4 Ch

lg_1 gs_2 b v_4

w_3

w_3 Ch

lg_1 gl_2 w_3 Ch

ws_3 lg_1 gl_2 B

ws_3 lg_1 gl_2 b

ws_3 lg_1 gl_2 b v_4

ws_3 lg_1 gl_2 b fl_1 v_4

ws_3 lg_1 gl_2 B sk

ws_3 lg_1 gl_2 b sk

wt

mn

Chromosome 3

A_1 ga_7; A_2 C R

A_1 sh_2; A_2 C R

A^d-31; A_2 C R

A^d-31; A_2 C R Dt_1

A^d-31 sh_2; A_2 C R

a^P et; A_2 C R Dt_1

a_1^P et; A_2 C R dt_1

a_1; A_2 C R B Pl dt_1

a_1 et; A_2 C R Dt_1

a_1^m et; A_2 C R dt_1

a_1 sh_2; A_2 C R Dt_1

a_1 sh_2; A_2 C R Dt_1 B Pl

a_1 sh_2; A_2 C R dt_1

a_1^{st} Sh_2; A_2 C R Dt_1

a_1^{st} sh_2; A_2 C R Dt_1

a_1^{st} sh_2 et; A_2 C R Dt_1

a_1^{st} et; A_2 C R Dt_1

ba_1

Cg

cl_1

cr_1

cr_1 d_1 Lg_3

d_1

d_1 pm_1

d_1 Lg_3

Chromosome 3 (Continued)

d_1 Rg lg_2

d_1 ts_4 lg_2

d_1 Rg ts_4 lg_2

d_1 Rf_1 ts_4 lg_2

d_1 ts_4 lg_2 a_1; A_2 C R Dt_1

gl_6 lg_2 A^b et; A_2 C R Dt_1

gl_6 lg_2 a_1 et; A_2 C R Dt_1

gl_7

lg_2 A^b Sh et; A_2 C R Dt_1

lg_2 a_1 et; A_2 C R Dt_1

lg_2 a_1 et; A_2 C R dt_1

lg_2 a_1 sh_2 et; A_2 C R Dt_1

lg_2 a_1^{st} et; A_2 C R Dt_1

lg_2 a_1^{st} sh_2; A_2 C R Dt_1

lg_2 pm

Lg_3

Lg_3 Rg

na_1

na_1 lg_2

pm

ra_2

ra_2 lg_2

ra_2 lg_2 pm

ra_2 Rg

ra_2 Rg lg_2

Chromosome 3 (Continued)

Rg

ra_2 Rg ts_4 pm_1 lg_2

rt

ts_4

ts_4 na_1

ys_3

pg_2

vp_1

Primary trisomic 3

Chromosome 4

bm_3

bt_2

bt_2 gl_4

c_2; A_1 A_2 C_1 R

fl_2

Ga_1 Su_1

Ga_1^s Su_1

gl_3

gl_3 dp

la su_1 gl_3

la su_1 Tu gl_3

lw_4; lw_3

o_1

st

su_1

Chromosome 4 (Continued)

su_1^{am}

su_1 bm_3

su_1 gl_3

su_1 gl_3 ra_3

su_1 gl_4

su_1 ra_3

su_1 Tu

su_1 Tu gl_3

su_1 zb_6

su_1 zb_6 Tu gl_3

su_1 zb_6 Tu

su_1 o_1

Ts_5

Ts_5 su_1

Ts_5 su_1 zb_6

Ts_5 st

Ts_5 st su_1

Tu gl_3

zb_6

v_8

dp

j_2

Primary trisomic 4

Chromosome 5

a_2; A_1 C R

a_2 bm_1 bt_1 bv_1 pr; A_1 C R

a_2 bm_1 bt_1 pr; A_1 C R

a_2 bm_1 pr v_2; A_1 C R

a_2 bm_1 pr ys_1; A_1 C R

a_2 bt_1 b_3 Pr; A_1 C R

a_2 bt_1 pr; A_1 C R

a_2 bt_1 pr ys_1; A_1 C R

a_2 v_3 pr; A_1 C R

a_2 pr; A_1 C R

ae

ae td

bm_1 pr; A_1 A_2 C R

bm_1 pr v_2 A_1 A_2 C R

bm_1 pr ys_1; A_1 A_2 C R

bm_1 pr ys_1 v_2; A_1 A_2 C R

bt_1 pr; A_1 A_2 C R

bt_1 pr ys_1; in A_1 A_2 C R

gl_5

gl_8

gl_{17}

gl_{17} bt_1

gl_{17} v_2

lw_2

lw_3; lw_4

Chromosome 5 (Continued)

na_2

na_2 pr

pr; A_1 A_2 C R

pr ys_1; A_1 A_2 C R

ys_1

v_3 pr; A_1 A_2 C R

v_{12}

vp_2 gl_8

vp_2 pr; A_1 A_2 C R

vp_7

ps = allele of vp_7

vp_7 pr; A_1 A_2 C R

eg

lu_1

sh_4 lu_1

sh_4

yg_1

Primary trisomic 5

Chromosome 6

Y_1 rgd

at = allele of si_1

Bh

po Y_1 pl

po y_1 pl

Pt

si_1

Chromosome 6 (Continued)

wi

y_1

w^m = allele of y_1

pb_1 = allele of y_1

y_1 l_{10}

y_1 l_{4120}

y_1 l_{4920}

y_1 pb_4 pl

y_1 pb_4 Pl

Y_1 pg_{11}; Wx pg_{12}

Y_1 pg_{11}; wx pg_{12}

y_1 pg_{11}; wx pg_{12}

y_1 Pl Bh

y_1 pl Bh

Y_1 Pl sm

Y_1 Pl sm py; A_1 A_2 b P^{RR}

Y_1 pl su_2

y_1 pl su_2

y_1 Pl

y_1 Pl w_1

Dt_2; a_1 A_2 C R

w_1

ms-si = allele of si

orobanche

w_{8657}

Primary trisomic 6

Chromosome 7

Bn

bd

g_2

gl_1

gl_1^m

gl_1 o_5

gl_1 g_2

gl_1 ij bd

gl_1 sl

gl_1 Tp_1

gl_1 g_2 Tp_1

Hs

ij

ij bd

in; pr A_1 A_2 C R

in gl_1; pr A_1 A_2 C R

o_2

o_2 bd

o_2 gl_1 sl

o_2 ra_1 gl_1

o_2 ra_1 gl_1 ij

o_2 ra_1 gl_1 Tp

o_2 v_5 ra_1 gl_1

o_2 v_5 ra_1 gl_1 Hs

o_2 v_5 ra_1 gl_1 Tp_1

ra_1 gl_1 ij bd

Chromosome 7 (Continued)

Tp_1

vp_9 gl_1

Dt_3; a_1 A_2 C R

Primary trisomic 7

Chromosome 8

gl_g

v_{16} j_1

v_{16} ms_8 j_1

necrotic 6697

sienna 7748

Primary trisomic 8

Chromosome 9

Bf_1

Bf_1 bm_4

bm_4

bp Wx; P^{RR}

C Ds wx

C sh_1 Wx; A_1 A_2 R

C sh_1 wx; A_1 A_2 R

c sh_1 wx; A_1 A_2 R

c sh_1 ms_2; A_1 A_2 R

C wx; A_1 A_2 R

C Wx bz_1; A_1 A_2 R

C wx ar; A_1 A_2 R

c sh_1 wx gl_{15}

Chromosome 9 (Continued)

c sh_1 wx gl_{15} Bf_1

c sh_1 wx bk_2

c Wx; A_1 A_2 R

c wx; A_1 A_2 R

c wx v_1

c wx Bf_1; A_1 A_2 R

Dt_1; a_1^m A_2 C R

gl_{15}

gl_{15} Bf_1

gl_{15} bm_4

C_1^I Ds Wx

C_1^I wx; A_1 A_2 R B

K_9^L C sh_1 wx; A_1 A_2 R

l_6

l_7

ms_2 sh_1; A_1 A_2 C R

sh_1 bp wx; P^{RR}

sh_1 wx gl_{15}

sh_1 wx l_7

sh_1 wx v_1

wx Bf_1

wx Bf_1 bm_4

wx bk_2

Wx bk_2 bm_4

wx bk_2 bm_4

Chromosome 9 (Continued)

wx d_3

wx l_6

Wc

Wx pg_{12}; y_1 pg_{11}

wx pg_{12}; Y_1 pg_{11} pl

wx pg_{12}; y_1 pg_{11}

wx^a

yg_2 c sh_1 wx; A_1 A_2 R

yg_2 c sh_1 bz_1 wx; A_1 A_2 R

yg_2 c sh_1 wx gl_{15}; A_1 A_2 R

yg_2 C sh_1 bz_1 wx; A_1 A_2 R

wd

lo

Primary trisomic 9

Chromosome 10

bf_2

du_1

g_1

g_1 Tp_2

g_1 r^g; A_1 A_2 C

g_1 r^{ch}

g_1 r; A_1 A_2 C wx

g_1 $R^r sr_2$; A_1 A_2 C

g_1 R^g sr_2; A_1 A_2 C

g_1 r sr_2; A_1 A_2 C

Chromosome 10 (Continued)

l_1

l_1; w_1

li g_1 R; A_1 A_2 C

li g_1 r; A_1 A_2 C

nl_1 g_1 R; A_1 A_2 C

Og R; A_1 A_2 C B Pl

oy

r^g; A_1 A_2 C

r^r; A_1 A_2 C

r^r E^j; A_1 A_2 C

r K^{10}; A_1 A_2 C

R^r K^{10} g_1; A_1 A_2 C

R^g sr_2; A_1 A_2 C

r^r sr_2; A_1 A_2 C

r^g wx; A_1 A_2 C

R^r:Boone; A_1 A_2 C

R^{mb}; A_1 A_2 C

R^{nj}; A_1 A_2 C

R^{st}; A_1 A_2 C

R^r Lc; A_1 A_2 C

v_{18}

w_2

w_2 l_1

zn_1

Primary trisomic 10

Unplaced Genes

dv

dy

el

gl_{12}

gl_{14}

gl_{16}

h

l_3

l_4

ms_6

ms_{12}

ms_{13}

Rs_1

v_{13}

w_{11}

ws_1 ws_2

ub

zb_1

zb_2

zb_3

zn_2

l_{4923}

"necrotic 8376" (seedling)

Multiple Gene Stocks

A_1 A_2 C R^r Pr B Pl

A_1 A_2 C R^g Pr B Pl

A_1 A_2 C R Pr

A_1 A_2 C R Pr wx

A_1 A_2 C R Pr wx gl_1

A_1 A_2 C R Pr wx y_1

A_1 A_2 C R pr

A_1 A_2 C R pr y_1 gl_1

A_1 A_2 C R pr y_1 wx

A_1 A_2 C R pr y_1 wx gl_1

A_1 A_2 c R Pr y_1 wx

A_1 A_2 C r Pr y_1 wx

bm_2 lg_1 a_1 su_1 pr y_1 gl_1 j_1 wx g_1

colored scutellum

lg_1 su_1 bm_2 y_1 gl_1 j_1

su_1 y_1 wx a_1 A_2 C R^g pr

y_1 wx gl_1

hm_1 hm_2

Popcorns

Amber Pearl

Argentine

Black Beauty

Hulless

Ladyfinger

Ohio Yellow

Popcorns (Continued)

Red

South American

Strawberry

Supergold

Tom Thumb

White Rice

Exotics and Varieties

Black Mexican Sweet Corn (with B-chromosomes)

Black Mexican Sweet Corn (without B-chromosomes)

Knobless Tama Flint

Knobless Wilbur's Flint

Gourdseed

Maiz chapolote

Papago Flour Corn

Parker's Flint

Tama Flint

Zapaluta chica

Tetraploid Stocks

p^{RR}

p^{VV}

Ch

B

a_1 A_2 C R Dt_1

su_1

Tetraploid Stocks (Continued)

pr; A_1 A_2 C R

y_1

gl_1

ij

Y_1 sh_1 wx

sh_1 bz_1 wx

wx

A_1 A_2 C R

A_1 A_2 C R B P1

Cytoplasmic Steriles and Restorers

WF9 - (T)	rf_1 rf_2
N6 (S)	
WF9	rf_1 rf_2
N6	rf_1 Rf_2
R213	Rf_1 rf_2
Ky21	Rf_1 Rf_2

These combinations are also available in other inbred backgrounds.

V. RECENT MAIZE PUBLICATIONS

Alimova, G. K. and L. F. Andreeva. A comparative autoradiographic investigation of DNA synthesis in the course of meiosis in the microsporocytes and the cells of the tapetal layer of the anthers in fertile and sterile forms of maize. Bot. J. Moskva 54:1705-1716. 1969.

Amosu, J. O. and A. L. Hooker. Weight loss of mature corn stalk tissue induced by twelve fungi. Phytopath. 60:1790-1793. 1970.

Arias G., C. M. Inheritance of various endosperm mutants in maize, Z. mays L. Agron. Trop., Venezuela 19:3-18. 1969.

Arny, D. C., G. L. Worf, R. W. Ahrens and M. F. Lindsey. Yellow leaf blight of maize in Wisconsin: its history and the reactions of inbreds and crosses to the inciting fungus (Phyllosticta sp). Plant Dis. Reptr. 54:281-285. 1970.

Ashman, R. B. The compound structure of the R^{st} allele in maize. Genetics 64:239-245. 1970.

Ayers, J. E., R. R. Nelson, C. Koons, and G. L. Scheifele. Reactions of various maize inbreds and single crosses in normal and male-sterile cytoplasm to the yellow leaf blight organism (Phyllosticta sp.). Plant Dis. Reptr. 54:277-280. 1970.

Baker, L. H. and R. N. Curnow. Choice of population size and use of variation between replicate populations in plant breeding selection programs. Crop Sci. 9:555-560. 1969.

Banerjee, U. C. Electron micrographs of grass pollen (Tripsacum and Zea) and chlamydospores (Tilletia). Jour. Palynology 5(1): i-vi. 1969.

Banerjee, U. C. and E. S. Barghoorn. Electron microscopy of the tapetal membranes in grasses and the control of the Ubisch bodies (orbicules) on mature exine pattern. Int. Cong. Pollen and Pollen Phys. Pullman, Washington. 1969. (Abstr.)

Banerjee, U. C. and E. S. Barghoorn. Reinvestigation of the ektexine pattern of the pollen grains of cultivated cereal grasses. Amer. Jour. Bot. 57:742. 1970. (Abstr.)

Bansal, R. K. A study of the factors associated with resistance to stalk rot, Diplodia zeae (Schw.) Lev., in corn (Zea mays L.). Diss. Abstr. 29:2712B. 1969.

Barlow, P. W. Mitotic spindle and mitotic cell volumes in the root meristem of Zea mays. Planta 91:169-172. 1970.

Bartlett, A. S., E. S. Barghoorn, and R. Berger. Fossil maize from Panama. Science 165:389-390. 1969.

Bhat, B. K. and N. L. Dhawan. Cytoplasmic variation in geographical races of maize and its effect on quantitative characters. Indian J. Genet. 30:446-450. 1970.

Bhat, B. K. and N. L. Dhawan. Threshold concentration of plasmonsensitive polygenes in the expression of quantitative characters of maize. Theoret. Appl. Genet. (in press)

Bhat, B. K. and N. L. Dhawan. The role of cytoplasm in the manifestation of quantitative characters of maize. Genetica 42. (in press)

Bianchi, A., M. R. Parlavecchio, and F. Restaino. Behavior and linkage relationship of gametophyte factors in chromosome 9 of maize. Genet. Agr. 22:345-361. 1970.

Bianchi, A., F. Salamini, and M. R. Parlavecchio. On the origin of controlling elements in maize. Genet. Agr. 22:335-344. 1970.

Bljandur, O. V. A study of mutations isolated in the second generation from the combined action of ionizing radiation and chemical mutagens. Trans. Kisinev Agric. Inst. 45:158-163. 1966.

Borovskij, M. I. and A. E. Kovarskij. Intergeneric hybridization of maize with Tripsacum (T. dactyloides). Bull. Acad. Sci. Moldav. SSR No. 11:25-35. 1967.

Brink, R. A., J. L. Kermicle, and N. K. Ziebur. Derepression in the female gametophyte in relation to paramutant R expression in maize endosperms, embryos, and seedlings. Genetics 66:87-96. 1970.

Brown, A. H. D. and R. W. Allard. Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. Genetics 66:133-145. 1970.

Byrka, S. G. Ultrasonic waves as a mutagenic factor. Trans. Kisinev Agric. Inst. 46:102-104. 1967.

Cameron, J. W., H. Johnson, O. C. Taylor, and H. W. Otto. Differential susceptibility of sweet corn hybrids to field injury by air pollution. Hort. Science 5:217-219. 1970.

Carlson, W. R. Cytogenetic studies with translocations involving the B chromosome of maize. Diss. Abstr. 29:3620B-3621B. 1969.

Carlson, W. R. Nondisjunction and isochromosome formation in the B chromosome of maize. Chromosoma 30:356-365. 1970.

Cerny, J. The effect of chronic irradiation of plants and acute irradiation of dry seeds on the variability of Z. mays L. Genetika a Slechteni 5:263-270. 1969.

Chang, S. C. Studies on the inheritance of resistance to Sclerospora sacchari in maize. Rep. Corn Res. Cent. Tainan DAIS No. 6:1-6. 1968. (Chinese)

Chang, S. C. Location of genes for resistance to Sclerospora sacchari in maize by the use of chromosome-translocation stocks. Rep. Corn Res. Cent. Tainan DAIS No. 7:1-6. 1969. (Chinese)

Chi, R. K., S. A. Eberhart, and L. H. Penny. Covariances among relatives in a maize variety (Zea mays L.). Genetics 63:511-520. 1970.

Costa-Rodrigues, L. Races of maize in Portugal. Agronomia lusit. 31. 1969.

Craig, J. and J. M. Fajemisin. Inheritance of chlorotic lesion resistance to Helminthosporium maydis in maize. Plant Dis. Reptr. 53:742-743. 1969.

Dankov, T. and M. Stoilov. On the problem of the genetic nature of cytoplasmic male sterility in maize and its use in hybrid seed production. Genet. and Pl. Breed. 2:369-383. 1969. (Bulgarian)

Das, L. V. D. Chromosome associations in diploid and autotetraploid Zea mays Linn. Cytologia 35:259-261. 1970.

Das, L. D. V. Intergeneric crosses between sugarcane and maize: embryological studies. J. Hered. 61:288-290. 1970.

De Wet, J. M. J., R. J. Lambert, J. R. Harlan, and S. M. Naik. Stable triploid hybrids among Zea-Tripsacum-Zea backcross populations. Caryologia 23:183-187. 1970.

Dollinger, E. J., W. R. Findley, and L. E. Williams. Resistance inheritance to maize dwarf mosaic virus in maize (Zea mays L.). Crop Sci. 10:412-415. 1970.

Dunn, G. M. and T. Namm. Gene dosage effects on monogenic resistance to northern corn leaf blight. Crop Sci. 10:352-354. 1970.

Dzjuba, V. A. Study of the collection of tetraploid maize. Trans. Appl. Bot. Genet. Pl. Breed. 41:80-89. 1969.

Eastin, J. A. Leaf position and leaf function in corn--carbon-14 labeled photosynthate distribution in corn in relation to leaf position and leaf function. Proc. 24th Ann. Corn and Sorghum Res. Conf., A. S. T. A. 1969.

Eastin, J. A. C-14 labeled photosynthate export from fully expanded corn (Zea mays L.) leaf blades. Crop Science 10:415-418. 1970.

Eberhart, S. A. Yield stability of single cross genotypes. Proc. 24th Ann. Corn and Sorghum Res. Conf. 24:22-35. 1969.

Efron, Y. Genetic research of acid phosphatase isozymes in maize. Res. Rep. Hebrew Univ. Jerusalem 1:542-543. 1969. (Abstr.)

Efron, Y. Tissue specific variation in the isozyme pattern of the AP_1 acid phosphatase in maize. Genetics 65:575-583. 1970.

Efron, Y. Alcohol dehydrogenase in maize: genetic control of enzyme activity. Science 170:751-753. 1970.

Efron, Y. Differences between maize inbreds in the activity level of the AP_1 controlled acid phosphatase. Biochem. Genetics 5:33-44. 1971.

Feist, W. A. and R. J. Lambert. Changes in six different opaque-2 genotypes of Zea mays L. during successive generations of backcrossing. Crop Sci. 10:663-665. 1970.

Ficsor, G., F. C. Janca, and C. Van Hook IV. Tests for mutagenicity using nitrosoguanidine, DDT, and captan in maize. Newsletter of the Environmental Mutagen Society 3:38. 1970. (Abstr.)

Finnegan, D. J. Genetically controlled electrophoretic variants of a starch-degrading enzyme in Zea mays. Austr. J. Biol. Sci. 22:1055-1059. 1969.

Francis, C. A. Modification of the Ozalid paper technique for measuring integrated light transmission values in the field. Crop Sci. 10: 321-322. 1970.

Francis, C. A. Effective day lengths for the study of photoperiod sensitive reactions in plants. Agron. J. 62:790-792. 1970.

Francis, C. A., D. Sarria V., D. D. Harpstead, and C. Cassalett D. Identification of photoperiod insensitive strains of maize (Zea mays L.) II. Field tests in the tropics with artificial lights. Crop Sci. 10:465-468. 1970.

Fujii, T. Whole and partial mutations induced in the developing embryo of maize by X-rays. Rep. Kihara Inst. Biol. Res. No. 20:55-61. 1968.

Galinat, W. C. The cupule and its role in the origin and evolution of maize. Mass. Agr. Exp. Sta. Bul. 585. 1970.

Galinat, W. C. Maize from the Blain site. In Blain Village and the Fort Ancient Tradition in Ohio (O. H. Prufer and O. C. Shane III, Eds.) Kent State Univ. Press. 1970.

Galinat, W. C., T. R. Reinhart, and T. R. Frisbie. Early eight-rowed maize from the Middle Rio Grande Valley, New Mexico. Harvard Univ. Bot. Mus. Leaflets 22:313-331. 1970.

Gardner, C. O. Mutation studies involving quantitative traits. Gamma Field Symposia 7:57-77. 1968.

Gardner, C. O. Genetic variation in irradiated and control populations of corn after ten cycles of mass selection for high grain yield. In Induced Mutations in Plants, Int. Atomic Energy Agency, Vienna, Austria. pp. 469-477. 1969.

Gardner, C. O. The role of mass selection and mutagenic treatment in modern corn breeding. Proc. 24th Ann. Corn and Sorghum Res. Conf., A. S. T. A. 24:15-21. 1969.

Gardner, C. O. Influence of mass selection and thermal neutron irradiation on means and cumulative gene effects in a variety of corn (Zea mays L.). Diss. Abstr. 29:2263B. 1969.

Garwood, D. L., E. J. Weber, R. J. Lambert, and D. E. Alexander. Effect of different cytoplasms on oil, fatty acids, plant height, and ear height in maize (Zea mays L.). Crop Sci. 10:39-41. 1970.

Gelinas, D. A. A study of indoleacetic acid oxidase inhibitors in normal and knotted maize plants. Diss. Abstr. 29:4060B. 1969.

Gelinas, D. and S. N. Postlethwait. IAA oxidase inhibitors from normal and mutant maize plants. Plant Physiol. 44:1553-1559. 1969.

Ghobrial, H. K. G. Studies of recombination in heterozygotes for single and multiple chromosomal interchanges in maize (Zea mays L.). Diss. Abstr. 29:2755B. 1969.

Glover, D. V. Location of a gene in maize conditioning a reduced plant stature. Crop Sci. 10:611-612. 1970.

Goldman, S. L. An investigation into the nature of the mechanism of genetic recombination in maize. Diss. Abstr. 29:2755B. 1969.

Goldman, S. L. and E. H. Coe, Jr. Region-specific recombination in the an_1-bz_2 interval in maize. Canad. J. Genet. Cyt. 12:882-891. 1970.

Golynskaja, E. L., N. F. Bezrucko, T. M. Grigorenko and N. Koman. Possibility of prognosis of heterosis on the basis of a study of hybrid embryos in maize. Cytology and Genetics, Kiev 1(3):38-50. 1967.

Grogan, C. O. The inheritance of cold resistance in plants. Soc. for Cryobiology. Proc. 7th Ann. Mtng, Los Angeles, Calif. 1970.

Grogan, C. O. Genetic variability in maize (Zea mays L.) for germination and seedling vigor at low temperatures. Proc. 25th Ann. Corn and Sorghum Ind.-Res. Conf. 1970.

Grogan, P. W. and D. S. Tealke. Resistance of some Australian inbred maize lines to maize dwarf mosaic disease. Austr. J. Exp. Agric. Anim. Husb. 9:541-546. 1969.

Guthrie, W. D., J. L. Huggans and S. M. Chatterji. Influence of corn pollen on the survival and development of second-brood larvae of the European corn borer. Iowa St. J. Sci. 44:185-192. 1969.

Hallauer, A. R. Genetic variability for yield after four cycles of reciprocal recurrent selections in maize. Crop Sci. 10:482-485. 1970.

Hallauer. A. R. Corn breeding--the key to the future. Proc. 22nd Ann. Fertilizer and Ag. Chem. Dealers Conf. 22:10.1-10.10. 1970.

Hallauer, A. R. Zygote selection for the development of single-cross hybrids in maize. Adv. Frontiers of Plant Sci. 25:75-81. 1970.

Hallauer, A. R. and S. A. Eberhart. Reciprocal full-sib selection. Crop. Sci. 10:315-316. 1970.

Hanson, G. P. B-chromosome-stimulated crossing over in maize. Genetics 63:601-609. 1969.

Harlan, J. R., J. M. J. De Wet, S. M. Naik and R. J. Lambert. Chromosome pairing within genomes in maize-Tripsacum hybrids. Science 167: 1247-1248. 1970.

Harris, J. W. Genetic and biochemical studies on isozymes of the E_4 esterase in maize. Diss. Abstr. 29:3209B. 1969.

Helm, J. L., D. V. Glover, and M. S. Zuber. Effect of endosperm mutants on pericarp thickness in corn. Crop Sci. 10:105-107. 1970.

Hooker, A. L., D. R. Smith, S. M. Lim, and J. B. Beckett. Reaction of corn seedlings with male-sterile cytoplasm to Helminthosporium maydis. Plant Disease Reptr. 54:708-712. 1970.

Hooker, A. L., D. R. Smith, S. M. Lim, and M. D. Musson. Physiological races of Helminthosporium maydis and disease resistance. Plant Disease Reptr. 54:1109-1110. 1970.

Hornbrook, A. R. and C. O. Gardner. Genetic study of "necrotic leaf spot" mutation induced in an inbred line of maize (Zea mays L.) by thermal neutron irradiation of seed. Radiation Botany 10:113-117. 1970.

Jellum, M. D. Plant introductions of maize as a source of oil with unusual fatty acid composition. J. Agr. Food Chem. 18:365-370. 1970.

Jellum, M. D. Developmental changes in fatty acid composition of oil in kernel fractions of corn (Zea mays L). J. Am. Oil Chemists' Soc. 47:245-248. 1970.

Jellum, M. D. Fatty acid composition of oil from four kernel fractions of corn (Zea mays L.). Cereal Chem. 47:549-558. 1970.

Jellum, M. D. and C. W. Kuhn. Yield performance and disease evaluation of corn hybrids in Georgia. Georgia Agr. Exp. Sta. Res. Report 93. 18 pp. 1970.

Jimenez, J. R. The effect of the opaque-2 and floury-2 genes on the production of protein in maize endosperm. Diss. Abstr. 29:4048B. 1969.

Johnson, G. R. Analysis of genetic resistance to maize dwarf mosaic disease. Crop Sci. 11:23-25. 1971.

Judin, B. F. The effect of autotetraploidization on the capacity of maize for apomixis and polyembryony. News Siber. Sect. USSR Acad. Sci. 3:125-126. 1967.

Kermicle, J. L. Pleiotropic effects on seed development of the indeterminate gametophyte gene in maize. Amer. J. Bot. 58:1-7. 1971.

Kermicle, J. L. Somatic and meiotic instability of R-stippled, an aleurone spotting factor in maize. Genetics 64:247-258. 1970.

Kermicle, J. L. Dependence of the R-mottled aleurone phenotype in maize on mode of sexual transmission. Genetics 66:69-85. 1970.

Kirby, L. T. and E. D. Styles. Flavonoids associated with specific gene action in maize aleurone, and the role of light in substituting for action of a gene. Canad. J. Genet. Cyt. 12:934-941. 1970.

Klun, J. A., W. D. Guthrie, A. R. Hallauer, and W. A. Russell. Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy 2H-1, 4-benzoxazin-3(4H)-one and resistance to the European corn borer in a diallel set of eleven maize inbreds. Crop Sci. 10:87-90. 1970.

Konarev, V. G., J. V. Peruanskij, and A. J. Rubcenja. Zein of the opaque-2 maize mutant and the possibilities of manifestation of the mutant gene in hybrids. Rep. All-Un. Lenin Acad. Agric. Sci. No. 9:28-31. 1969.

Koul, A. K. Pollen polymorphism in Zea mays L. Sci. and Cult. 35:103-104. 1969.

Kovacs, I. (Editor) Some methodological achievements of the Hungarian hybrid maize breeding. Akademiai Kiado, Budapest. pp. 385. 1970.

Kravcenko, A. N. and L. A. Kulakova. The initial effect of high doses of γ rays and certain chemical mutagens on the morphological characteristics of maize. Trans. Kisinev Agric. Inst. 45:138-141. 1966.

Kuhn, C. W. and M. D. Jellum. Evaluations for resistance to corn stunt and maize dwarf mosaic diseases in corn. Georgia Agr. Exp. Sta. Res. Bull. 82. 37 pp. 1970.

Lambert, R. J. and D. E. Cochran. Use of R alleles in obtaining the modified protein double mutant (opaque-2, floury-2) in Zea mays L. Crop Sci. 10:715-716. 1970.

Lim, S. M., A. L. Hooker, and J. D. Paxton. Isolation of phytoalexins from corn with monogenic resistance to Helminthosporium turcicum. Phytopath. 60:1071-1075. 1970.

Lorenzoni, C. Fattori gametofitici nelle popolazioni di mais italiane. Genet. Agr. 22:309-321. 1970.

Lysikov, V. N. and A. N. Kravcenko. A study of certain maize forms obtained as a result of γ irradiation. Trans. Kisinev Agric. Inst. 45:112-125. 1966.

Lysikov, V. N. and Z. A. Orinstejn. Comparative efficiency of action of mutagenic treatments on susceptibility of maize to maize smut in the year of treatment. Trans. Kisinev Agric. Inst. 45:199-202. 1966.

Lysikov, V. N., P. L. Brik and Ju. M. Bezgubov. Electron-microscopic research on some reproductive and vegetative organs of a triploid-like mutant of maize. Trans. Kisinev Agric. Inst. 46:40-48. 1967.

Lysikov, V. N., A. I. Konotop and O. V. Bljandur. First findings on the feasibility of utilizing chemomutant sublines in a synthesis of simple hybrids in maize. Trans. Kisinev Agric. Inst. 46:141-148. 1967.

Lysikov, V. N., A. N. Konotop, O. V. Bljandur, and S. G. Byrka. The breeding of inbred lines of maize with cytoplasmic male sterility by experimental mutagenesis. Trans. Kisinev Agric. Inst. 46:65-71. 1967.

Maguire, M. P. Non-random metaphase I orientation of the chromosomes of a trivalent. Genetica 41:361-368. 1970.

Maguire, M. P. Heterogeneity in interallelic recombination in maize. Genetics 64:40. 1970. (Abstr.)

Maguire, M. P. Induced abnormalities of synapsis, chromosome contraction and loss of chiasmatic association following crossing over. J. Cell Biol. 47:126a. 1970. (Abstr.)

Maleckij, S. I. A new allele of the gametophyte gene in the fourth chromosome of maize. Genetika, Moskva 6:14-20. 1970.

Mangelsdorf, P. C. Cryptic genes for "tripsacoid" characteristics in Maiz amargo of Argentina and other Latin American varieties. Bol. Soc. Arg. Bot. 12:180-187. 1968.

Manoliu, M. and T. Pirvu. Investigations on linkage and factorial recombination in maize. Lucrar. sti Inst. Agron. N. Balcescu, Bucuresti 10:49-58. 1967.

Manoliu, M. and T. Pirvu. Examination of the phenomenon of linkage and factorial exchange in maize. II. Lucrar. sti. Inst. Agron. N. Balcescu, Bucuresti 11:43-53. 1968.

Marques-Sanchez, F. and A. R. Hallauer. Influence of sample size on the estimation of genetic variances in a synthetic variety of maize. I. Grain yield. Crop Sci. 10:357-361. 1970.

Marques-Sanchez, F. and A. R. Hallauer. Influence of sample size on the estimation of genetic variances in a synthetic variety of maize. II. Plant and ear characters. Iowa State J. Sci. 44:423-436. 1970.

Matosko, I. V. and N. V. Turbin. Some biochemical properties of heterotic maize plants. Dokl. Akad. Nauk BSSR 10(6):417-420. 1966.

Michel, K. E. A test for "affinity" in maize. Heredity 25:133-137. 1970.

Michel, K. E. and C. R. Burnham. The behavior of nonhomologous univalents in double trisomics in maize. Genetics 63:851-864. 1969.

Miku, V. E. A new form of male sterility in maize: absence of tassel. Genetika, Moskva 6:21-27. 1970.

Mock, J. J. and K. J. Frey. Leaf number--an index of meiosis in corn (Zea mays L.). Crop Sci. 10:659-661. 1970.

Molot, P. M. Studies on the resistance of maize to diseases due to Helminthosporium and Fusarium. II. Resistance factors. Ann. Phytopath. 1:353-366. 1969.

Mottinger, J. P. The effects of X-rays on the bronze and shrunken loci in maize. Diss. Abstr. 29:3223B-3224B. 1969.

Mottinger, J. P. The effects of X-rays on the bronze and shrunken loci in maize. Genetics 64:259-271. 1970.

Mouli, C. Mutagen-induced dichotomous branching in maize. J. Hered. 61:150. 1970.

Mouli, C., N. K. Notani, and A. R. Gopal-Ayangar. A case of aberrant transmission of genetic factors on the short arm of chromosome 9 in maize. Canad. J. Genet. Cyt. 12:259-264. 1970.

Mouli, C. and N. K. Notani. Absence of a detectable change in Ds at the A_1 locus in maize following mutagenic treatments. Canad. J. Genet. Cyt. 12:436-443. 1970.

Murray, H. T. Differentiation of the metaxylem vessels of the wilted mutant of Zea mays. Diss. Abstr. 29:4064B. 1969.

Namm, T. Gene dosage effects on monogenic chlorotic lesion resistance to northern corn leaf blight. Diss. Abstr. 29:2305B. 1969.

Nass, H. G. and P. L. Crane. Effects of endosperm genes on dry matter accumulation and moisture loss in corn (Zea mays L.). Crop Sci. 10:276-280. 1970.

Nass, H. G. and P. L. Crane. Effect of endosperm mutants on germination and early seedling growth rate in maize (Zea mays L.). Crop Sci. 10:139-140. 1970.

Nass, H. G. and P. L. Crane. Effect of endosperm mutants on drying rate in corn (Zea mays L.). Crop Sci. 10:141-144. 1970.

Oberst, V. M. and T. S. Calyk. Production of chimerical plants of maize by means of colchicine. Cytology and Genetics, Kiev 2:510-516. 1968.

Paez, A. V., J. P. Ussary, J. L. Helm, and M. S. Zuber. Survey of maize strains for lysine content. Agron. J. 61:886-889. 1969.

Palilova, A. N., N. V. Turbin, and N. V. Atrasenok. Electron-microscope study of the ovules of inbred lines of maize with different types of cytoplasm. Rep. Acad. Sci. USSR 176:202-204. 1967.

Paterniani, E. Selection for reproductive isolation between two populations of maize, Zea mays L. Evolution 23:534-547. 1969.

Peterson, P. A. Controlling elements and mutable loci in maize: their relationship to bacterial episomes. Genetica 41:33-56. 1970.

Pfahler, P. L. In vitro germination and pollen tube growth of maize (Zea mays) pollen. III. The effect of pollen genotype and pollen source vigor. Canad. J. Bot. 48:111-115. 1970.

Piva, G., F. Salamini, and E. Santi. Protein characteristics and nutritive value of maize which is homozygous for the opaque-2 allele, isolated from an Italian population. Ann. Fac. Agrar. Bari 8:175-188. 1968.

Pollacsek, M. Effect of a dominant suppressor gene on the expression of opaque-2 gene in maize. Ann. de l'Amelioration des Plantes 20(3): 337-345. 1970.

Poneleit, C. G. and L. F. Bauman. Diallel analysis of fatty acids in corn (Zea mays L.) oil. Crop Sci. 10:338-342. 1970.

Rakha, F. A. Production and genetic analysis of new A-B translocations in maize. Diss. Abstr. 29:3212B. 1969.

Rakha, F. A. and D. S. Robertson. A new technique for the production of A-B translocations and their use in genetic analysis. Genetics 65:223-240. 1970.

Rautou, S. Test rapide d'appreciation de la teneur en lysine du grain de mais "opaque 2". Eucarpia, Cinquieme Reunion de la Section "Mais et Sorgho". Budapest-Martonvasar, Sept. 1969.

Reddy, G. M. and B. V. Ramasastri. Amino-acid composition of Indian maize varieties. Indian J. Genet. 27:461-464. 1967.

Roth, L. S., G. C. Marten, W. A. Compton, and D. D. Stuthman. Genetic variation of quality traits in maize (Zea mays L.) forage. Crop Sci. 10:365-368. 1970.

Russell, W. A. and S. A. Eberhart. Effects of three gene loci in the inheritance of quantitative characters in maize. Crop Sci. 10: 165-169. 1970.

Salamini, F., B. Borghi, and C. Lorenzoni. The effect of the opaque-2 gene on yield in maize. Euphytica 19:531-538. 1970.

Salamini, F. and C. Lorenzoni. Genetical analysis of glossy mutants of maize. III. Intracistron recombination and high negative interference at the gl_1 locus. Molec. Gen. Genetics 108:225-232. 1970.

Sastry, G. R. K. Paramutation and mutation of R^{ch} in maize. Theoretical and Applied Genetics 40:185-190. 1970.

Sastry, G. R. K. Instability of the Pl gene in maize. Heredity 25:498. 1970. (Abstr.)

Schwartz, D. An example of gene fixation resulting from selective advantage in suboptimal conditions. Amer. Nat. 103:479-481. 1969.

Scott, G. E., E. E. Rosenkranz, and L. R. Nelson. Host reaction to maize dwarf mosaic virus from Mississippi and Ohio. Plant. Dis. Reptr. 53:933-935. 1969.

Semenov, V. J., E. V. Semenova and L. F. Kapicyna. A study of the nature of chromosome pairing and of aneuploidy in connection with fertility in autotetraploid maize. Genetika, Moskva 5:67-83. 1969.

Sereverja, N. I. and N. I. Ekkerman. Heterosis in maize in connection with double fertilization in higher plants. Cytology and Genetics, Kiev 2:119-126. 1968.

Shih, K. L. Effects of ionizing irradiation on paramutation at the R locus in maize. Diss. Abstr. 29:891B. 1968.

Shih, K. and R. A. Brink. Effects of x-irradiation and gamma irradiation on paramutation in R heterozygotes in maize. Genetics 65:473-482. 1970.

Sidenko, L. E. Pathogenicity of the fungus causing maize smut. Trans. Har'kov Agric. Inst. 50:54-57. 1966.

Singleton, W. R. Mutations induced by treating maize seeds with thermal neutrons. from "Induced Mutations in Plants". Int. Atomic Energy Agency, Vienna. 1969.

Sleper, D. A. and W. A. Russell. Interrelationships among several stalk characteristics in maize and their significance in resistance to natural stalk breakage. Iowa State J. Sci. 45:197-209. 1970.

Smith, D. R., A. L. Hooker, and S. M. Lim. Physiologic races of Helminthosporium maydis. Plant Disease Reptr. 54:819-822. 1970.

Sreeramulu, C. and L. F. Bauman. Yield components and protein quality of opaque-2 and normal diallel of maize. Crop Sci. 10:262-265. 1970.

Sreeramulu, C., L. F. Bauman, and G. Roth. Effect of outcrossing on protein quality, kernel weight, and related characters in opaque-2 and floury-2 maize (Zea mays L.). Crop Sci. 10:235-237. 1970.

Strel'cuk, S. I. Combined use of induced mutations and polyploidy in breeding plants. Cytology and Genetics, Kiev 2:501-506. 1968.

Stroup, D. Genic induction and maternal transmission of variegation in Zea mays. J. Hered. 61:139-141. 1970.

Stuber, C. W. and R. H. Moll. Epistasis in maize (Zea mays L.). II. Comparison of selected with unselected populations. Genetics 67: 137-149. 1971.

Styles, E. D. Functionally duplicate genes conditioning anthocyanin formation in maize. Canad. J. Genet. Cyt. 12:397. 1970. (Abstr.)

Styles, E. D. Paramutation of the R locus in maize trisomics and comparison of paramutation systems in maize and tomato. Canad. J. Genet. Cyt. 12:941-947. 1970.

Suman, Ju. M. Obtaining an original maize mutant as a result of strychnine treatment of the intergeneric hybrid maize x teosinte. Trans. Kisinev Agric. Inst. 45:142-144. 1966.

Thomas, J. P. The inheritance of modifier genes influencing amylose synthesis in maize. Diss. Abstr. 29:2764B. 1969.

Tyrnov, V. S., V. A. Lavrovskij, and N. H. Enaleeva. The level of spontaneous chromosome aberrations in maize haploids. Genetika, Moskva 5:150-151. 1969.

Van Dyke, C. G. and A. L. Hooker. Ultrastructure of host and parasite in interactions of Zea mays with Puccinia sorghi. Phytopath. 59:1934-1946. 1969.

Vera, G. A. and P. L. Crane. Effects of selection for lower ear height in synthetic populations of maize. Crop Sci. 10:286-288. 1970.

Wanjari, M. R. and N. W. Widstrom. Chromosome knobs and resistance to Heliothis zea in maize inbreds. J. Hered. 61:171-173. 1970.

Warner, R. L. Inheritance of nitrate reductase activity and characterization of nitrate reductase in Zea mays. Diss. Abstr. 29:3629B. 1969.

Weber, D. F. An attraction between nonhomologous univalent chromosomes and further tests of distributive pairing in Zea mays. Genetics 64:s65. 1970. (Abstr.)

Widstrom, N. W., W. J. Wiser and L. F. Bauman. Recurrent selection in maize for earworm resistance. Crop Sci. 10:674-677. 1970.

Wilkes, H. G. Teosinte introgression in the maize of the Nobogame Valley. Bot. Mus. Leaflets 22:297-311. 1970.

Zoludzeva, V. P. and A. M. Palilava. A complex study of the genotype, cytoplasm and factors maintaining sterility in the formation of cytoplasmic male sterility in maize. News Acad. Sci. BSSR No. 1: 44-51. 1966.

Zuber, M. S., M. L. Fairchild, A. J. Keester, V. L. Fergason, G. F. Krause, E. Hilderbrand, and P. J. Loesch, Jr. Evaluation of 10 generations of mass selection for corn earworm resistance. Crop Sci. 11:16-19. 1971.

RECENT MAIZE PUBLICATIONS FROM USSR

(Prepared by M. I. Hadjinov and V. S. Shcherbak)

Alimova, G. K. and A. F. Andreeva. Comparative autoradiographic studies of DNA synthesis in microsporocyte meiosis and in the cells of tapetum of pollen sacs in maize fertiles and steriles. Journal of Botany, No. 11:1705. 1969.

Belousova, N. I. Maize hybridization with Tripsacum for experimentally obtaining apomixis in maize. Apomixis and Breeding Sci., pp. 191-196. 1970.

Borovsky, M. I. Apoximis in maize x Tripsacum hybrids. Apomixis and Breeding Sci., pp. 196-210. 1970.

Chalyk, T. S. Development and test of sterile counterparts of maize lines got by androgenesis. Apomixis and Breeding Sci., pp. 276-283. 1970.

Galeev, G. S., N. I. Kissel and L. A. Taova. Fertility and use of maize opaque-2 in breeding. Rep. All-Un. Lenin Acad. Agr. Sci. No. 10: 5-8, 1969.

Grishina, E. V. and M. I. Zaytseva. Dependence of haploid formation frequency in maize on conditions of fertilization and a level of homozygosity. Apomixis and Breeding Sci., pp. 247-251. 1970.

Khokhlov, S. S. and Zaytseva, M. I. Height of haploid plants obtained from cross-pollinated and selfed maize diploids. Apomixis and Breeding Sci., pp. 251-255. 1970.

Khotyleva, L. V. and L. A. Tarutina. Estimation of quantitative character inheritance in maize populations based on diallelic crosses. Problems in Mathematical Genetics, Minsk. 1969.

Khvatova, M. N. Experimental obtaining of haploids in maize and homozygous lines out of them. Apomixis and Breeding Sci., pp. 270-276. 1970.

Konarev, V. G., Y. V. Peruansky and A. Y. Rubchenya. Zein in the maize mutant opaque-2 and possibilities of mutant gene manifestation in hybrids. Rep. All-Un. Lenin Acad. Agr. Sci. No. 9:28-31. 1969.

Koptar, S. G. and M. I. Borovsky. Cytological studies of maize x Tripsacum hybrids in F_2, F_3 and F_4. Methods of maize breeding and seed production in Moldavia, pp. 171-175. Kishinov, 1970.

Maletsky, S. I. A new allele of a gametophyte gene in maize chromosome IV. Genetics 6(2):14-20. 1970.

Miku, V. E. Genetic analysis of some spontaneous mutations of tassel seed type in maize. News of the Moldavian Academy of Science, Biology and Chemistry No. 5:27-30, 1969.

Nemienko, N. E. Seed protein inheritance in maize interline single crosses. Agri. News, pp. 34-42. 1969.

Nhuen Van Thuet, and S. I. Strelchuk. Meiosis characteristics in tetraploid maize progenies depending on the type of pollination of females. Cytology and Genetics, Acad. Sci. USSR, No. 3, 1970.

Obersht, V. and T, Chalyk. Heterosis in maize on a tetraploid level. Agr. of Moldavia, No. 1. 1970.

Oryol, L. I. A study of pollen development in the maize of the Texas and Moldavian type with male sterility (in vivo). Bul. All-Union Plant Breeding Inst. 15:47-52. 1970.

Palilova, A. N. CMS in plants. Sci. and Engineering, Minsk. 1969.

Palilova, A. N., V. P. Deeva, N. V. Aprashenok, and E. I. Lyulkina. Functional features of mitochondrions extracted from ovules of maize plants varying in a cytoplasm type. Rep. BSSR Acad. Sci. 13(5). 1969.

Palilova, A. N., N. V. Turbin and V. P. Zholudeva. CMS manifestation in maize under effect of environment and nucleus genetic constitution. Agr. Biology 4(3). 1969.

Palilova, A. N. and V. P. Zholudeva. Pollen fertility restoration in maize hybrids developed on a sterile base. Cytology and Genetics 3(1). 1969.

Pashnar, T. E. Cytological research of apomixis forms of maize x Tripsacum hybrids. Apomixis and Breeding Sci., pp. 210, 213. 1970.

Rostovtseva, T. S. Chromosome structure in early selfed maize lines. Genetics 5(9). 1969.

Rotar, A. I., S. I. Pashkar, T. S. Chalyk and V. M. Obersht. Biochemical and morphological characteristics of pollen and seeds in tetraploid maize developed experimentally. Cytology and Genetics, Acad. Sci. USSR, No. 1. 1970.

Semyonov, V. I., E. V. Semyonova and L. F. Kapitsina. A study of the character of chromosome pairing and aneuploidy in connection with autotetraploid maize fertility. Genetics 5(10):67-83. 1969.

Shumny, V. K., L. A. Pshenitsyn and A. N. Bychkov. Studies of a gametophyte gene of chromosome IV in diploid and tetraploid maize. Cytology and Genetics, No. 2:147. 1970.

Sotchenko, V. S. Environmental influence on variation in common and specific combining ability of maize lines. Rep. All-Un. Lenin Acad. Sci., No. 2:14-16. 1970.

Turbin, N. V., L. V. Khotyleva and L. A. Tarutina. Genetic analysis of some quantitative characters in maize. Problems in Mathematical Genetics, Minsk, 1969.

Turbin, N. V., L. V. Khotyleva and L. A. Tarutina. Analysis of dominance in the system of diallelic crosses. Problems of Genetics and Breeding, Minsk. 1970.

Turbin, N. V., A. N. Palilova, N. V. Atrashenok and E. I. Lyulkina. A study of some microsporogenesis stages in pollen of steriles and fertiles by an electron microscope. Rep. USSR Acad. Sci. 195(5). 1970.

Turbin, N. V., A. N. Palilova, A. V. Smolskaya and L. S. Serpoktylova. Sterile cytoplasm influence on microsporogenesis in maize lines. Rep. USSR Acad. Sci. 188(5). 1969.

Yudin, B. F. Experimental polyploidy and an apomixis study in maize. Apomixis and Breeding Sci., pp. 66-72. 1970.

Zosina, N. F. A study of tassel seed mutations from the genetic collection of maize. Articles of post-graduates and young research workers of the All-Union Plant Breeding Inst., pp. 178-181. 1970.