SHERRET S. CHASE
Iowa State College

Chapter 25

*Monoploids in Maize**

Haploid sporophytes have been reported in jimson weed (Blakeslee *et al.*, 1922), cotton (Harland, 1920), tobacco (Chipman and Goodspeed, 1927), evening primrose (Gates, 1929), maize (Randolph, 1932a, 1932b), wheat (Gaines and Aase, 1926), rice (Ramiah *et al.*, 1933) tomato (Lindstrom, 1929), pepper (Christensen and Bamford, 1943), and in many other genera which have been subjects of cytogenetic study.

A haploid organism, strictly speaking, is one which has only one set of chromosomes, that is, one genome per cell. In the common usage of botanists, geneticists, and others, a sporophyte originating by reduced parthenogenesis or by an equivalent process, and consequently carrying the reduced or gametic complement of chromosomes in each cell instead of the normal zygotic complement, is referred to as a *haploid.*

Thus the term, as applied to a sporophyte, has come to carry the connotations of both parthenogenetic origin and gametic chromosome number, and the actual genomic condition tends to be ignored. Many so-called haploids are actually diploids or polyploids. Thus the haploids of common wheat are triploids, since the parent species, *Triticum vulgare,* is a hexaploid. To emphasize the fact that the haploids of maize carry only one set of chromosomes per cell, that is, only one chromosome of each type instead of the normal pair, the alternate term *monoploid* is used here to designate these aberrant plants.

In normal sexual reproduction in maize the pollen tube penetrates the eight nucleate embryo sac. One of the two male gametes released fuses with the egg nucleus to form the zygote, while the other fuses with the two polar nuclei to form the primary endosperm nucleus. In the abnormal type of reproduction giving rise to monoploid sporophytes the processes apparently are the same except that for some reason the *first* male gamete fails to fuse with the egg nucleus and is lost. The egg nevertheless is activated and devel-

* Journal Paper No. J-1906 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1201.

ops into an embryo. Evidence for this is indirect—monoploid embryos are found in kernels having normal (3n) endosperm. It is possible that some or all monoploids arise from reduced cells of the embryo sac other than the egg, from the synergids perhaps.

As tools for experimental research monoploids offer many possibilities: in the cytological field for studies of the meiotic distributions of unpaired chromosomes, *non-homologous* synaptic relations of the chromosomes and mechanics of chromosome doubling; in the genetic field for direct observation of mutational effects, measurement of mutation rates, studies of cytoplasmic effects, and biochemical investigations; in the agronomic field for the production of diploid, homozygous stocks directly from the monoploids. The following discussion is concerned primarily with my own investigations into the latter possibility.

A monoploid carries in each of its cells, or nuclei, only one chromosome of each type. Thus if the chromosome complement of any cell can be doubled, the affected cell and any derivative of it will consequently be both diploid and homozygous. If such homozygous diploid sectors include the reproductive tissues, meiosis should then be normal and the gametes produced functional. Thus such plants can produce diploid progeny—homozygous diploid progeny if the individual is successfully self pollinated—since every gamete of the plant is genetically equivalent to every other gamete. In a monoploid without diploid sectors, since the chromosomes lack synaptic mates, meiosis is highly irregular. Only rarely are functional gametes carrying the full complement of chromosomes produced. If two of these rare functional gametes from a single monoploid do fuse in syngamy, the zygote produced will be diploid, and homozygous, unless the gametic chromosomes were subject to chromosomal aberration during the irregular meiosis.

Production of homozygous diploid progeny from monoploids results in the fixation of a single gametic complex. In any population, desirable gametes are more frequent than desirable zygotes. For example, if one has on hand an individual heterozygous for three pairs of genes and wishes to obtain from it a definite homozygous product by selfing, *one* individual in *sixty-four* of the immediate self progeny (S_1) will, on the average, carry the desired genotype. *One* gamete in *eight*, extracted as a monoploid and then converted into a homozygous diploid, will furnish the same genotype (see Fig. 25.1).

Successful production of homozygous diploids in quantity from monoploids depends upon the adequate solution of two main problems. The first of these is the production and recognition in the seedling, or in earlier stages, of large numbers of monoploids. This problem has been solved to the extent that thousands of monoploids can be produced with relatively small expenditure of effort. The second problem is that of deriving self, and consequently homozygous, diploid progeny from the monoploids isolated. This problem also has a practical, though partial, solution.

MONOPLOIDS IN MAIZE

It has been known for some time that monoploids occur naturally in maize in measurable frequency. Data of Randolph (Randolph and Fischer, 1939) and of Einset (1943) suggest that monoploids occur spontaneously at a rate of about one per thousand. Data of Stadler (unpublished) indicate a rate of about one per hundred in a genetic stock. At the start of the studies reported

♀ \ ♂	*ABC*	*ABc*	*AbC*	*Abc*	*aBC*	*aBc*	*abC*	*abc*
ABC	$\frac{ABC}{ABC}$	$\frac{ABc}{ABC}$	$\frac{AbC}{ABC}$	$\frac{Abc}{ABC}$	$\frac{aBC}{ABC}$	$\frac{aBc}{ABC}$	$\frac{abC}{ABC}$	$\frac{abc}{ABC}$
ABc	$\frac{ABC}{ABc}$	$\frac{ABc}{ABc}$	$\frac{AbC}{ABc}$	$\frac{Abc}{ABc}$	$\frac{aBC}{ABc}$	$\frac{aBc}{ABc}$	$\frac{abC}{ABc}$	$\frac{abc}{ABc}$
AAbbCC...AbC...AbC*	$\frac{ABC}{AbC}$	$\frac{ABc}{AbC}$	$\frac{AbC^*}{AbC}$	$\frac{Abc}{AbC}$	$\frac{aBC}{AbC}$	$\frac{aBc}{AbC}$	$\frac{abC}{AbC}$	$\frac{abc}{AbC}$
Abc	$\frac{ABC}{Abc}$	$\frac{ABc}{Abc}$	$\frac{AbC}{Abc}$	$\frac{Abc}{Abc}$	$\frac{aBC}{Abc}$	$\frac{aBc}{Abc}$	$\frac{abC}{Abc}$	$\frac{abc}{Abc}$
aBC	$\frac{ABC}{aBC}$	$\frac{ABc}{aBC}$	$\frac{AbC}{aBC}$	$\frac{Abc}{aBC}$	$\frac{aBC}{aBC}$	$\frac{aBc}{aBC}$	$\frac{abC}{aBC}$	$\frac{abc}{aBC}$
aBc	$\frac{ABC}{aBc}$	$\frac{ABc}{aBc}$	$\frac{AbC}{aBc}$	$\frac{Abc}{aBc}$	$\frac{aBC}{aBc}$	$\frac{aBc}{aBc}$	$\frac{abC}{aBc}$	$\frac{abc}{aBc}$
abC	$\frac{ABC}{abC}$	$\frac{ABc}{abC}$	$\frac{AbC}{abC}$	$\frac{Abc}{abC}$	$\frac{aBC}{abC}$	$\frac{aBc}{abC}$	$\frac{abC}{abC}$	$\frac{abc}{abC}$
abc	$\frac{ABC}{abc}$	$\frac{ABc}{abc}$	$\frac{AbC}{abc}$	$\frac{Abc}{abc}$	$\frac{aBC}{abc}$	$\frac{aBc}{abc}$	$\frac{abC}{abc}$	$\frac{abc}{abc}$

* Desired homozygous individual. $\frac{1}{8}$ of gametes and $\frac{1}{64}$ of zygotes.

FIG. 25.1—Efficiency of Monoploid Method Compared with Selfing to S_1 for Obtaining Homozygous Individual *AAbbCC* from Heterozygous Parent *AaBbCc*.

here it was assumed that naturally occurring monoploids would furnish a sufficient supply at a rate of occurrence of the order of one in one or two thousand plants of a progeny provided the bulk of the diploids could be screened out by some simple device during the seed or seedling stages. This has proven feasible.

It was also assumed that some method for inducing doubling of the monoploid chromosome complement would have to be developed. Though this still appears desirable and possible, artificial induction of chromosome doubling has not been necessary in order to obtain diploid self progeny from a portion of the monoploids. The reason for this is that the fertility of the plants is in-

creased naturally by spontaneous doubling of the chromosome complement. About 10 per cent of untreated monoploids have yielded successful self progeny, largely as a result of this spontaneous somatic diploidization.

Since monoploids are for the most part of maternal origin, these plants should resemble their seed parents. Thus the search for monoploids is greatly facilitated if one looks for them among the progeny of markedly dissimilar parents. If one crosses a purple maize stock as pollen parent onto plants which lack this color and then finds non-purple seedlings in the progeny, one has reason to think these aberrant plants may be monoploids. In practice, the marker phenotype is used to indicate the diploid plants. These are discarded as recognized. Morphological and cytological tests are used for positive recognition of the monoploids.

In brief, the techniques used in isolating monoploids are as follows. The stock from which one wishes to obtain monoploids is pollinated with pollen from a genetic marker stock. The marker may carry the purple plant color genes ($A_1\ A_2\ B\ Pl\ R$) or brown ($a_1\ A_2\ B\ Pl\ R$), purple plumule ($A\ Pu_1\ Pu_2$), or any suitable complex of marker genes not carried by the seed stock. The ears at harvest are checked for kernels resulting from accidental self or cross pollinations. This check is made possible by using marker stocks which carry endosperm marker genes as well as plant marker genes. The markers which have been used, as appropriate, are purple aleurone ($A_1\ A_2\ A_3\ C\ R\ i\ Pr$), red aleurone ($A_1\ A_2\ A_3\ C\ R\ i\ pr$), starchy endosperm ($Su$), and yellow endosperm (Y).

The kernels *not showing* the endosperm marker phenotype are discarded (if the pollinations have been carefully made few discards are necessary). Then the kernels saved are germinated and a check made of the embryos or seedlings for the plant marker phenotype. All *showing* this character are discarded. The remainder are transplanted after first taking from each a root tip or two for cytological study. A second screening off of diploids is carried out after the first seedling leaves of the putative monoploids are fully extended. Those having the first leaf as long as the comparable leaf of the seed parent are almost without exception diploid and are therefore discarded. The true monoploids are then recognized by chromosome number determinations. Errors in classification at each stage result primarily in loss of monoploid plants. Consequently monoploid frequencies as reported are likely to be less than the actual frequencies of occurrence.

The putative monoploids screened off as a result of the genetic check include the actual monoploids and also diploids of the following types: diploid hybrids mutant for marker genes, hybrids carrying strong color suppressor genes, hybrids in which disease (generally fungus infection) has suppressed the development of the color phenotype, and a few maternal diploids. Occasionally paternal monoploids also are produced. These may be recognized

when the hybrid phenotype is unlike that of either the pollen or the seed parent, as is the case in crosses in which the brown marker stocks are used as pollen parents. In such crosses, maternal monoploids of the progeny should resemble the seed parent. Paternal monoploids should be brown (green at early stages) and the hybrids purple. The particular brown stocks used carry recessive markers, liguleless or japonica. These also serve to mark the very rare paternal monoploids.

When the monoploids reach the reproductive stage the practice has been to self these plants if any self pollen is shed, to cross them by other monoploids shedding excess pollen, or to pollinate them by diploids if self pollen is lacking.

FERTILITY OF MONOPLOIDS

The estimate of the fertility of monoploids, based on the assumption of 10 chromosomes distributed independently at meiosis, is one normal egg in 1024. That is, if abundant normal pollen were used in pollination these plants should set one good kernel in 1024 ovules. Actual fertility of the monoploids studied has been much higher than this, in spite of the fact that the amount of pollen used has often been scant. Little is known of the mechanics of meiosis in maize monoploids. Studies of the reactions of unpaired chromosomes at meiosis suggest that monoploid meioses may produce some functional gametes with structurally altered chromosomes (Kostoff, 1941). A proportion of the syngamic products in such cases would consequently be structurally heterozygous. If the reproductive tissue of a monoploid becomes diploidized before meiosis is initiated the gametes produced should all be structurally normal and strictly equivalent genetically. Some progenies were checked to determine the extent of chromosome aberration. The percentage of nonviable (actually, non-stainable) pollen produced by the monoploid derivatives was used as an indication of chromosome abnormalities. Among the progenies of diploid seed parents by monoploid pollen parents about 1 per cent had 10 per cent or more bad pollen. Among the progenies of monoploid seed parents by diploid pollen parents about 8 per cent had 10 per cent or more bad pollen. Among the progenies of monoploid by monoploid, 17 per cent had 10 per cent or more bad pollen. In the latter two classes, both of those in which monoploids were used as the seed parents, the monoploids thus used were those which had shown no evidence of diploidization in the tassels.

In a group of 298 monoploids, 282 matured. Of these 139 shed some pollen, 68 formed kernels, and 34 yielded successful self progeny. The fertility of this group of plants and of the whole series to date was far in excess of that expected of maize monoploids on theoretical grounds. The difference can be ascribed largely to spontaneous doubling of the chromosome complement in cells giving rise to reproductive tissue (Chase, 1949b).

PARTHENOGENESIS

A number of interesting facts have come out of studies of the frequency of reduced parthenogenesis in maize. One unanticipated fact has been that of the effect of the male (pollen) parent on parthenogenesis. Although this parent does not contribute its genes to the maternal monoploid, the particular pollen parent used in any cross does have an effect on the rate of occurrence of maternal monoploids (Chase, 1949a). In Table 25.1 the results of

TABLE 25.1

FREQUENCIES OF OCCURRENCE OF MONOPLOIDS FROM SEVERAL INBREDS AND HYBRIDS WHEN INBREDS A385 AND 38-11 WERE USED AS THE POLLEN PARENTS

Seed Parents	A385 as Pollen Parent				38-11 as Pollen Parent			
	Number of Progeny	No. *n*	Freq. per Thousand	Av.	Number of Progeny	No. *n*	Freq. per Thousand	Av.
Os420	1,715	1	.58	.29	4,909	9	1.84	1.83
M14	2,074	0	.00		2,738	5	1.83	
WF9	1,792	0	.00	.00	5,065	6	1.19	1.24
W22	1,839	0	.00		2,322	3	1.29	
Os420/M14	6,238	0	.00	.19	6,648	11*	1.66	2.52
WF9/W22	5,148	2	.38		3,554	12	3.38	
(Os420/M14)/(WF9/W22)	5,068	1	.20		4,868	2*	.41	
Averages			.17				1.66	
Golden Cross Bantam	12,324	2	.16		6,638	20	3.01	

* Known to be too low.

paired crosses involving two different pollen parents, inbreds A385 and 38-11, are summarized. Both of these inbreds carry the purple plumule marker system. From the genetic point of view A385 is the more satisfactory of the two. That is, in its hybrids the marker phenotype is generally well developed. In the hybrids of 38-11 the phenotype is often obscure. Consequently few monoploids were lost by misclassification in the progenies of A385, whereas a considerable number may have been lost in those of 38-11. In spite of this the data show 38-11 to be ten or more times as effective as A385 as a *stimulator* of parthenogenesis. This effect seems to be general. That is, the several dent stocks and also the sweet corn hybrid show about the

same proportionate effect of the pollen parents. Other data involving other crosses and data taken in other seasons are in agreement with those summarized here.

Data summarized in Table 25.2 are presented to show the variation in monoploid frequency dependent on the seed parent. Summaries are given of frequencies in crosses in which a single pollen parent, a brown marker, was used. The differences, expressed in terms of frequencies per 1000 seedlings and also as the frequency per seed parent, are quite striking. The rate of parthenogenesis seems to be roughly proportional to the intensity of agronomic selection to which these various stocks have been previously subjected.

TABLE 25.2

FREQUENCIES OF OCCURRENCE OF MONOPLOIDS IN SOME DENT STOCKS WHEN CROSSED BY A UNIFORM POLLEN PARENT

Seed Parent	Pollen	Number Progeny	No. *n*	Freq. per Thousand	Freq. per Seed Parent
Lancaster	N*	10,173	4	.39	.12
Reid's	N	14,650	11	.75	.38
Stiff Stalk Synthetic (SSS_0)†	N	91,125	90	.98	.37
Early Synthetic (ES_0)	N	8,226	10	1.13	.36
Dent Inbreds and Hybrids‡	N	.....	.....	1.35	.....
Stiff Stalk Synthetic (SSS_1)†	N	121,764	176	1.45	.51
Averages	.....	.....	.....	1.01	.35

* Brown, liguleless stock, *a B Pl C R^g Pr lg;* Randolph 43687-1.
† Original and first cycle Stiff Stalk Synthetic.
‡ 1947 data (Chase 1949), averages of frequencies per thousand.

Other data, including that from sweet corn varieties, hybrids and inbreds, bear out this relation. A likely explanation, other things being equal, is that the frequency of occurrence of *viable* monoploids is correlated inversely with the frequency of lethal genes in the source stocks. That the frequency of lethal genes in a stock is not the sole basis of differences between stocks becomes evident when one compares stocks which have been subject to an equivalent degree of selection.

It also becomes evident that there is another genetic basis for differences in rates of parthenogenesis when one analyzes the frequency of occurrence of monoploids as a function of the individual seed parent plants. In Table 25.3 summaries are given of the numbers of monoploids per seed plant in crosses in which the Stiff Stalk Synthetic variety was used as the seed parent. The distribution of none, one, two, and three monoploids per parent is about what one might expect on a chance basis. But the likelihood of getting five, six, and seven monoploids per seed plant by chance in three, three, and two cases respectively in a sample of 1065 parent plants is remote. The likeliest expla-

nation is that certain genotypes favor parthenogenesis. Whether this is a function of the sporophyte or of the gametes is not certain. It appears more likely that the effect originates in the individual gametes (eggs).

Emerson (unpublished) and Lindstrom (unpublished) and others have attempted to stimulate parthenogenesis in maize by the application of hormones and other chemicals to the ovules before or during fertilization. The results were uniformly discouraging. Randolph (1932b) found a number of

TABLE 25.3

DISTRIBUTION OF MONOPLOIDS PER SEED PARENT, STIFF STALK SYNTHETIC

Number of Monoploids per Seed Parent	Number of Seed Parents in Each Class
0	776
1	195
2	60
3	19
4	7
5	3
6	3
7	2
Total	1,065

TABLE 25.4

MONOPLOID FREQUENCIES AMONG THE PROGENIES OF MONOPLOID DERIVATIVES

Seed Parents	Pollen Parent	Number of Progeny	Number of Monoploids	Freq. per Thousand
H159	V	1,716	15	8.70
(H15/H25), S_1	V	1,792	14	7.81
(H19/H25), S_1	V	537	5	9.34
(H152/H143)	V	550	10	18.18

monoploids in material which had been subjected to heat treatments designed to induce polyploidy. Though it is a question whether the heat induced parthenogenesis, this type of treatment should be repeated on material in which the natural rate of parthenogenesis is known. In connection with the general monoploid study reported here a number of special treatments have been tried. Among these are hormone treatments, X-radiation of pollen, intergeneric crosses, pollination with pollen from tetraploid maize, and delayed pollination. These experiments are incomplete.

There is presently available one method by which high rates of parthenogenesis can be had. This is by selection of the pollen and seed parents used in

a cross. As shown in Table 25.4, monoploid derivatives are particularly favorable parthenogenetic stocks. In this series of crosses the stock *V* used as the pollen parent is a purple marker which is better than average as a stimulator of parthenogenesis. The seed parent in each case was a monoploid derivative; either a homozygous diploid (H159), or a single cross hybrid between two monoploids (H152/H143), or an advanced generation of such a hybrid.

The average frequency per 1000 for the stock from which H159 was derived (the Stiff Stalk Synthetic) is about 1.21. In each case the frequency of parthenogenesis is higher than that of the stock or stocks from which the monoploid derivatives were obtained. The hybrid H152/H143 and the frequency of monoploids in its progeny are particularly interesting in that H152 was a monoploid extracted from Inbred P39 and H143 a monoploid from Inbred P51. Thus the cross of the two is the single cross hybrid Golden Cross Bantam, based on monoploid parents. Normal Golden Cross Bantam crossed by marker stock *V* has a monoploid frequency of about 4.00 per thousand. A high rate of parthenogenesis is characteristic not only of the four stocks listed in Table 25.4 but of all monoploid derivatives adequately tested.

H159 not only has a high rate of parthenogenesis among its progeny but also a high degree of fertility among the monoploids produced. Of the 15 monoploids obtained from the cross with stock *V*, 12 were grown to maturity. *All* of these had one or more diploid sectors in the tassel and *all* set good seed.

On the average about one monoploid in ten is self fertile—in the sense that it yields a successful homozygous diploid progeny. One would like to obtain diploid self progeny from all monoploids. Since any increase in the rate of somatic diploidization should result in increased fertility, a number of treatments with polyploidizing agents have been tried. Colchicine, as used, brought about an increase in fertility but injury to the plants killed so many that no over-all gain was effected. In these treatments, solutions of approximately .5 per cent aqueous colchicine were injected into the scutellar nodes of the monoploid seedlings. It is possible that use of more dilute solutions injected repeatedly would be more effective.

Podophyllin, as a saturated aqueous solution, produced drastic stunting and inhibition of the development of the ears and tassels. Heat treatment, tried on a very minor scale, seemed to be about as effective as colchicine and had the same disadvantage. In this problem, as in that of increasing the rate of parthenogenesis, genetic methods seem to offer the best available solution. That is, stocks derived from self fertile monoploids are better sources of self fertile monoploids than the stocks from which the original monoploids were obtained.

Synthetic varieties that combine high monoploid frequency, high monoploid fertility, and high general agronomic desirability can probably be developed from homozygous diploids, both sweet and dent, already on hand.

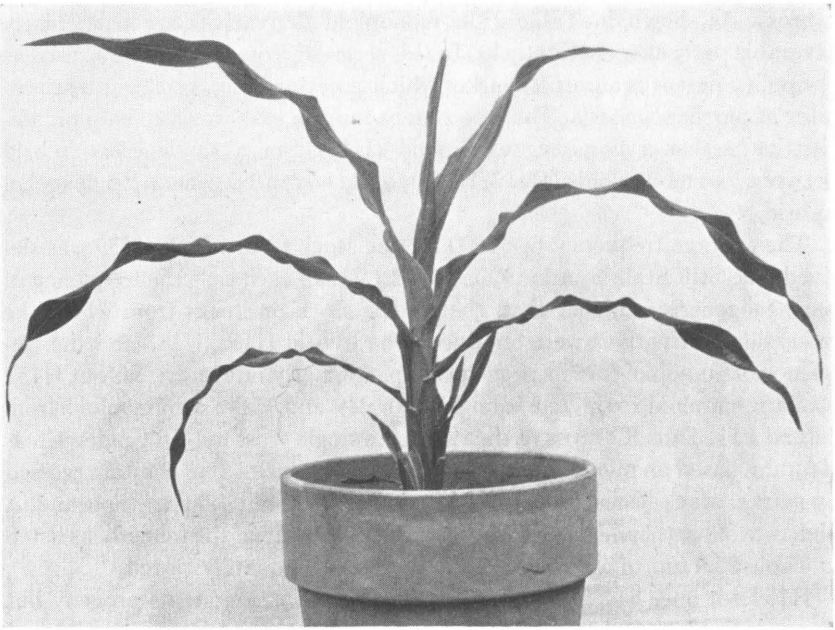

FIG. 25.2—Sweet corn monoploid sporophyte derived from Golden Cross Bantam.

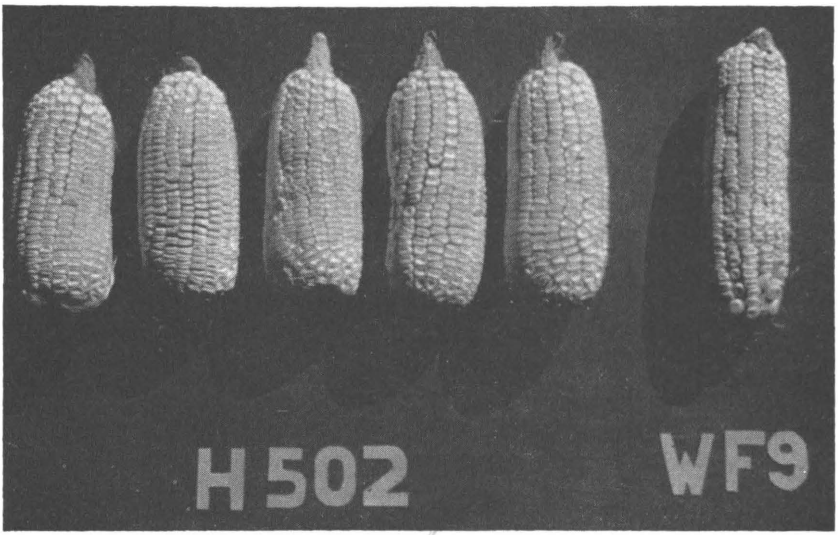

FIG. 25.3—Ears of homozygous diploid dent (H502) and inbred WF9. H502 is a Stiff Stalk Synthetic derivative. The ears shown are from plants of the first diploid generation.

Such synthetic varieties should be 10–20 times more effective as sources of new homozygous diploid lines than the better heterozygous stocks already tested.

About fifty homozygous sweet corn diploid stocks and about fifty homozygous dent stocks have been developed at Ames during the past two years of exploratory work. These are being tested for combining ability in comparison with related inbred lines. Though there is no reason *a priori* to expect that these lines will be better than average combiners, there is reason to think they should carry well balanced genetic systems, since passage through the sporophyte phase as a monoploid involves drastic selection against lethal and sublethal genes. In appearance the homozygous lines seem better than average unselected inbreds in general vegetative vigor.

CONCLUSIONS

It has been demonstrated that homozygous diploid stocks of maize can be produced from monoploid sporophytes. The method as now developed is practical from the point of view of the plant breeder as an alternate to inbreeding for the production of homozygous lines. As a method of gamete selection it offers unique possibilities. Improvements now being attempted should increase the efficiency of the procedure very considerably. It is not known yet whether the homozygous lines produced will prove to be better or poorer or equal to unselected advanced generation inbred lines on the average in respect to combining ability.