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Connecticut Agricultural Experiment Station

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**The Genetics and Morphology of Some
Endosperm Characters in Maize**

P. C. MANGELSDORF

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The Genetics and Morphology of Some Endosperm Characters in Maize*

P. C. MANGELSDORF

INTRODUCTION

When Nawaschin in 1898 discovered the phenomenon of double fertilization in *Lilium* it was generally believed by botanists that such a peculiar mechanism was confined to this species and perhaps a few closely related ones. Later investigations have shown that it is widely distributed and that the endosperm of angiosperms, with perhaps a few exceptions, is the product of a sexual fusion, quite apart from that which gives rise to the embryo, and differing from the latter in that one male nucleus combines with two or more female nuclei, while the embryo results from a fusion in which both parents contribute equally.

Thus the endosperm of angiosperms is unique in several respects. It resembles its near relative, the embryo, in its sexual origin, but differs from the latter in structure, in capacity for continued development, and in ability to reproduce.

This unique sporophyte, if indeed, it may be called a sporophyte, achieves its highest development in the cereals, in which it constitutes the part which makes these plants of such great economic importance, and in which it gives evidence of its sexual origin by the expression of the hereditary factors which it receives from its two parents.

Mendelian characters which have their expression in the endosperm have been found in wheat, rye, barley, rice and maize. They are apparently most numerous in maize and a number of characters which affect the color of the endosperm or aleurone layer and the texture of the endosperm tissue of this species have long been familiar, and have played an important part in the researches leading up to the re-discovery of Mendel's Law

*Parts I-IV of a thesis submitted to the Faculty of the Bussey Institution of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Science, June, 1925. Part V, on "Genetic Factors Which Affect the Development of the Gametophyte and Their Relation to Some Endosperm Characters," has been combined with researches by Dr. D. F. Jones on the same subject and will appear in *Genetics*, Vol. XI, under the title "The Expression of Mendelian Factors in the Gametophyte of Maize."

in 1900 and in the accumulation of a vast amount of genetic evidence since its re-discovery.

The endosperm characters of maize are of unusual value to the geneticist because, like all endosperm characters, they are visible sooner than those which affect other parts of the plant, and because they are readily studied in large numbers without the necessity of devoting a great amount of land or labor to the purpose. The average ear of maize bears from several hundred to a thousand seeds and one pollination on a single plant produces a large population which is readily classified because the environment has been remarkably alike for all its members.

In recent years the widespread application of a new method of corn improvement which involves the extensive inbreeding of this crop by artificial self-pollination, has brought to light many new characters which influence the development of the endosperm. Because of their possible phylogenetic significance, and because they represent new material which may prove of value in charting the germplasm of this important species, these characters merit a thorough study. The following pages are devoted to the preliminary investigations of a number of these new characters, their breeding behavior, morphology, effect upon development, and their relation to each other and to other characters.

DEFINITIONS

Two terms are used so frequently throughout the pages which follow that they deserve to be defined and limited.

The *endosperm generation* is the period beginning with fertilization and ending with the disappearance of the endosperm through absorption or digestion. In the cereals, the endosperm persists until the germination of the seed but in some plants it is almost completely lost in the early stages of development. This period has also been termed the *xenia* generation by some writers. In crosses the F_2 endosperm generation is borne on F_1 plants.

An *endosperm character* is any character which has its expression in the endosperm generation. The term does not imply that the character in question affects only the endosperm, in fact some of the endosperm characters produce their major effect upon the embryo.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. E. M. East, under whose supervision this investigation was made and these pages written, for his helpful advice and kindly criticism. A word of appreciation is due the Connecticut Agricultural Experiment Station for an arrangement enabling the writer to complete these studies while a member of its staff, and particularly to Dr. D. F.

Jones for his suggestion of the problem, his generous provision of material including a number of preliminary crosses, and his constant encouragement throughout. Acknowledgment is also due Dr. Florence McCormick for advice in regard to the preparation of material for histological studies, to Helen Parker Mangelsdorf, for assistance in the statistical work, and to numerous investigators who have contributed material.

PART I

DEFECTIVE SEEDS

Defective seeds are lethal or semi-lethal characters which affect the development of the endosperm and embryo between the time of fertilization and maturity. These characters were first reported by Jones (1920) who described them as "aborted seeds with either entirely empty pericarps or badly shrivelled seeds, completely lethal in some cases and partially so in others." He found these characters to be inherited as simple Mendelian recessives.

Previous to 1920 aborted seeds had frequently been noted on open-pollinated ears of maize but had generally been regarded as due to imperfect pollination or other external factors.

Self-pollinated ears in which approximately one fourth of the seeds were aborted, had also undoubtedly appeared in the cultures of many investigators before 1920 but these lethal characters were not noted or were not regarded as heritable.

In an early edition of Bailey's "Plant Breeding" appears a photograph of two ears grown shortly after the re-discovery of Mendel's Law, illustrating the alternative inheritance of the starchy and sugary conditions of the endosperm. One of these ears is clearly segregating for defective seeds in addition to the other two characters. The segregation is so well defined that the normal and aborted seeds on three of the rows of grain can be counted from the photograph. Seventy-five normal and 23 defective seeds are noted. The investigator who pollinated these ears to prove or disprove to his own satisfaction the newly re-discovered Law of Mendel, had more evidence of its correctness than he probably realized.

Richey (1923) found several defective seeds on an ear of maize, believed to be many centuries old, unearthed from an Indian graveyard in Peru. He concludes from this discovery that defective seeds are characters of considerable antiquity. Although his conclusion is probably correct, it is scarcely justified from this evidence alone, since it is equally possible that the few aborted seed on this ancient ear are of the non-hereditary types described in Part II.

In the past few years many experiment stations in this country, Canada, South America and Europe have undertaken projects for the improvement of corn by the method outlined by East and Jones (1919) and by Jones (1920) and known as "Selection in Self-fertilized Lines." Thousands of self-pollinations in many varieties have been made every year, and this extensive inbreeding of a naturally cross-fertilized species has brought to light many recessive variations previously covered up by the remarkable heterozygosity which exists in the average variety of maize.

Among these variations have been a large number of defective seed types.

The writer (Mangelsdorf, 1923) has noted defective seeds in self-pollinated ears of more than 30 representative American varieties as well as several from Spain, Italy, China and Peru. Since 1920, defective seeds have been reported under various names by numerous investigators. Lindstrom (1920, 1923) has described "abortive," "flint defectives," and "sweet defectives." Demerec (1923) has reported a condition which he calls "germless," Eyster (1922) a peculiar defect to which he gives the term

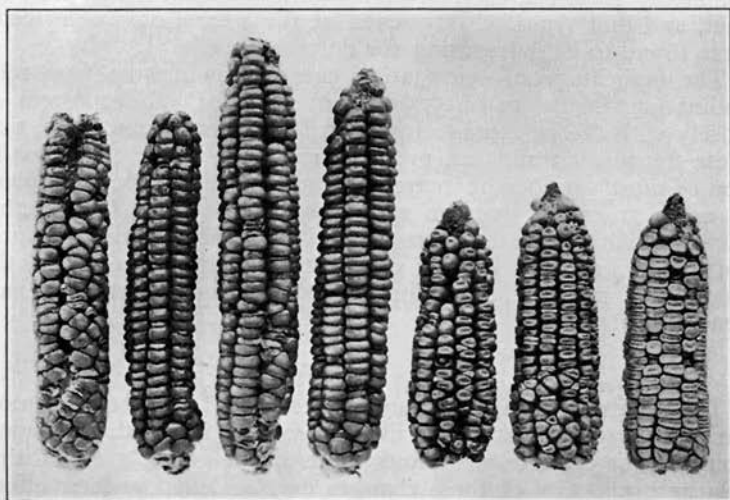


FIG. 51.—Self-pollinated ears of three New England varieties which are segregating for defective seeds. The third, sixth and seventh ears from the left represent the original ears of stocks de_1 , de_2 and de_3 respectively.

"scarred" and Wentz (1924) a type known as "miniature germ." Garber and Wade (1924) report a semi-lethal type of defective seed in their cultures.

All of these characters may be considered as variations of the "defective" condition since all of them represent a seed development considerably below normal and most of them are lethal or semi-lethal in a homozygous condition.

WIDESPREAD DISTRIBUTION IN GERMPLASM

Some conception of the frequency with which defective seeds occur may be gained from the following figures taken from self-pollinations made in typical American varieties.

Defective seeds were first noted in a lot of 86 self-pollinated ears of four New England varieties. Thirteen of these ears, or 15.0 per cent, were segregating.

In 1922, 575 self-pollinated ears of six regional strains of Sanford White Flint were examined for these variations. Nineteen of these ears, or 3.3 per cent, were found to be segregating.

Hutchison (1922), in making a systematic search for variations of all sorts, self-pollinated 2,110 ears representing 468 different lots of seed which had been obtained from seed companies and experiment stations. These lots contained most of the varieties commonly grown in the Northern states and included sweet, pop, dent, and flint types. Sixty-seven of these ears, or 3.2 per cent, were found to be segregating for defective seeds.

The percentage of segregating ears, following the first self-pollination, in the lot of 575 ears of Sanford White, agrees so closely with the percentage found in Hutchison's 2,110 ears, that these figures, 3.3 and 3.2, probably represent the average condition of most varieties of maize. In other words, about one plant in every 30 in the average variety is heterozygous for a lethal factor which causes defective seeds. In some varieties this proportion is probably higher, depending to some extent on the amount of natural self-pollination which has occurred in past generations.

ORIGIN BY MUTATION

The relatively high frequency of these lethal characters in most varieties suggests that maize, like *Drosophila*, is constantly undergoing factor changes at various points in the germplasm and that a large proportion of these changes may be lethal in their effect. Muller and Altenburg (1919), in a study to determine the frequency of mutation in the X chromosome of *Drosophila*, find that characters which are lethal or semi-lethal are the most frequent to occur. They estimate that the X chromosome in *Drosophila* produces a new lethal, on the average, about once in every 100 generations. Recent evidence from homozygous inbred strains of maize indicates that the frequency of lethal mutations in this species may be as high or perhaps considerably higher.

In 1921 an inbred strain of maize which had been self-pollinated for thirteen generations and which was apparently homozygous for its genetic factors, as demonstrated by a test made by Jones (1924), began to segregate for defective seeds. The sudden appearance of this new character was clearly due to a germinal change since nothing of this kind had previously been noted, although a careful search for new variations in all inbred strains had been constantly maintained. Nor could this new character have been the result of a segregation following accidental crossing since outcrossing with unrelated stocks is immediately apparent

by the increased vigor and productiveness of the hybrid plants. With the exception of the segregation for defective seeds, the mutant stock differed in no detail from the original inbred strain.

This new character originating by mutation in a homozygous stock is a typical defective seed, is completely lethal in its effect

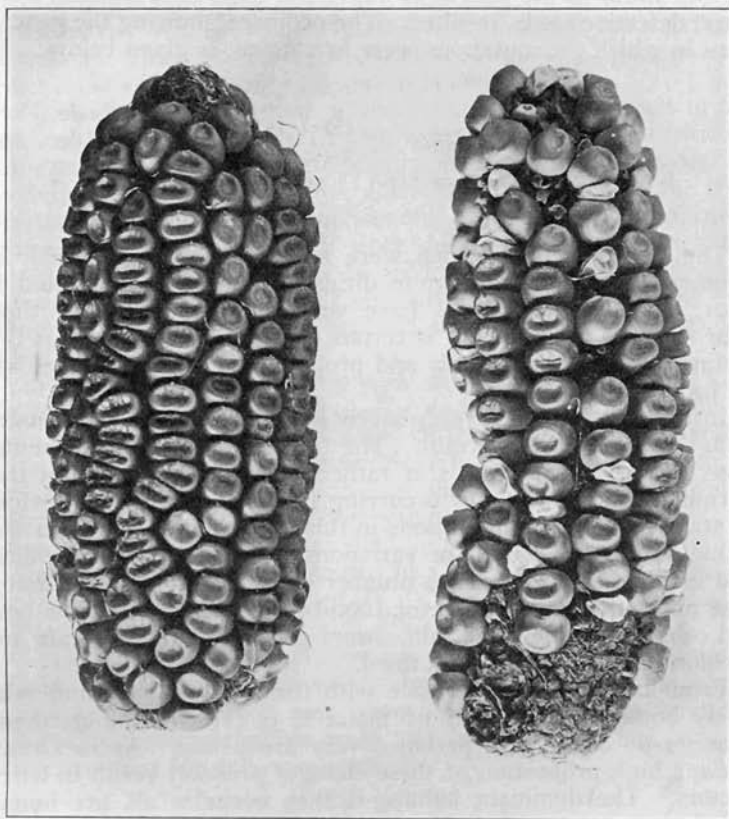


FIG. 52.—Ears of a strain of Leaming which mutated to defective seeds after thirteen generations of inbreeding. The ear at the right is segregating for the mutant character.

and is inherited as a simple Mendelian recessive. Ears of this inbred strain which are segregating for defective seeds are shown in Fig. 52.

A sister strain separated from this one after seven generations of inbreeding also showed defective seeds in the thirteenth generation when grown by H. A. Wallace at Des Moines, Iowa. This

same strain, though of slightly different pedigree, produced defective seeds on two ears in 1924 after 17 generations of inbreeding. The parental ears of both segregating progenies were normal in 1923 so two separate mutations must have occurred. In other words, four separate mutations have appeared in the germplasm of this strain in the past four years and each time a lethal character, defective seeds, resulted. The pedigree, showing the generations in which the mutations were first noted, is given below :

$$1-6-1-3-4-4-4-2 < \begin{matrix} 4-4-2-1 \\ 5-5-2-1-1-(1) \end{matrix} < \begin{matrix} 1-1-1-1-1-1 \\ 3-(1) \end{matrix} < \begin{matrix} (1) \text{ seg. de.} \\ (2) \text{ seg. de.} \end{matrix}$$

The two defectives which were noted in 1924 are similar in appearance but both are quite different from the one found in 1921. Though no crosses have yet been made between these four separate mutations, it is certain that they are of at least two distinct types phenotypically and probably genetic differences will be found as well.

In the past four years only a few ears have been self-pollinated each season from this strain. The fact that four separate mutations have been noted in this rather small sample indicates that germinal changes are now occurring rather frequently. Previous to 1921, however, no mutations in this stock had ever been noted, though an active search for variations was constantly maintained and in some seasons a large number of ears were self-pollinated. The only other mutations ever found in inbred strains have been red cobs in a white cob strain, dwarf plants in another strain and a chlorophyll deficiency in a third.

From the limited experience with these long-inbred and relatively homozygous strains of maize it is evident that germinal changes do occur and, perhaps, very frequently. As in *Drosophila*, a high proportion of these changes probably result in lethal factors. The dominant lethals, if they occur at all, are immediately lost, because individuals which carry them do not live to reproduce. The recessive factors, unless they have a marked deleterious effect in the heterozygous condition, may be carried along for generations. It is not at all surprising, therefore, to find these lethal factors in almost every variety of maize. Inbreeding brings them to light and demonstrates that about one plant in every thirty is heterozygous for one or more of them. How many genetically distinct lethal seed factors there are in maize can not be estimated at the present time, but some indication of the enormous number which probably exists may be gained from the following pages.

SOURCE OF MATERIAL

As already mentioned, defective seeds were noted by the writer in more than 30 varieties of maize. It soon became evident, that in order to make a thorough investigation it would be impossible to study the breeding behavior of more than half this number. Accordingly only those stocks in which defective seeds had appeared at least two successive generations, and in which the segregating ears gave clear-cut 3:1 ratios, were continued. Fourteen stocks met these requirements and were retained.

It is almost certain that, in confining the investigation to those strains in which simple 3:1 ratios were obtained, lethals which are due to duplicate or triplicate factors were eliminated. It was considered best, however, to study first the inheritance of the simple recessives without the complications brought in by duplicate or triplicate factors, especially since defectives which segregate in 15:1 or 63:1 ratios would be difficult to distinguish from the various types of non-hereditary defectives which occur in small numbers on almost every ear and which are discussed in some detail in Part II.

The source of the fourteen types of defective seeds used in this investigation is given below. The *de* numbers under which they are discussed were assigned after all the types had been arranged in a series on the basis of their "defectiveness." Those with high numbers such as *de*₁₃ and *de*₁₄ are the most defective in appearance, while those with low numbers, such as *de*₁, *de*₂, and *de*₃ most nearly approach the normal condition and are only semi-lethal in effect, being sometimes obtained in a homozygous condition.

*de*₁

This type appeared following the first self-pollination of Gold Nugget, an eight rowed, large-eared, yellow, flint variety. Twenty self-pollinated ears were obtained of which three were segregating for defective seeds. Only one strain has been kept heterozygous for defective seeds.

*de*₂

This defective was obtained from a self-pollinated ear of an eight-rowed yellow flint type grown by Dr. E. G. Anderson, then at Cornell University. This is probably the same stock in which Lindstrom found his "flint defective" and, if so, was obtained originally from Dr. W. E. Castle of Harvard University.

*de*₃

Defective seeds appeared on a self-pollinated ear of Century Dent, a many-rowed, medium early, yellow, New England dent variety. Five ears out of 18 which were self-pollinated, segre-

gated for defective seed, but only this and one other, de_8 , have been continued.

 de_4

This type was first noted in the third generation of inbreeding in two ears of a strain of Beardsley's Leaming, a many-rowed, fairly late, yellow dent variety. No defectives had been noted in this strain in the first two generations and their appearance in the third may have been due to mutation.

 de_5

This stock originated from a segregating self-pollinated ear of Reid's Yellow Dent received from Dr. J. R. Holbert of Bloomington, Illinois.

 de_6

A self-pollinated ear of Luce's Favorite, a large eared, New England dent variety received from Dr. R. A. Emerson, segregated for defective seeds of this type.

 de_7

A mutation in an inbred strain of Chester's Leaming which had been self-pollinated for thirteen successive generations and was apparently homozygous, gave rise to this defective.

 de_8

This stock originated from another ear of the same lot of self-pollinated ears of Century Dent as de_3 .

 de_9

This type was found on a self-pollinated ear of Cornell No. 12, a selection of Funk's 90 Day, obtained from Dr. R. A. Emerson.

 de_{10}

This defective appeared in a stock of "fine striped" which had been obtained some years previously from Cornell University.

 de_{11}

The source of this stock was a self-pollinated ear of Clarage Dent, a typical Western yellow dent variety received from Professor M. T. Meyers, Ohio University.

 de_{12}

Seeds of this type appeared in a self-pollinated ear of Burbank's "Rainbow," a novelty purchased from Peter Henderson Co., New York.

*de*₁₃

This type was isolated from a cross made by Mr. H. A. Wallace of Des Moines, Iowa. The seed parent was a hybrid combination of four inbred strains; the pollen parent a plant of "Illinois Two Ear" which Mr. Wallace believed to be homozygous for defective seeds.

*de*₁₄

This defective appeared in the second generation of inbreeding of a strain of Beardsley's Leaming, the same variety which gave rise to *de*₄. Defective seeds were not noted in this strain in the first generation of inbreeding.

SIMPLE MENDELIAN RECESSIVES

Except for the fact that there is often a slight deficiency of recessives, whereas an excess might be expected because of the regular occurrence of non-hereditary defectives on almost every ear, all of these fourteen types appear to be inherited as simple Mendelian recessives.

CROSSING EXPERIMENTS

The first defectives studied, *de*₁, *de*₂, *de*₃, and *de*₈, showed slight phenotypical differences, the first three being "partial" defectives; the last a "complete" defective. Crosses of these four strains made by Dr. D. F. Jones, the later generations of which were classified by the writer, indicated that these four defectives were genetically distinct. The next step was to determine the number of factors involved in the remaining ten stocks.

METHOD OF CROSSING

Throughout the investigation the general method of crossing two stocks has been as follows: A number of tassels, five or more, of the strain to be used as pollen parent were bagged. When pollinations were made the pollen from all of the bagged plants was collected, combined and mixed. Theoretically two-thirds of the plants in any segregating stock are heterozygous for the lethal factor and one-third are homozygous for the dominant allelomorph. Half of the pollen grains of the heterozygous plants should carry the lethal factor while the remaining half, as well as all of the pollen from the homozygous plants, should lack the lethal factor. Assuming an equal production of pollen by the heterozygous and homozygous plants, a composite collection of pollen made in this way should contain, on the average, one-third of the pollen grains carrying the lethal factor and two-thirds carrying its dominant allelomorph.

The seed parent of the cross should, like the pollen parent, produce heterozygous and homozygous plants in the proportion of 2:1. When the composite mixture of pollen is applied to homozygous plants, only normal seeds should be produced. When applied to the heterozygous plants, of which half the ovules carry the lethal factor, one-sixth of the seeds should be defective if the two stocks which are crossed are alike in their lethal factors, but all of the seeds should be normal if the lethal factors of the two parental stocks are unlike.

Since on the average two-thirds of the plants of a segregating stock are heterozygous for the lethal factor, the odds against obtaining no heterozygous plants when pollinations are made by this method are as follows:

No. Ears Pollinated	Odds
1	2:1
2	8:1
3	26:1
4	80:1
5	242:1

In order to be reasonably certain of including at least one heterozygous plant in every cross and to allow for failure to secure seed, it was customary to pollinate five ears. The pollen was always collected from five or more plants and it is practically certain that some pollen from heterozygous plants was always included in the mixtures.

This method of making the crosses between stocks, rather than between individual plants, has the advantage of being very rapid, a large number of pollinations being made from a single collection of pollen.

In several cases in which crosses were made between strains which regularly bear two ears, one of the ears was self-fertilized to determine the composition of the plant, the other was crossed. At harvest, only the crosses between known heterozygotes were retained. This method requires so much additional time and labor that its possible advantages are offset by the fact that only a limited number of crosses can be made in a season. The same objections were found to the method used by Demerec (1923) who, in making crosses between white seedling stocks, pollinated the ears with a mixture of own and foreign pollen and separated the selfed and crossed seeds by the effects of xenia. Only stocks which differ in their endosperm color or texture can be crossed by this method.

That the method of crossing at random without determining the composition of the plants used, gives the results which are theoretically expected, is shown by the following experiment, which incidentally shows that these lethal factors retain their genetic

identity as do any other Mendelian characters. A stock in which defective seeds had appeared for three successive generations was crossed with another stock originally from the same source, but which had been crossed with an entirely unrelated strain and the defective seeds recovered in the second generation. The pedigree of these two stocks is shown below :

$$105-9-7 \begin{cases} \text{I-I} = \text{Stock A} \\ \text{Cross-9-I} = \text{Stock B} \end{cases}$$

Six ears of Stock A were pollinated by a mixture of pollen collected from six plants of B. Four of the six ears proved to be segregating for defective seeds. A count of the normal and defective seeds on these four segregating ears is shown in Table I.

TABLE I. Ratios Resulting when Plants Heterozygous for a Lethal Factor are Pollinated with a Composite Mixture of Pollen from Homozygous and Heterozygous Plants.

Ear No.	Normal	Defective
448	184	22
449	116	32
450	151	32
451	164	43
Total	615	129
Ex. 5:1	620	124
Deviation	5	

The agreement of the actual results with the theoretical expectation is surprisingly good. Exactly two-thirds of the ears proved to be segregating and exactly one-sixth of the seeds on these ears were defective.

RESULTS OF CROSSES

It was believed that the most rapid progress in determining the total number of lethals involved in the fourteen stocks, would be made by crossing the first four, which were apparently all different, with the remaining ten, on the assumption that some of the untested stocks would prove to be carrying the same lethal factors as the first four and these could then be eliminated from further investigation.

Thirty-nine crosses were made in 1922 with the astonishing results that the F_1 seeds were normal in every case. This indicated that not one of the ten stocks carried the same genetic factors for defective seeds as the four original strains by which they had been crossed,

The next step was to cross the stocks in all combinations among themselves. This program involved a total of 91 crosses $\frac{n^2-n}{2}$

of which 45 had already been made. Since the remainder could not all be made in a single season, it was decided to cross first only the stocks in which the defective seeds showed some resemblance. In appearance the fourteen types range from complete defectives in which the caryopsis consists of little more than the flattened, transparent, pericarp to the partial defectives in which the recessive seeds are about half the size of normal seeds. Between these two extremes are all gradations and within each type there is a certain amount of variation. Several representative types of defectives are shown in Fig. 53. The fourteen types were arranged in a series on the basis of the average appearance of the recessive seeds. The plan was to cross each type with the two or three others nearest to it in the series. Although it was realized that resemblance in appearance did not necessarily mean genetic relationship, it seemed only reasonable to suppose that types resembling each other phenotypically were more likely to be alike genetically than those which were wholly different in appearance.

Twenty-seven crosses between types close together in the series were made in 1923 and again every cross produced only normal seeds.

Four additional crosses were made in 1924 and eight more in 1925; these, with the six preliminary crosses of 1920 and 1921, bring the total number to 84. Two of the crosses were reciprocals, however, so that the actual number of distinct combinations is only 82. This leaves 9 of the possible 91 crosses which are not yet made.

Of all these crosses, only one, a combination of de_5 and de_{11} , gave defective seeds in F_1 . This shows that de_5 and de_{11} are genetically identical, and that the results of any crosses made with one of these stocks applies as well to the other. Taking this fact into consideration only seven combinations remain to be made.

THE F_2 GENERATION

In order to be certain that segregating plants had always been included in making the crosses, and to obtain additional evidence that the two types entering the cross were genetically distinct in each case, F_2 progenies of a large proportion of the crosses have been grown.

Since the heterozygous crossed ears could not be distinguished from the homozygous ones, all of the ears of a cross were combined by counting off an equal number of seeds from each. As

one-third of the pollen grains of a composite collection of pollen are expected to carry the lethal factor of the pollen parent, and

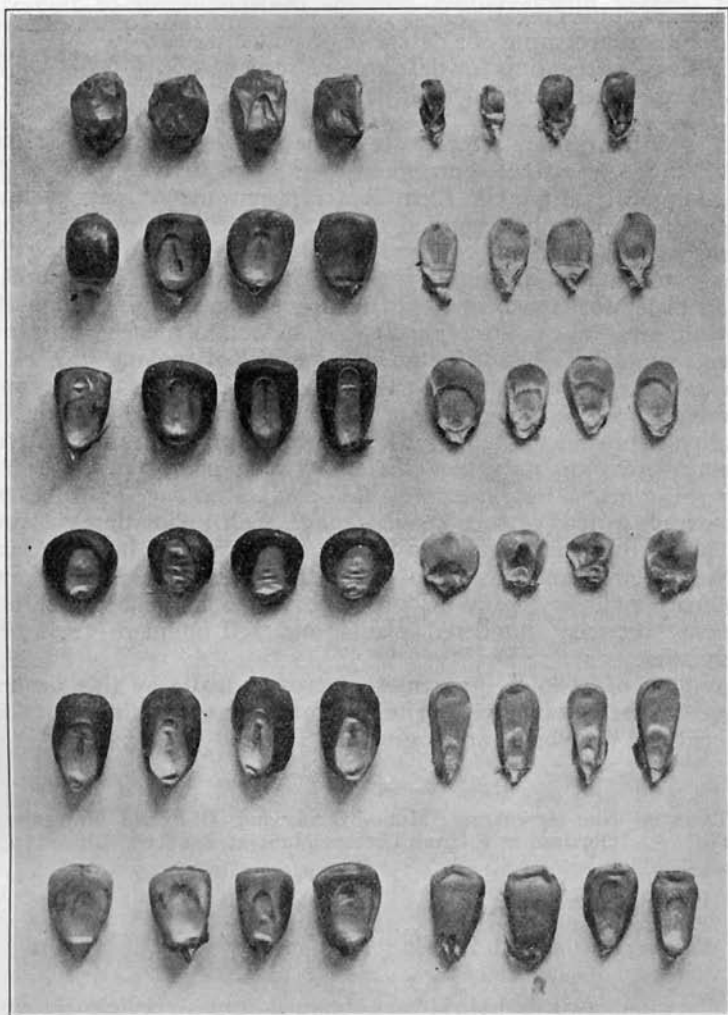


FIG. 53.—Six types of defective seeds showing the gradation from complete to partial defectives. Normal seeds from same ears are shown at left.

one-third of the ovules on a composite collection of ears the lethal factor of the seed parent, then a mixture of seed obtained from

such crosses would be expected to give on the average the following F_2 progenies:

- 4 Ears not segregating.
- 2 Ears segregating for defective of pollen parent.
- 2 Ears segregating for defective of seed parent.
- 1 Ear segregating for both defectives.

The di-hybrid ears are expected only once in nine times. However, if the defectives contributed by the two parents show slight phenotypical difference, then the reappearance of two distinct types in F_2 may be regarded as fairly conclusive evidence that heterozygous plants of both parents were included in making the cross and that the parental types are therefore genetically unlike.

In order to have better than an even chance of obtaining di-hybrid ears, it was customary to self-pollinate fifteen to twenty plants of each cross. Though this method of making crosses in a wholesale manner and self-pollinating F_2 progenies on the same prodigious scale, may appear to entail unnecessary labor, in reality, it proved to be the most economical procedure. It is true that by growing only the crosses between plants known to be heterozygous, di-hybrid ears would be expected once out of every four trials instead of once in every nine. Self-pollinating vigorous F_1 plants on a large scale can be done very rapidly, however, and it has been found easier to make the crosses at random and pollinate twice as many F_2 progenies, than to make individual crosses between numbered plants and self-pollinate fewer F_2 progenies.

A total of 1089 F_2 progenies of crosses made by this method have been self-pollinated. The ratio of non-segregating to mono-hybrid and di-hybrid ears is given in Table 2.

TABLE 2. Non-segregating, Mono-hybrid, and Di-hybrid Progenies Obtained in F_2 from Crosses Made at Random.

	Found	Expected	Deviation
Ears not segregating	428	484	-56
Ears segregating one type	552	484	68
Ears segregating both types	109	121	-12

The number of di-hybrid ears obtained agrees very closely with the theoretical expectation. There is a significant excess of the mono-hybrid ears, however. These are expected only as frequently as the non-segregating ears, actually they have appeared in considerable excess. A greater production of pollen by the heterozygous plants in some of the stocks, or the occurrence of heterozygous plants more frequently than two out of three, might account for this.

SUMMARY OF CROSSES

The diagram in Fig. 54 gives a picture of the situation with respect to these fourteen stocks.* Squares with vertical cross hatching represent crosses in which the F_1 seeds were normal. Those with horizontal cross hatching represent crosses in which

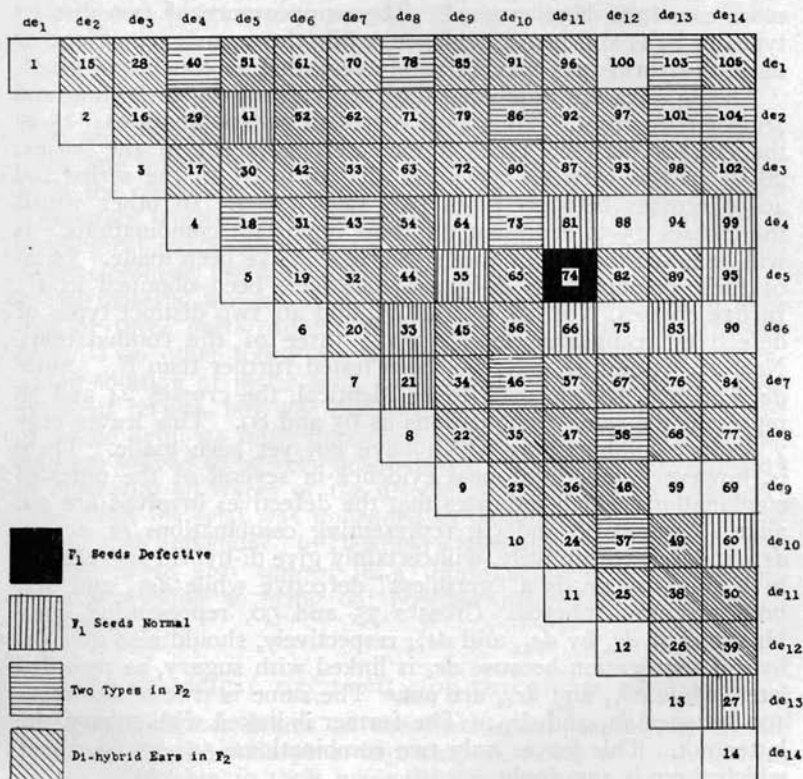


FIG. 54.—Diagram showing the crosses which have been made among the fourteen defective seed stocks. The types were arranged in the order of their "defectiveness."

the F_1 seeds were normal and two distinct types of defectives appeared in F_2 though no di-hybrid ears were obtained. Squares with diagonal cross hatching represent crosses in which the F_1

* For reasons of economy the detailed data showing the segregation in the individual ears of the fourteen parental stocks and their crosses are not included. Any marked deviations from expectation are noted, however, and are discussed in this and other papers.

seeds were normal and one or more di-hybrid ears were obtained in F_2 , while the single case in which defective seeds appeared in F_1 is shown by a solid square.

As has already been pointed out, the production of only normal seeds in F_1 is fairly good evidence that the two types entering the cross are genetically distinct providing that three or more ears have been crossed. The reappearance of two distinct types in F_2 is still better evidence, while the occurrence of one or more di-hybrid ears may be safely regarded as conclusive proof.

The squares are numbered diagonally from top to bottom and from left to right. Thus 1-14 represent self-pollinations; 15-27 the crosses between types immediately adjacent in the series; 28-39 crosses between types one degree apart in the series and 40-50 crosses between types two steps apart. In other words the crosses 15-50 represent the most important combinations. It will be noted that all of these 36 crosses have been made. In 27 of these combinations di-hybrid ears have been obtained in F_2 . In five crosses, Nos. 18, 37, 40, 43, and 46, two distinct types of defectives reappeared in F_2 , while three of the combinations, Nos. 21, 33 and 41, have not been tested further than F_1 . Since de_5 and de_{11} have proven to be identical, the crosses 24 and 38 represent the same combinations as 65 and 89. This leaves only 7 distinct combinations which have not yet been made. There is, however, some additional evidence in several of the untested combinations which indicates that the defectives involved are not alike. Crosses 88 and 94, representing combinations of de_4 by de_{12} and de_{13} respectively, will certainly give di-hybrid segregation in F_2 because de_4 is a "germless" defective while de_{12} and de_{13} both produce embryos. Crosses 75 and 90, representing combinations of de_6 by de_{12} and de_{14} respectively, should also give di-hybrid segregation because de_6 is linked with sugary, as recorded later, while de_{12} and de_{14} are not. The same is true of the cross 100 between de_1 and de_{12} . The former is linked with sugary, the latter not. This leaves only two combinations, 59 and 69, about which there is any doubt.

The evidence, then, is almost conclusive in indicating that thirteen distinct genetic factors for defective seeds are involved in the fourteen stocks tested. There is, of course, the possibility that one of the two doubtful combinations will reveal an additional case of two stocks which are genetically identical, even though these combinations are between defectives which differ decidedly in appearance. It must be remembered that two characters may be genetically identical in one main factor and yet differ phenotypically because of minor modifying factors. This is shown by the cross between de_5 and de_{11} which proved these two defectives to be identical although they were far apart in the arbitrary series which had been arranged on the basis of external appearance.

These results give some indication of the enormous number of distinct defective seed types which probably occur in maize. A sample of fourteen types was taken at random from the grab bag which constitutes the germplasm of maize, and thirteen of these proved to be genetically distinct. The total number of distinct lethal seed factors in the germplasm of this species can only be conjectured, but it probably equals or exceeds the number of distinct varieties of maize which are now grown.

THE MORPHOLOGY OF DEFECTIVE SEEDS

In order to determine the irregularities in development which cause one-fourth of the seeds on a segregating ear to be defective while the remainder are normal, and to find, if possible, constant differences which distinguish some of the types from others, a histological examination of all fourteen types, in various stages of development, has been made.

The material was killed and fixed in Carnoy's solution, a mixture of three parts of absolute alcohol to one of glacial acetic acid. Two other fixing agents, Benda's solution and a concentrated solution of picric acid, were also tried. The former gave excellent results, but was discontinued because of the high cost of osmic acid, its most important constituent. The picric acid solution proved to be very unsatisfactory because of the difficulty of removing all traces of the discoloration from such large sections. All material was imbedded in paraffin, cut in sections of ten microns and stained in Delafield's haematoxylin. Some of the sections were also stained in a dilute solution of iodine and potassium iodide to bring out possible differences in the starch grains.

THE CYTOLOGICAL MECHANISM OF ENDOSPERM FORMATION

The cytological details of the mechanism leading up to the formation of the endosperm in maize are fairly well established. When Nawaschin, in 1898, made the discovery that the endosperm of *Lilium* is the product of a sexual fusion entirely apart from that which gives rise to the embryo, three investigators, DeVries (1899), Correns (1899), and Webber (1900), simultaneously and independently reached the conclusion that this mechanism was probably responsible for the phenomenon of xenia in maize, although it was not until 1901 that Guignard furnished the cytological evidence of double fertilization in this species.

More recently Weatherwax (1919) and Miller (1919) have independently repeated Guignard's researches and both have given detailed descriptions and illustrations of the entire process leading up to fertilization. In most respects the accounts of these two writers agree very well, though Miller believed that all four

megaspores functioned while Weatherwax observed the disintegration of three megaspores, with only one persisting. In an earlier paper, however, Weatherwax (1917) also had made the observation that all four megaspores functioned and his discovery that only one persisted was made only after attention was called to the disagreement between his earlier cytological observation and certain well established facts regarding the genetic behavior of the endosperm.

When the pollen tube enters the micropyle, two identical sperm are emptied into the embryo sac. One of these fuses with the egg and an embryo is produced; the other fuses with one of the polar nuclei which lie close together in the embryo sac. Almost immediately the fusing nuclei are joined by the second polar nucleus, this process constituting the "triple fusion" characteristic of many angiosperms. It is of importance to note, in connection with the possible explanation of some of the forms of non-hereditary defectives described later, that both Weatherwax and Miller, in repeated observations, never found the two polar nuclei fusing before fertilization of one of them had occurred.

The endosperm of angiosperms is unique in that it is the product of a fusion in which the two parents do not contribute equally. Two maternal nuclei, with their assortment of chromosomes bearing the hereditary factors, combine with one male nucleus. The female parent, therefore, contributes two sets of chromosomes and a double dose of the assortment of hereditary factors while the pollen parent contributes only one set of chromosomes and a single dose of factors.

This peculiar situation enabled Hayes and East (1915) to demonstrate the fallacy of the "presence and absence" conception of dominant and recessive factors. These writers found that in crosses between flint and flour varieties the inheritance was always apparently maternal, a double dose of the maternal condition being always dominant to a single dose of the alternative condition. In other words, two "absences" were dominant to a single "presence."

DEVELOPMENT AFTER FERTILIZATION

The general features of the development of the endosperm and embryo in the cereals are fairly well established. Details of development which distinguish maize from other grasses are gradually being added as special phases are investigated. True (1893) and Poindexter (1903) studied the general development of the caryopsis. Reed (1904) has investigated the secreting cells of the scutellum of maize. Sargent and Robertson (1905) have made a very thorough study of the anatomy of the scutellum. The aleurone layer has been the subject of cytological studies especially by Lüdtkke (1890), Haberlandt (1890) and Groom

(1893). The successive stages in the development of the embryo have been described and figured by Weatherwax (1920).

The general development of the caryopsis in maize is briefly as follows: The endosperm fusion nucleus begins division almost at once and the rapidly growing endosperm soon fills the embryo sac. The embryo nucleus does not divide immediately after fusion and the first division usually does not occur until after the nuclei of the endosperm number 20 or more. (Miller, 1919.) The nucellus soon begins to disintegrate and is partly absorbed, the remainder being compressed into a thin integument between the pericarp and endosperm.

By the time that the early milk stage is reached, the endosperm occupies the entire space within the pericarp and exerts considerable pressure. (See Plate XXI, Fig. 1.) The embryo on the other hand is still rather rudimentary. From this point on, the embryo develops more rapidly than the endosperm, the latter undergoing only slight additional increase in size while the former grows rapidly, pushing further and further into the endosperm tissue.

Small starch grains are found in the outer cells of the endosperm in the early milk stage, which in the writer's material occurred at about 15-20 days after pollination. By the time that the late milk stage is reached at about 25 days to four weeks after pollination, the cells in the upper part of the endosperm are completely packed with starch grains, although those in the lower part are still relatively clear. In material fixed after this stage, the contents of the cells drop out in sectioning and in most cases no histological studies of further changes have been made.

The aleurone layer is present in most specimens in the early milk stage, though no color can be detected in this layer in unsectioned material at this period.

DEVELOPMENT OF DEFECTIVE SEEDS

In preliminary experiments of 1922, seeds were fixed at intervals of 1, 2, 4, 7, 10, 20 and 30 days after pollination. The defective seeds could not be distinguished from the normal seeds in the early stages and it was necessary to examine sections from a large number of seeds in order to be certain that defectives were included. In 1923 no material was collected until the normals and defectives on segregating ears could be distinguished from each other. This point was usually reached in the blister or early milk stage, or at about fifteen to twenty days after pollination in most of the types. Some of the partial defectives, however, could not be distinguished from normal seeds on the same ears until later.

Defectives and normal seeds were always removed in pairs for comparison and the seeds were usually taken from the middle of

the ear to avoid differences due to unequal development at butts and tips of the ears. Before dropping the seeds into the fixing agent, as much tissue as possible on either side of the embryo was removed to permit a more rapid penetration of the solution.

REGULAR DEVELOPMENT IN EARLY STAGES

The inheritance of all of the defective seed types as simple Mendelian recessives pointed to a regular functioning of the fertilization mechanism and the fusion of male and female gametes. The specimens collected in the early stages bore out this assumption. All of the types of which the early stages after pollination were studied showed the normal beginning of endosperm and embryo formation and no marked differences between normal and defective seeds were noted.

In specimens fixed after the blister or early milk stage, however, the differences between normal and defective seeds were very striking. The various types of defectives differed from each other, however, only in general development and, with the exception of one type, no specific morphological differences, which always distinguish one type from another, have been found. A general account of the development of these various types of aborted seeds follows.

THE PERICARP

No matter how defective the endosperm and embryo may be, the pericarp usually attains a normal or almost normal development. This affords a striking illustration of the complete independence of these two tissues which, though borne on the same plant, represent distinct sporophyte generations. The pericarp is maternal in its origin and with the exception of the stimulus from pollination which sets off its development, it does not appear to be influenced by the hereditary composition of the new sporophyte which it encloses.

In normal seeds, the pericarp is constantly distended by the pressure of the growing endosperm. In the defectives, there is always a space between these tissues. In early stages this space is filled, partly with nucellar tissue and partly with a clear watery solution. In later stages the walls of the ovule are pushed together by the pressure of the normal seeds on either side and the space disappears as shown in Plate XXI, which shows three successive stages in the development of the de_{14} type of defective seeds.

THE NUCELLUS

In normal seeds the nucellus rapidly disintegrates following fertilization. Part of it is probably absorbed while the remainder is compressed into a thin integument between the endosperm and

pericarp and soon loses its identity as a separate tissue. (Plate XXI.) The nucellus, like the pericarp, is of maternal origin and is not influenced by the sporophyte with which it is in constant contact, except that in the absence of a vigorous and rapidly growing endosperm it is permitted to persist as a distinct tissue for longer periods than it does in normal seeds.

THE ENDOSPERM

The endosperm of the defective seeds differs from that of normal seeds in degree rather than in kind. In no case does it attain the size of the normal endosperm, but in details of development no marked differences are noted. With regard to the development of the endosperm, the defectives of these fourteen stocks form a continuous series ranging from the de_{13} and de_{14}

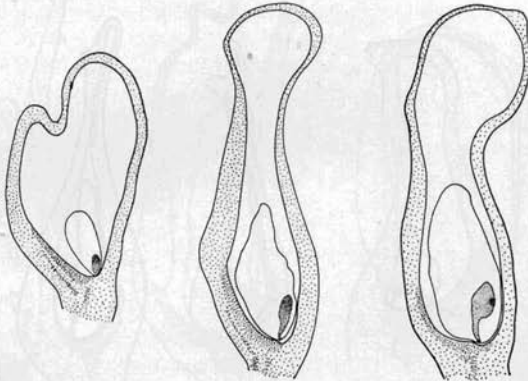


FIG. 55.—Three successive stages of development of defective seeds of de_{10} . No aleurone layer is found at any stage. (Figures 55-58 represent a magnification of approximately 6.5 diameters.)

types in which only a small mass of tissue is present, to the de_1 and de_3 types, in which the endosperm is fully half size.

With regard to the rate at which the endosperm increases in size from week to week the same gradation is found. In the de_{14} stock, for example, the defective seeds at the early stage have only a small mass of endosperm tissue. At the late milk stage no increase in size is noted, and at the early dough stage the development of the endosperm still remains practically the same, as shown in Plate XXI. In partial defectives, such as those of the de_8 stock, for example, the endosperm gradually increases in size from week to week though its rate of growth is considerably retarded as compared to the endosperm of normal seeds and its

final size is considerably less than that of a normal endosperm. (Plate XXII.)

The order of the fourteen stocks of defective seeds on the basis of their endosperm development as shown by these histological studies is not exactly the same as the arrangement made on the basis of external appearance alone. In general, however, the two series agree very well.

STARCH GRAIN FORMATION

The same general differences noted in size of endosperm are also found with regard to the formation of the starch grains. In the defectives at the lower end of the series no starch grains were found at any stage examined. Types further up in the series produce small starch grains in the periphery of the endosperm in later stages, while in the partial defectives, starch grains are found

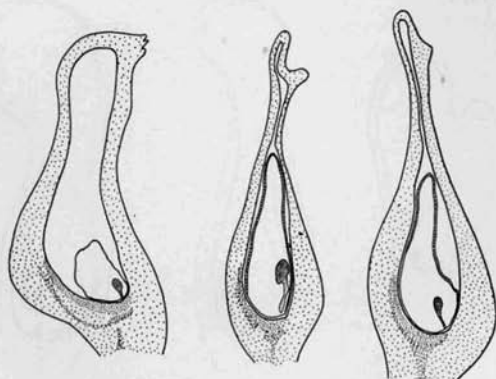


FIG. 56.—Defective seeds of *de*₁₄ at early milk stage; *de*₇, late milk; *de*₁₂, late milk.

in every stage examined, and in later stages the cells of the endosperm are packed with starch grains which are apparently normal in size and structure.

No characteristic differences in the structure of starch grains were noted with the exception of the defective seeds of *de*₄. In the recessive seeds of this strain the starch grains have the appearance of undergoing hydrolysis many of them being completely broken up.

THE ALEURONE LAYER

The defectives at the foot of the series produce no aleurone layer at any stage examined and this might be expected since an aleurone layer is usually formed only in later stages of normal development. Beginning with *de*₁₁, however, an aleurone layer

is found in the later stages examined. In some cases this layer extends only part way around the endosperm, in others it completely surrounds this structure except, of course, at the base where no aleurone layer is found even in normal seeds. Drawings in which the aleurone layer has been enlarged out of proportion to the other structures are shown in figures 55-58. In defectives higher in the series than de_{10} , an aleurone layer is found in almost all stages examined.

THE EMBRYO

With the exception of de_4 , all of the types were found to contain embryos. This type is similar to the "germless" seeds reported by Demerec (1923) which at maturity contain no embryo.

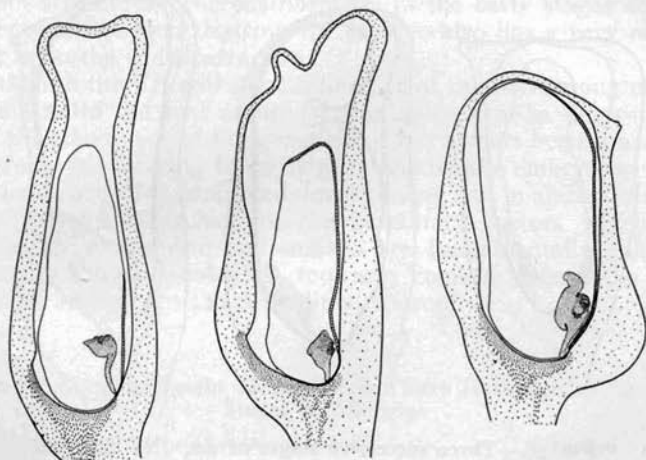


FIG. 57.—Defective seeds of de_{11} , de_3 , and de_8 at approximately the same stage, showing difference in degree of development of embryo, endosperm, and aleurone layer.

No paraffin sections of this defective were secured because the recessive seeds are distinguishable from the normals only shortly before maturity, at which time the seeds are too corneous to be sectioned by the paraffin method. Free-hand dissection in early stages of seeds from ears later proven to be segregating for this character, showed that an embryo was present in all the seeds. Apparently the embryo is digested and absorbed later. The cavity which remains on the germinal side of the seed is partly filled with a hard brittle mass of substance with no definite structure. As already mentioned, the endosperm of this type also shows evidence of digestion.

In general, there is a marked correlation between the development of the embryo and that of the endosperm. The complete defectives in which the endosperm remains practically stationary have very rudimentary, undifferentiated embryos which show no further development after the early milk stage. (Plate XXI.) Defectives higher in the series show some increase in size from week to week, but no clear differentiation of various parts of the embryo is apparent though a "growing point" is indicated by a greater concentration of nuclei in certain regions. Beginning with *de*₈, a scutellum, coleptile and several rudimentary leaves are distinguished, while in some of the partial defectives the development of the embryo is fairly normal.

The embryos of the defective seeds often show considerable dis-

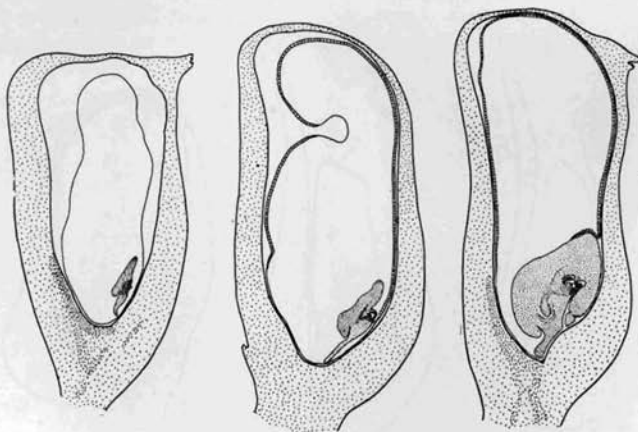


FIG. 58.—Three successive stages of *de*₈. No aleurone layer is found in the early stage but a partial or complete layer is formed later. Note the distortion of the embryo in the final stage.

ortion in shape. In later stages this may be attributed to the pressure exerted by the normal seeds on either side as shown in Plate XXIII, but in some cases a distortion is noted even when the defective seeds have not been under pressure. The embryo tends to be short and "blocky" as shown in Plate XXII, which may be compared to Plate XXIV which shows the successive stages in the development of the normal embryo.

GENERAL ASPECTS

Aside from the interesting demonstration that the pericarp, once its development is stimulated by pollination, proceeds quite independently of the tissues which it encloses, the most outstanding

feature of the morphology of the defective seeds is the marked correlation between the development of the endosperm and embryo.

An external examination of some of the defective seeds had led to the assumption that these, in many cases, contained no tissue whatever. It was surprising, therefore, to find distinct, though rudimentary, endosperm and embryo at some stage in every type examined. It was even more surprising to find that the deleterious influence of the lethal factors, which they carried, affected both of these structures to almost the same degree. It might be supposed that some factors would affect specifically the embryo, permitting the endosperm to continue in a more or less normal fashion. Others might be expected to inhibit particularly the endosperm. This has not been found to be the case. Even in the *de*₄ type, which lacks an embryo at maturity, the development of both structures proceeds normally in the early stages and the influence which later destroys the embryo also has a very marked effect upon the endosperm.

Although there is still some difference of opinion among morphologists as to the real nature of the endosperm in angiosperms, from the standpoint of the geneticist it has always been considered a sporophyte, differing from its near relative the embryo, in structure, in capacity for continued development, and in ability to reproduce. In the expression of the hereditary factors which they receive, the endosperm and embryo are fundamentally alike and eventually the morphologists, too, may come to regard the endosperm of angiosperms as a modified sporophyte.

TABLE 3. Average Weight of Seeds from Ears Harvested at Successive Stages of Maturity.

Days after Pollination	Av. wt. in mg.				Relative Development
	1	2	3	Av.	
14	5	5	5	5	1.5
21	25	27	37	30	9.1
28	82	86	71	80	24.2
35	111	97	126	111	33.6
41	159	166	189	171	51.8
51	234	222	226	227	68.8
75	374	319	306	330	100.0

PHYSIOLOGY OF DEFECTIVE SEEDS

Histological examinations of the normal and defective seeds were confined largely to a rather brief period beginning with the blister stage and ending when the seeds had reached the dough stage. In order to determine the differences between normal and defective seeds in later stages of development, a comparison of their rate of growth, final dry weight, percentage of germination

and effect upon the sporophyte and gametophyte in the haploid condition was made.

RATE OF GROWTH, FINAL WEIGHT AND GERMINATION OF
NORMAL SEEDS

The rate of growth of normal seeds of maize was determined as follows: A large number of plants of an F_1 hybrid of homozygous inbred strains were grown under uniform soil conditions. These plants, all being genetically alike, came into silk and were pollinated at about the same time. At two weeks after pollination, and at intervals thereafter until maturity, three ears were taken at random from these plants. The ears were reduced to an air dry condition after which the kernels were removed, counted, weighed "en masse" and the average weight at each stage determined by simple division.

It is quite possible that in ears harvested at various stages of maturity in this way there is a transfer of materials from the cob to the kernels during the drying out process. Such an exchange, if it occurs at all, would probably be proportionate for the various stages and is not regarded as a serious source of error.

TABLE 4. Percentage Germination of Corn Seeds Harvested at Successive Stages of Maturity.

Days After Pollination	Relative Development	Percent Germination
14	1.5	0
21	9.1	28
28	24.2	56
35	33.6	72
41	51.8	92
51	68.8	96
75	100.0	98

The average weights in milligrams and the relative weights, as compared to the final weight at maturity, of these seeds harvested at various stages of development, are given in Table 3. Figures 1 and 2 of Plate XXV show respectively a representative ear at each stage and 50 seeds from each ear.

The 50 seeds representing each stage of development were planted in sand in the greenhouse with the results shown by the photograph in Plate XXV, Figure 3. Some germination occurred in every lot except the first which was harvested at fourteen days after pollination. The percentage of germination of each lot and the relative weight of the seeds from which it was grown are given in Table 4.

The normal seeds of maize, like those of barley (Harlan and Pope, 1922), are apparently capable of some germination at all stages of development but the very earliest.

RATE OF GROWTH, FINAL WEIGHT, AND GERMINATION OF DEFECTIVE SEEDS

The rate of growth of defective seeds as compared to normal seeds from the same ears was determined for one type, de_{10} . Ears from an F_1 hybrid of this stock crossed with an unrelated inbred strain were harvested when the segregation on the ears first became apparent and at intervals thereafter until maturity. When all the ears had been reduced to dryness, the kernels were shelled off, the normal and defective seeds separated, counted and weighed, and the average weight of each class determined. The growth curves of the normal and defective seeds from ears segregating for de_{10} are shown in Figure 59. Although the rate of

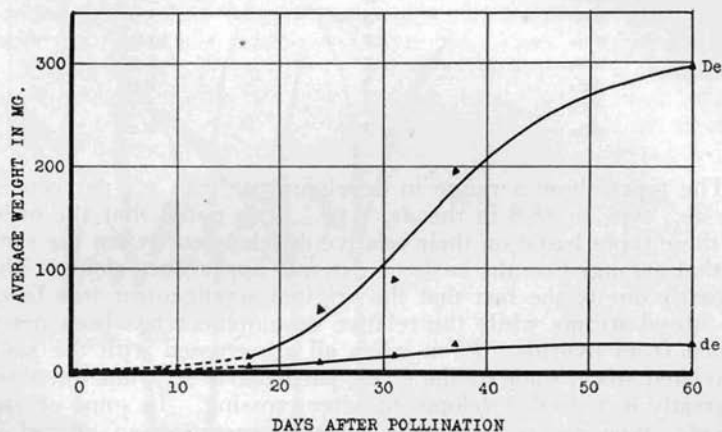


FIG. 59.—Growth curves of normal and defective seeds from the same segregating ears of the de_{10} stock.

increase in dry weight is very low for the defective seeds, the two curves appear to be of the same general type.

In determining the relative development at maturity of the fourteen types, dry weight was used as a criterion. Obviously, the relative development cannot be determined with any high degree of accuracy because it is influenced to some extent by the environment and considerably by the hereditary constitution of the stocks, certain of the types showing more development in crosses than in inbred strains. In order to make the determinations as nearly comparable as possible, the figures on relative development have, with one exception, been taken from crosses which were all identical with respect to one of the parents, and all grown the same season. In this way the defectives are compared under conditions in which hereditary and environmental differences are reduced to a minimum.

The relative development of defective seeds at maturity of each of the fourteen stocks is shown in Table 5.

TABLE 5. Relative Development in Defective Seeds of Fourteen Stocks as Compared to Normal Seeds on Same Ears.

Stock	No. Ears	Relative Development
de_1	6	50.4
de_2	3	58.8
de_3	3	29.9
de_4	3	37.3
de_5	2	18.0
de_6	3	34.0
de_7	3	18.8
de_8	3	15.0
de_9	3	5.9
de_{10}	2	13.7
de_{11}	3	7.3
de_{12}	3	4.0
de_{13}	2	4.5
de_{14}	3	2.4

The types show a range in development from 2.4 per cent in the de_{14} type to 58.8 in the de_2 type. It is noted that the order of these types based on their relative development is not the same as that arranged on the basis of external appearance alone. This is partly due to the fact that the original arrangement was based on inbred strains while the relative development has been determined from hybrids. Even when all are crossed with the same unrelated stock, some of the types, particularly 2, 7, and 10, show a greatly increased development after crossing. In some crosses the de_2 type reappears in the second generation so altered in appearance that it is scarcely recognized. The recessive seeds are almost equal to normal seeds in size and weight, and differ from the latter only in a paler color and a mottled appearance.

Whether this increase in development which follows crossing is due to the greater vigor of the plants on which they are borne, or to the action of modifying factors contributed by one or both parents, is not known. That some of the defectives are influenced by modifying factors, is almost certain. The de_5 and de_{11} types, for example, are genetically alike, yet differed in appearance, not only in the original stocks, but in hybrids in which both were crossed to the same unrelated stocks.

Judging from the dry weight alone, the fourteen types of defectives correspond to various stages in the development of the normal seed. This is shown diagrammatically in Figure 73 (Part III) in which the relative development of each type is represented by a point on the normal growth curve of maize seeds.

At first glance, the defective seeds are comparable to normal seeds which have had their development arrested at an early stage,

as has occurred in the ears harvested at successive stages of development, shown in Plate XXV. When these various defective seeds are tested for germination, however, it is found that they are by no means equal to normal seeds of the same relative development. This is shown by the figures in Table 6, in which the germination of each type of defective is compared to the theoretical germination of immature normal seeds of the same relative development. The theoretical germination of the normal seeds was determined by interpolation of the figures in Table 4.

It is noted that the defective seeds in every case show a much lower germination than would be expected from normal seeds of

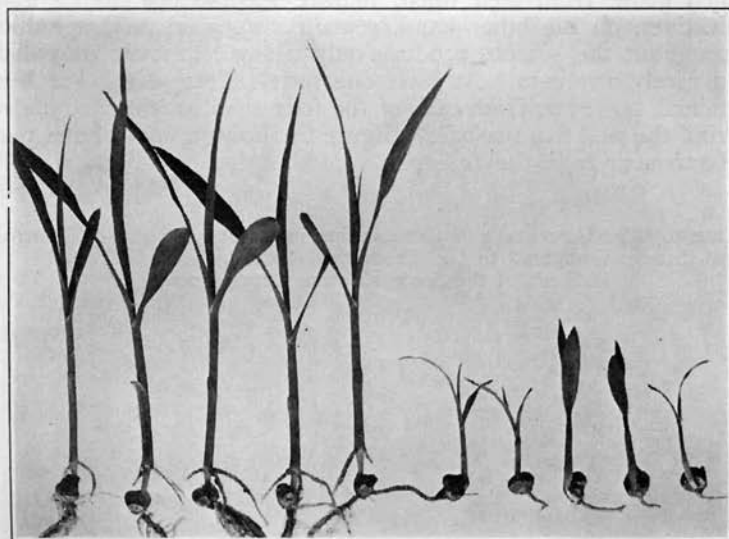


FIG. 60.—Seedlings from normal and defective seeds of de_2 . Only rarely do the seedlings from the recessive seeds survive more than a few weeks.

similar development. In fact, the types below de_7 showed no germination whatever in this test with the exception of one seed from a total of 131 seeds of the de_{11} type. Defective seeds of de_8 have also shown slight germination in some cases, but in the ears used in this particular test no germination occurred.

Not only are the defective seeds less able to germinate than normal seeds of the same development, but those which do succeed in sprouting produce very weak seedlings which are lacking in vigor and soon die. Seedlings from normal and defective seeds of the de_2 stocks are shown in Figure 60. By planting large numbers of seeds in the greenhouse and transplanting the most

vigorous of the seedlings to the field, it has been possible to obtain homozygous plants of stocks 1, 2, 3 and 6.

The behavior of the plants grown from homozygous defectives is in striking contrast to those which result when immature normal seed is grown. In appearance the two lots of seed are almost identical, both being badly shrivelled and aborted. Both types give a low germination and the seedlings are, in both cases, very weak and spindling. The plants which are genetically normal, however, soon recover from the handicap of the poor food supply which the aborted seed affords, and by flowering time are fully normal in stature, and yield practically as much grain as plants which grow from well filled, mature seed. The homozygous defectives, on the other hand, remain very weak and spindling throughout the season, produce only a small amount of pollen, and rarely any ears. At least one pure defective ear has been obtained, however, from each of the four stocks mentioned above, during the past five seasons. Figure 61 shows a normal and pure defective ear from the de_3 stock.

TABLE 6. The Percentage of Germination in Defective Seeds of Fourteen Stocks Compared to the Theoretical Germination of Normal Seeds of the Same Relative Development.

Stock	Defectives	Theoretical Normal
de_1	45.6	91.0
de_2	44.9	94.0
de_3	54.0	66.5
de_4	3.5	75.5
de_5	11.2	47.5
de_6	11.8	72.5
de_7	19.6	49.0
de_8	0	42.0
de_9	0	17.0
de_{10}	0	39.0
de_{11}	0.8	22.0
de_{12}	0	10.0
de_{13}	0	12.5
de_{14}	0	3.5

The fact that the germination of the defective seeds is considerably lower than that of immature normal seeds of the same relative development, that the seedlings of all types are extremely weak, and that even the most vigorous ones which are able to survive make a very feeble growth throughout the season, indicates that these lethal and semi-lethal factors do more than merely arrest the seeds at a certain stage; apparently these factors in a homozygous condition have a deleterious influence on the sporophyte at any stage in which they are given an opportunity for expression. In most of the types the deleterious influence is so

marked that the career of the new sporophyte is brought to an end in the seed stage and the lethal factors have no opportunity for further damage past this period.

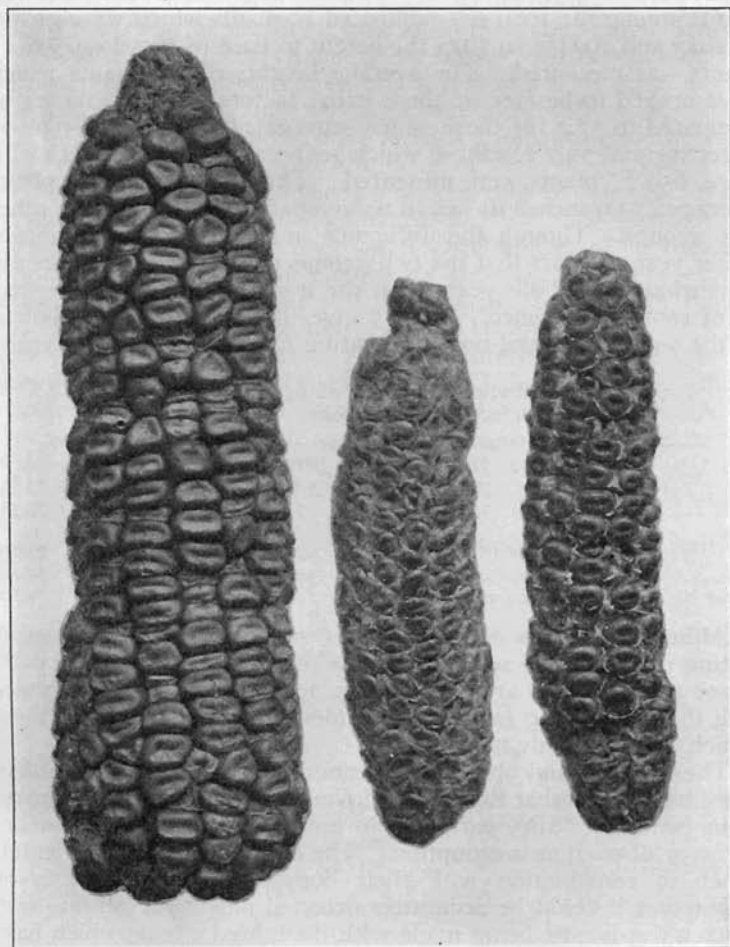


FIG. 61.—Left; ear segregating *des*. Center; self-pollinated ear from a plant homozygous for *des*. Right; open-pollinated ear from homozygous plant.

THE INFLUENCE OF LETHAL FACTORS IN HETEROZYGOUS CONDITION

Since the effect of the lethals is so marked when they are in homozygous condition, it might be questioned whether they have

not a similar unfavorable influence, though in a smaller degree, in the heterozygous condition.

The only evidence bearing on this question is that secured from a comparison of the height of segregating and non-segregating plants among the 1089 self-pollinated F_1 plants which were grown in 1923 and 1924. In 1923 the height to base of tassel on 398 F_1 plants was measured. The average heights of the plants which later proved to be free of these lethal factors was 77.9 inches as compared to 77.2 for those which segregated for a single type of defective and 73.7 for those which segregated for two types. In 1924, 659 F_1 plants were measured. The non-segregating plants averaged 73.9 inches in height as compared to 71.9 for the other two groups. Though the difference in height is not significant either year, the fact that the two groups of segregating plants are somewhat lower both years than the non-segregating plants may be of some significance. Table 7 gives the frequency distribution of the segregating and non-segregating plants for these two years.

TABLE 7. Frequency Distribution in Height of F_1 Plants from Crosses of Defective Seed Stocks.

Type of Plant	Mid-Class Values in Inches												Total	Mean	
	42	47	52	57	62	67	72	77	82	87	92	97			102
Segregating 3:1	3	7	9	22	51	72	86	117	98	52	12	2	..	531	73.85 ± .29
Segregating 9:7	1	6	9	15	23	20	17	9	100	73.30 ± .57
Total segregating plants	3	7	10	28	60	87	109	137	115	61	12	2	..	631	73.80 ± .36
Not segregating	..	5	8	21	29	45	78	89	69	54	15	2	1	416	74.90 ± .33
Difference in favor of non-segregating plants															1.10 ± .49

Although no data on the yield of segregating and non-segregating plants can be secured because the ears are artificially pollinated and full ears are seldom obtained, it has often been noted that the segregating ears have a tendency to be smaller than those which are uniformly normal.

These results and observations cannot be regarded as more than mere indications that the lethals do have an effect in the heterozygous condition; they certainly do not afford conclusive evidence in favor of such an assumption. The effect, if any, of the lethals when in combination with their dominant allelomorphs is so slight that it could be accurately detected only by a delicate test. Such a test is now being made with the inbred strains which have mutated to defective seeds, and which are presumably homozygous for all factors with the exception of a single pair involving the defective factor and its dominant allelomorph.

EFFECT UPON THE GAMETOPHYTE

The gametophyte generation is ordinarily assumed to be independent of the influences of the genetic factors distributed on the chromosomes which it carries. Recent evidence (Jones, 1924)

(Mangelsdorf and Jones, 1926) indicates, however, that there are exceptions to the rule and that in some cases, the gametophytes from the same plant are not all alike in their ability to reach the micropyle and accomplish fertilization. This brings up the question of the effect of the lethal factors on the gametophyte generation. Since these factors have such a marked deleterious influence on the sporophyte at all stages, might they not have some degree of expression in the gametophyte generation as well?

It has already been noted that there is a deficiency of recessive seeds on segregating ears of a number of these types and such a condition might be explained by slower rate of growth of the pollen tubes carrying the lethal factors.

In order to answer this question, a large number of segregating ears of the de_3 stock which regularly shows a deficiency of the recessives were divided arbitrarily into top and bottom halves and the normal and defective seeds in each half were counted separately. If there were a constant difference in the rate of growth between pollen tubes carrying the lethal factors and those carrying the dominant allelomorphs, then the greater difference which the pollen tubes were forced to travel in reaching the ovules at the base of the ears would act against those carrying the lethal factors and cause a greater deficiency of the defective seeds in the lower halves of the ears.

The results of making counts in the upper and lower half of fifteen ears segregating for de_3 are shown in Table 8.

TABLE 8. Normal and Defective Seeds on Top and Bottom Halves of Ears Segregating de_3 .

Ear No.	Top Half		Bottom Half		Percent Defective	
	Normal	Defective	Normal	Defective	Top	Bottom
10	165	60	204	50	26.7	19.7
29	146	48	158	48	24.7	23.3
30	154	44	145	39	22.2	21.2
33	89	24	78	36	21.2	31.6
45	190	57	207	54	23.1	20.7
46	210	63	200	65	23.1	24.5
51	156	44	178	36	22.0	16.8
55	155	45	185	55	22.5	22.9
56	138	40	172	60	22.5	25.9
58	145	56	171	50	27.9	22.6
120	119	32	144	48	21.2	25.0
135	119	32	79	31	21.2	28.2
139	145	47	96	31	24.5	24.4
148	125	41	145	42	24.7	22.5
158	115	46	112	41	28.6	26.8
Total	2171	679	2274	686	23.8	23.2
Ex 3:1	2137.5	712.5	2220	740		
Deviations	33.5		46			
P. E.	15.6		15.9			
Dev./P. E.	2.2		2.9			

Although the percentage of recessives in the bottom halves of these ears is lower than that in the top halves, the difference is not significant. When the ears are examined individually it is noted that the percentage of recessives is lower in the top half almost as frequently as in the bottom half.

From these results it may be concluded that the lethal factors have very little, if any, effect upon the rate of pollen tube growth. Even very slight differences in the ability of the pollen tubes to reach the micropyle would cause marked distortions in the ratios and should result in different proportions of defective seeds in the upper and lower halves of the inflorescences.

LINKAGE RELATIONS

Although no special study of the linkage relations of these characters has yet been made, it was to be expected that some indications of linkage would be encountered as by-products of the other investigations. This proved to be the case.

The first linkage to appear was that between de_2 and a factor for albino seedlings. This linkage has been briefly mentioned in a previous paper. (Mangelsdorf, 1922.)

TABLE 9. Seedlings from Normal and Defective Seeds Showing Linkage Between de_2 and w .

Ear No.	Normal		Defective	
	Green	White	Green	White
2	126	15	30	20
4	158	19	23	19
5	79	19	0	19
7	130	20	1	39
8	119	1	28	32
Total	612	74	82	129

As already noted, the de_2 character is peculiar in that it is greatly modified in certain crosses. In a cross between the de_1 and de_2 stocks the recessive seeds of the latter reappeared, so altered in appearance, and so well developed, that it was considered feasible to plant a row of them in the field in order to secure a stock homozygous for this factor. A week later when the seedlings had emerged, this row was easily the most conspicuous one in the field. With one exception all of the seedlings were albinos, and this solid row of pure white seedlings in striking contrast to the normal green plants on either side furnished a most striking demonstration of linkage.

The remaining ears of this cross were tested in the greenhouse and the results are given in Table 9. Figure 62 shows the seedlings grown from the normal and defective seeds from one of these ears.

The amount of crossing over between de_2 and w is 18 per cent as determined from the normal seeds and 21.5 per cent as determined from the recessive seeds.

These values are believed to be somewhat high because of possible inaccuracies in the classification of normal and defective seeds on these ears. A large number of F_3 progenies were therefore grown. In these the development of the defective seed was reduced, and the segregation so well defined that, it is believed,

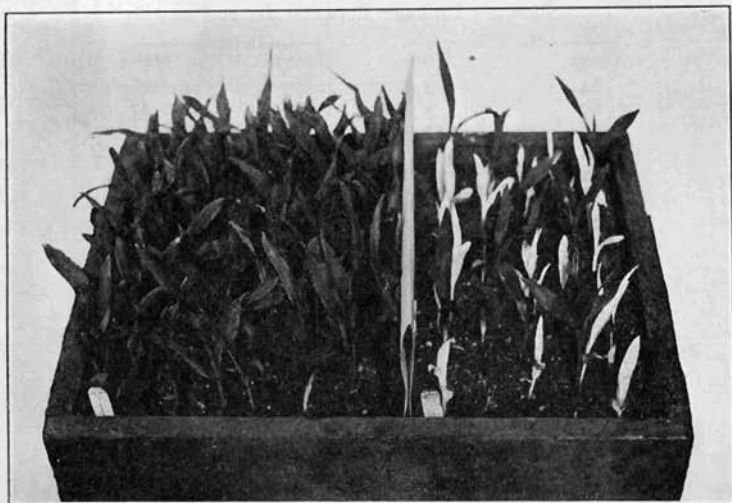


FIG. 62.—Seedlings from normal and defective seeds of de_2 showing linkage between defective seeds and a factor for white seedlings.

a fairly accurate separation was made. The results of planting the normal and defective seeds from 17 F_3 ears are given in Table 10.

The amount of crossing over as determined from the normal seeds is 11.7; from the defective seeds, 10.5.

As nearly as can be determined from the records the de_2 stock is the same one in which the w_2 factor for white seedlings was found. (Lindstrom, 1924.) The w_2 factor is known to belong to the second linkage group in maize and it is probable, therefore, that the de_2 factor is also a member of this group although further tests are necessary to substantiate such an assumption. Some additional evidence for it exists in the fact that Lindstrom (1923) has also found a case of close linkage between w_2 and a defective seed which answers the description of the de_2 type.

LINKAGE BETWEEN *Su* AND *de* FACTORS

The relation between the factor for sugary endosperm, a representative of the third linkage group, and the fourteen factors for defective seeds, may be determined from the crosses between *de*₇ and the thirteen remaining types. The *de*₇ stock, originally starchy, had been changed over to sugary before any crosses were made.

TABLE 10. Seedlings from Normal and Defective Seeds of F₂ Progenies Showing Linkage Between *de*₂ and *w*.

Normal		Defective	
Green	White	Green	White
84	3	5	28
89	5	16	22
79	17	0	12
83	9	2	32
91	1	27	32
54	7	1	16
58	2	1	2
42	0	0	6
8	3	0	2
63	3	1	25
21	2	3	2
84	8	1	26
81	12	12	33
51	0	0	4
74	9	1	11
68	4	0	15
59	2	2	15
1089	87	72	283

Since the defective seeds, with the exception of two or three types, cannot be accurately classified with regard to their endosperm texture, their linkage relations with sugary must be determined from the normal seeds alone. Linkage of sugary endosperm with any of the lethal factors would be indicated by a distortion of the normal 3 starchy: 1 sugary ratio. An excess of sugary seeds is expected when the sugary and defective seed factors enter the cross from opposite parents and the recessive factor of one is linked with the dominant allelomorph of the other. With complete linkage between *Su* and *de*, 33 $\frac{1}{3}$ per cent sugary seeds in the normal class are expected. Thus the range in crossing over from 50 per cent to 0, corresponds to a range in percentage of sugary seeds of 25 to 33 $\frac{1}{3}$. This is shown diagrammatically in Figure 63.

F₂ progenies of crosses between sugary endosperm and all of the defective seed types have been grown. The results of separating and counting the starchy and sugary seeds in the normal class, on ears from all of these crosses, are shown in Table 11.

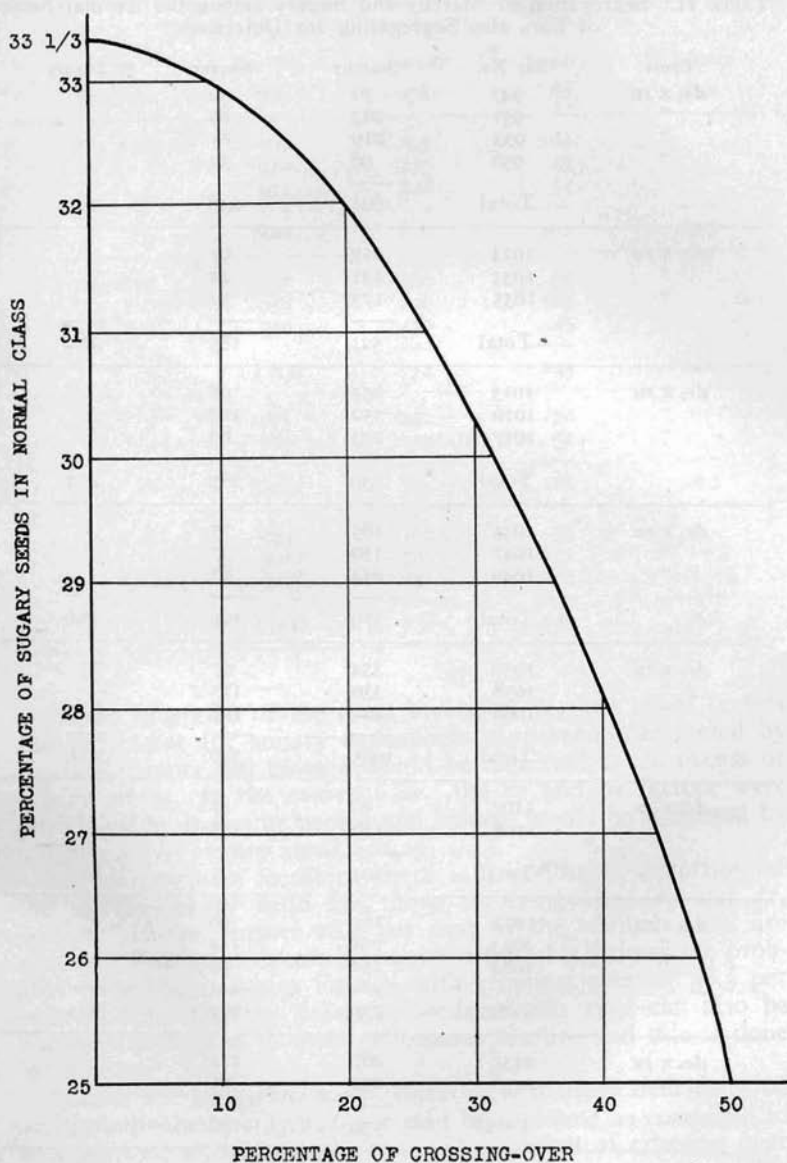


FIG. 63.—Diagram showing the theoretical distortion of the starch:sugary ratio among the normal seeds when a defective seed factor is linked with the normal allelomorph of sugary. With complete linkage $33\frac{1}{3}$ per cent sugary seeds are expected in the normal class.

TABLE II. Segregation of Starchy and Sugary among the Normal Seeds of Ears also Segregating for Defectives.

Cross	Ear No.	Starchy	Sugary	% Sugary
<i>de</i> ₁ × <i>su</i>	947	71	36	
"	951	213	89	
"	953	219	81	
"	956	98	32	
	Total	601	238	28.4
<i>de</i> ₂ × <i>su</i>	1024	217	91	
"	1034	131	42	
"	1035	173	52	
	Total	521	185	26.2
<i>de</i> ₃ × <i>su</i>	1013	255	98	
"	1016	352	100	
"	1017	223	89	
	Total	830	287	25.7
<i>de</i> ₄ × <i>su</i>	1045	185	75	
"	1047	150	37	
"	1049	244	87	
	Total	579	199	25.6
<i>de</i> ₅ × <i>su</i>	1056	334	97	
"	1058	330	115	
"	1068	421	128	
	Total	1085	340	23.0
<i>de</i> ₆ × <i>su</i>	1107	66	37	
"	1108	138	55	
	Total	204	92	31.1
<i>de</i> ₇ × <i>su</i>	1072	187	58	
"	1073	291	114	
"	1084	252	90	
	Total	730	262	26.4
<i>de</i> ₈ × <i>su</i>	2432	287	111	
"	2433	252	75	
	Total	539	186	25.7
<i>de</i> ₉ × <i>su</i>	1096	200	62	
"	1098	177	50	
"	1102	242	72	
	Total	619	184	22.9

TABLE II (cont'd). Segregation of Starchy and Sugary among the Normal Seeds of Ears also Segregating for Defectives.

Cross	Ear No.	Starchy	Sugary	% Sugary
<i>de</i> ₁₀ x <i>su</i>	996	278	88	24.0
<i>de</i> ₁₁ x <i>su</i>	969	295	104	
"	970	137	43	
"	971	228	54	
	Total	660	201	23.3
<i>de</i> ₁₂ x <i>su</i>	937	306	95	
"	938	298	105	
"	940	170	45	
	Total	774	245	24.0
<i>de</i> ₁₃ x <i>su</i>	1075	222	70	
"	1079	135	51	
	Total	357	121	25.3
<i>de</i> ₁₄ x <i>su</i>	981	162	55	
"	988	345	111	
"	992	231	83	
	Total	738	249	25.2

With the exception of the cross involving *de*₇, the lethal factors and the factor for sugary endosperm have been contributed by opposite parents and linkage would be indicated by an excess of sugary seeds. In the cross of *de*₇, the *de* and *su* factors were introduced by the same parent and linkage would be indicated by a deficiency of sugary seeds.

The only crosses in which there is a noticeable distortion of the starchy-sugary ratio are those involving the *de*₁ and *de*₈ factors. In the former 28.4 per cent of the normal seeds are sugary. This is an excess of sugary seeds of 3.4 times the probable error and indicates linkage with crossing over of 38.5 per cent. Fortunately the defective seeds of this type can also be readily classified as to their endosperm texture and this is done in Table 12.

Among the defective seeds there is a marked deficiency of sugary individuals, only 20.6 per cent being found as compared to 28.4 per cent in the normal class. The amount of crossing over as determined from the defective seeds is 39 per cent. This agrees very closely with the percentage as determined from the normal seeds. The evidence is fairly good, therefore, that the *de*₁ and *su* factors are linked.

TABLE 12. Segregation of Starchy and Sugary among the Defective Seeds from Ears of a Cross $de_1 \times su$.

Ear No.	Starchy	Sugary	% Sugary
947	42	10	19.2
951	75	27	26.5
953	85	18	17.5
956	45	9	16.7
Total	247	64	20.9
Ex. 3:1	233	78	25.0
Deviation	14	-14	4.4
Probable Error:	5.15		

In the cross of $de_6 \times su$, 31.1 per cent of the normal seeds were sugary. This is an excess of 3.6 times the probable error and indicates linkage with crossing over of 26 per cent. Unfortunately the defectives on these ears could not be classified with regard to their endosperm texture and it is not so certain that linkage between de_6 and su exists, although it is strongly indicated. An excess of sugary seeds of 3.6 times the probable error would be expected as a chance deviation only once in about 65 trials.

The cross between the de_1 and de_6 factors shows independent inheritance of these two characters as is shown in Table 13. This would be expected even though both are linked with the su factor providing that the loci of the two lethals were on opposite sides of the su locus.

TABLE 13. Segregation in F_2 of a Cross between de_1 and de_6 Showing Independent Inheritance.

Ear No.	Normal	Defective
166	124	103
168	117	83
175	161	142
178	195	131
Total	597	459
Ex. 9:7	594	462
Deviation	3	-3

LINKAGE OF DEFECTIVES WITH EACH OTHER

In most varieties of maize there are ten pairs of chromosomes. (Kuwada 1915, Kiesselbach and Petersen 1925.) Therefore, in crossing thirteen different factors in all combinations some cases of linkage are almost certain to occur. The difficulty lies in their detection. With independent inheritance two defectives when crossed give a 9:7 ratio in F_2 . With complete linkage these two defectives should give a 1:1 ratio. Thus the entire range of cross-

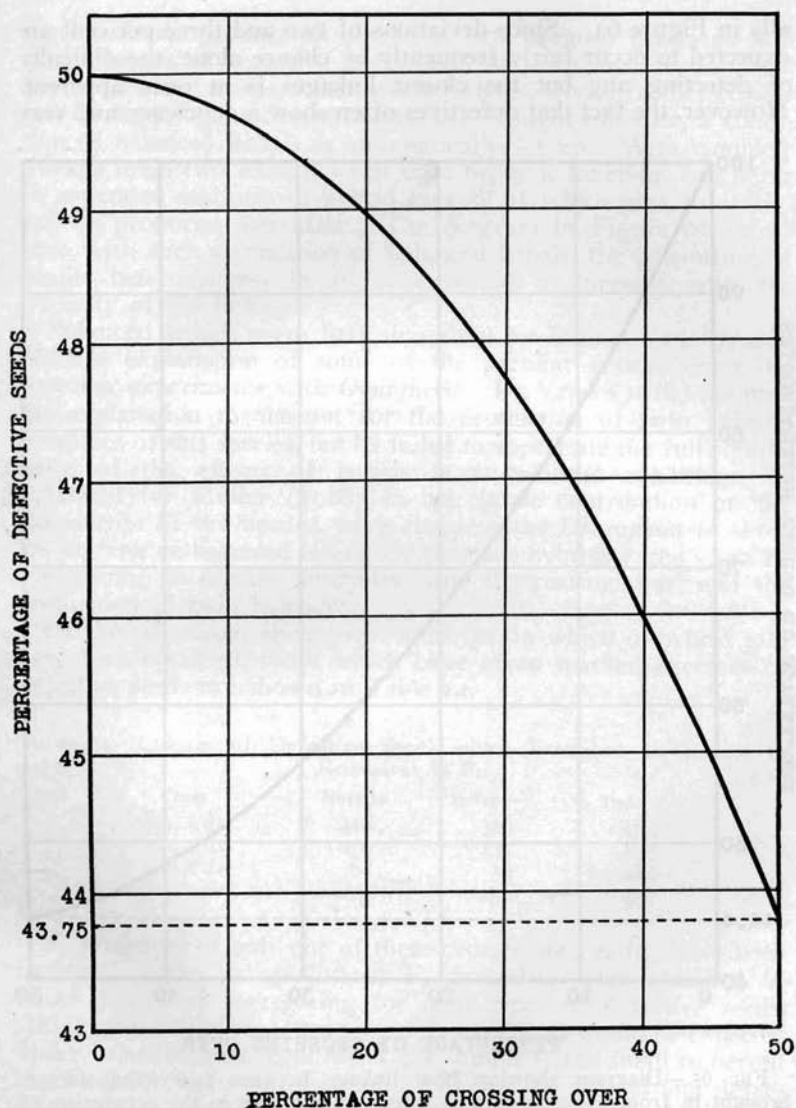


FIG. 64.—Diagram showing how linkage between two defective seed factors would distort the normal 9:7 di-hybrid ratio. If each defective is completely linked with the dominant allelomorph of the other, a 1:1 ratio is expected.

ing over from 50 per cent to 0 corresponds to a range of 43.75 to 50 in the percentage of defectives. This is shown diagrammatic-

ally in Figure 64. Since deviations of two and three per cent are expected to occur fairly frequently by chance alone, the difficulty of detecting any but the closest linkages is at once apparent. However, the fact that defectives often show a deficiency and very

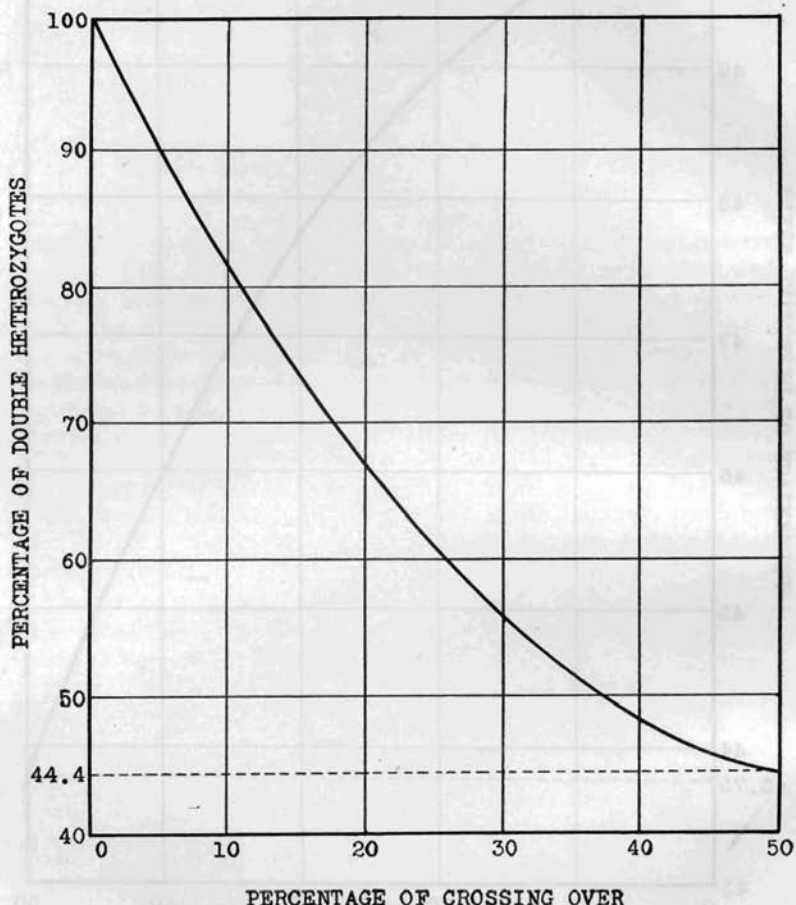


FIG. 65.—Diagram showing how linkage between two lethal factors brought in from opposite parents causes an increase in the proportion of heterozygotes.

rarely an excess, makes it necessary to regard any di-hybrid ears which produce more than 43.75 per cent defectives as possible cases of linkage. Further evidence must then be obtained by growing additional di-hybrid ears or by examining F_3 progenies. If the high percentage of defectives in F_2 is due to linkage, then

the F_3 should produce an excess of heterozygous plants as well as an excess of recessive seeds on a majority of the di-hybrid ears. In other words, when two defectives, whose factors occupy loci on homologous chromosomes, are brought together, a condition of balanced lethals is automatically set up. With complete linkage these two lethals, when once brought together, can never be separated and only di-hybrid ears all of which give 1:1 ratios will be produced thereafter. The diagram in Figure 65 shows how, with such a condition of balanced lethals, the proportion of double heterozygotes in F_3 is expected to increase with the intensity of the linkage.

Balanced lethals were first suggested by Renner (1916) as a possible explanation of some of the peculiar results from the breeding experiments with *Oenothera*. De Vries (1916) adopted the explanation to account for the production of twin hybrids in crosses of this species, but he failed to appreciate the full significance of the effects of lethals in a balanced condition. It remained for Muller (1918) in his classic contribution on the inheritance of the beaded wing character in *Drosophila* to show the bearing of balanced lethals on constant hybridity, the sporadic appearance of certain "mutants" due to crossing-over, and the production of twin hybrids.

Of the 59 crosses shown in Figure 54, in which di-hybrid ears have been obtained, those which have given marked excesses of defective seeds are shown in Table 14.

TABLE 14. Crosses of Defective Seeds which Produced an Excess of Recessives in F_2 .

Cross	Normal	Defective	% Defective
$de_{10} \times de_3$	418	376	47.4
$de_6 \times de_5$	270	235	46.5
$de_{10} \times de_6$	64	59	48.0
$de_{11} \times de_{11}$	275	251	47.7

F_3 progenies of only one of these crosses, $de_{10} \times de_3$, have been grown. Twelve self-pollinated F_3 progenies were obtained of which five were segregating for both types of defective seeds. Although the di-hybrid ears are not in excess, as would be expected under balanced lethal conditions, the sample is too small to permit any final conclusion on this point. When the normal and defective seeds on these five di-hybrid ears are counted, it is found that there is again an excess of the recessive seeds as is shown in Table 15.

When these five progenies are combined with the two F_2 progenies already shown, making a total of 1060 normal to 916 defectives, the deviation is 52 ± 13 . The average percentage of defective seeds is found to be 46.3, which indicates linkage with crossing over of 38.5 per cent.

TABLE 15. Normal and Defective Seeds from F_3 Progenies of a Cross of $de_{10} \times de_3$ Indicating Linkage between These Factors.

Ear No.	Normal	Defective	% Defective
858	112	99	46.9
859	130	100	43.5
862	132	112	45.9
867	125	105	45.7
868	143	124	46.4
Total	642	540	45.7
Expected	665	517	43.7
Deviation	-23		
P. E. = 11.5			

It should be mentioned, that with a condition of balanced lethals, occasional progenies are expected in F_3 in which there is a deficiency of recessives instead of an excess. This condition would be brought about through crossing over so that the two lethal factors, originally on homologous chromosomes, are now borne on the same chromosome. Thus unless the linkage between the two lethals were fairly close, so that the excesses and deficiencies within each progeny were sufficient to permit a separation, the two types of F_3 progenies would tend to balance each other and linkage would be almost impossible to detect.

LINKAGE OF DEFECTIVES WITH GROWTH FACTORS

The method of improving corn by selection in self-fertilized lines aims at the removal of all recessive abnormalities such as white seedlings and defective seeds. There seems to be a general belief that these factors have a deleterious effect, even in the heterozygous condition.

Lindstrom (1920) suggests that these recessive abnormalities, if they do have an unfavorable effect in the heterozygous condition, are permitted to persist in the germplasm only when they are linked with particularly good growth factors, and that in removing them by inbreeding, some of the best germplasm is lost. Jones and Mangelsdorf (1925) have shown, however, that inbred strains from which all recessive abnormalities have been eliminated, yield fully as well as sister strains which still carry one or more of these abnormal characters. Apparently nothing of value was lost through their elimination; neither was there any marked improvement when their supposedly unfavorable influence was removed. Still assuming that these factors have an influence in the heterozygous state, a probable explanation of these conflicting results is that the defective seeds and other lethal abnormalities are permitted to persist and accumulate, not because they are linked with especially good factors for development, as Lindstrom has suggested, but because their presence tends to keep short sections of the chromosomes which they occupy in a continued state of hetero-

zygosity. The increased vigor which results from such enforced heterozygosity of the accompanying growth factors enables the recessive abnormalities to survive in the germplasm even though they have an unfavorable influence in themselves.

Furthermore, when two such lethal factors which occupy homologous chromosomes are brought together, a condition of balanced lethals is set up which may so increase the vigor of the stock by keeping whole chromosomes or large sections of chromosomes in a continued state of heterozygosity, that the lethals are actually given an advantage and are able to survive even though they are linked with especially poor growth factors instead of particularly good ones.

Shull (1923) has pointed out that varieties of *Oenothera* which carry lethal factors are, in general, more vigorous than those which lack these characters. The mechanism of crossing over in *Oenothera* appears to be different from that in most species as is shown by both cytological and genetic studies; Shull (1923), Cleland (1925). All of the characters so far studied in this species fall into a single linkage group and the amount of crossing over between the members of the group is relatively low. It is possible, therefore, that lethal factors in *Oenothera* keep all of the chromosomes, with their hereditary factors for growth and development, in a continued state of enforced heterozygosity. If such is the case, then the increased vigor brought about in *Oenothera* by the presence of lethal factors is probably more marked than would occur in other species where there are as many linkage groups as chromosomes.

A PLANT CHARACTER FOR DEFECTIVE SEEDS

In addition to the thirteen endosperm characters which cause one-fourth of the seeds on segregating ears to be defective, a plant character which causes defectiveness in all the seeds on one-fourth of the plants has been found.

This character appeared in the de_{13} stock which was received from Mr. H. A. Wallace of Des Moines, Iowa. Mr. Wallace had found among the plants of the variety "Illinois Two Stalk" several which produced only aborted seeds, and which appeared to be homozygous for defective seeds. Pollen from one of these plants applied to a hybrid of inbred strains known to be free of hereditary defectives produced only normal seeds. The F_1 plants grown from these normal seeds were selfed and produced some ears which were segregating for defective seeds of the type which has already been described as de_{13} . Not all of the ears were segregating, however, as should have been the case, had one of the parents been homozygous for the defective factor. Nor did the recessive seeds on the segregating F_1 ears resemble the

aborted seeds of the pollen parent. The extracted recessives on the F_1 ears were completely aborted, appeared to have no endosperm tissue and showed no germination whatever. It was difficult to understand how this type could have been obtained in a homozygous condition, or why, if the pollen parent was homozygous for defective seeds, only part of the F_1 ears were segregating for the character.

This confusing situation was cleared up, however, when a large number of F_2 ears, which had been grown for another purpose, were harvested. A total of 201 F_2 ears were examined and of these 51, or almost exactly one-fourth, bore only aborted seeds and were identical in appearance to the ears of the grandparental pollen parent. The other 150 ears were normal in appearance although some were segregating for defective seeds and others were not.

Apparently the plant of "Illinois Two Stalk" which served as the pollen parent for this cross, in addition to being heterozygous for a recessive endosperm character de_{13} , was homozygous for a recessive plant character, to which the symbol de_{pl} may be given. On this hypothesis the genetic composition of the parental stocks and the F_1 seeds is as follows:

Pollen parent	De_{13}	de_{13}	de_{pl}	de_{pl}
Seed parent	De_{13}	De_{13}	De_{pl}	De_{pl}
F_1 Seeds	{	De_{13}	De_{13}	De_{pl}
		de_{13}	de_{13}	de_{pl}

Half of the F_1 seeds when selfed should produce ears segregating for the endosperm character de_{13} . The other half should give only normal seeds. Apparently these conditions have been met; of the five F_1 ears which had been selfed 3 were segregating for defective seeds and two were not.

That the new variation de_{pl} is actually a plant character is further demonstrated by the fact that the recessive plants produce only defective seeds regardless of the pollen which they receive. If it were an endosperm character cross pollination with normal plants should give only normal seeds.

The seeds on the defective ears show considerable variation in development, some being almost completely defective, others only partially so. None, however, are as fully developed as normal seeds, though many are capable of germination and when grown produce fairly vigorous plants which in turn bear only defective ears. Defective ears from such homozygous plants are shown in Figure 66.

There seems to be no relation between the defective seed type which appeared in the F_2 endosperm generation and the defective ears which came to light in the F_2 plant generation. The fact that both came out of the same cross is regarded merely as a

coincidence. The two have now been separated and stocks segregating for one and lacking the other have been obtained.

This variation is of interest in connection with the defective seeds because it produces practically the same effect in the individual seeds as do some of the endosperm characters.

The character of the endosperm is usually determined by the genetic constitution of the zygote* which results from the fusion

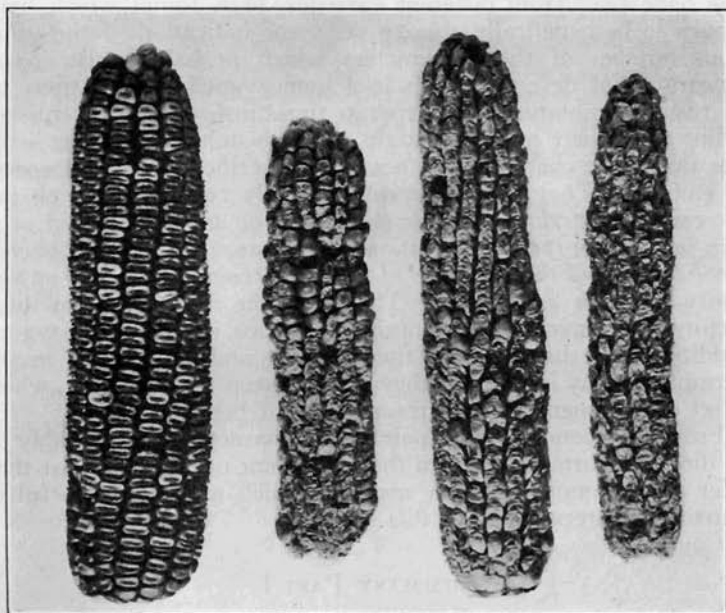


FIG. 66.—Normal and defective ears of the *dep1* stock. This factor causes all of the seeds on one-fourth of the plants to be defective regardless of the genetic constitution of the endosperm.

of the endosperm nuclei with a sperm nucleus. The defective ear, however, represents a condition in which the expression of the hereditary factors of the new sporophyte is prevented. Apparently there is present or lacking, in the recessive plants, something which causes all of their seeds to be aborted regardless of the genetic constitution of the seeds themselves. Such a situation might be compared to a population of plants in which all the plants were dwarfed because of a lack of moisture or fertility,

* To be strictly accurate, perhaps the term "zygote" ought not to be applied to the endosperm, but its use in this connection is probably less confusing than would be the adoption of a new term.

while in a better environment some of the plants would be tall and others dwarfed, because of the hereditary factors which they carried.

DISCUSSION

The fact that defective seeds have been noted in almost every variety of maize which has been examined and that only in one case have two, from different varieties, been found which have proven to be genetically alike, gives some indication of the enormous number of these characters which probably exist. The appearance of defective seeds in a homozygous inbred strain, as the result of mutation, four separate times in four years, furnishes a hint as to their probable origin. The histological studies indicate that these characters do not affect specifically the endosperm or embryo. *They are, apparently, merely variations which are extremely deleterious in their influence and their major effect is seen in the endosperm generation only because the period between fertilization and the maturity of the seed provides the first opportunity for their expression.* There is some indication that these factors also have an unfavorable influence in the heterozygous condition and the fact that they survive and accumulate in the germplasm may imply that they tend to keep other factors, which affect development, in a continued state of heterozygosity.

From the economic standpoint these characters are probably of no direct importance. From the standpoint of the geneticist they offer a vast amount of new material which may prove useful in charting the germplasm of this species.

SUMMARY PART I

1. Defective seeds are lethal or semi-lethal characters which affect the normal development of the seed.
2. These characters have been noted in many varieties of maize and it is estimated that one plant in every thirty, on the average, is heterozygous for defective seeds.
3. Defective seeds have been the most frequent variations to arise by mutation in homozygous inbred strains of maize.
4. Fourteen stocks segregating for defective seeds have been crossed in 82 combinations. Only two of these which are genetically alike have been found.
5. Histological studies show that normal fertilization apparently occurs in the formation of these seeds, and that both endosperm and embryo are produced. These structures develop very slowly, however, and remain rudimentary. Both are affected to almost the same degree by the influence of the lethal factors.
6. The relative development of the fourteen stocks of defective seeds as compared to normal seeds on the same ears ranges

from 2.4 per cent to 59 per cent, based upon the dry weight of the seeds.

7. The following indications of linkage have been encountered:

The de_2 factor with a factor for white seedlings, probably the w_2 factor, which is a representative of the second linkage group. Crossing over is about 11 per cent.

The de_1 and de_6 factors with the factor for sugary endosperm which is a representative of the third linkage group. Crossing over in the first case is 39 per cent, in the second 26 per cent.

Linkage between two different defectives is indicated in four crosses.

8. In addition to the 13 endosperm characters which cause one-fourth of the seeds to be defective, a plant character which causes all of the seeds on one-fourth of the plants to be defective, has been found.

PART II

NON-HEREDITARY DEFECTIVE SEEDS

Almost every ear of maize bears some aborted seeds. On most ears only a few of these are found scattered at random throughout the inflorescence; on others, entire regions of the ear produce only defective kernels. These undeveloped seeds have commonly been attributed to "imperfect pollination" though it has never been clear just what conditions were included by this term. It is now evident that many of these abortive seeds are lethal or semi-lethal characters, inherited as simple Mendelian recessives, but it must also be recognized that there are, in addition to the hereditary characters, various other types of defective seeds which do not appear to have an hereditary basis or at any rate are not inherited as recessive endosperm characters.

These non-hereditary types are formed regardless of whether the ear is self-pollinated or cross-pollinated. They are found in all varieties and occur as frequently in homozygous inbred strains as in ordinary heterozygous varieties. In appearance they are practically identical to some of the hereditary types, and in separating dominant and recessive individuals from a segregating ear, they are included with the latter. Because they sometimes represent a significant source of error in classification, it has been considered important to determine the frequency of their occurrence, the conditions causing their production and, if possible, some means of distinguishing them from the Mendelian characters which they resemble so closely.

A microscopic examination of sectioned material from various sources showed that there are at least four morphologically distinct types of non-hereditary defective seeds and that these are probably produced as the results of the following conditions:

1. Stimulus resulting from pollination without fertilization. (Parthenocarpy.)
2. Arrested development due to competition, dominance or other physiological conditions.
3. Irregularities in the mechanism of fertilization.

PARTHENO-CARPIC DEFECTIVES

The first of these four types was found on an ear which had been bagged as a pollination check. After a period of several weeks, the silks grew to such a length that some of them protruded from below the bag and became exposed to pollen. As a result, a few of the ovules on this ear developed slightly. These were not normal in appearance and contained, instead of the usual milky fluid, only a clear watery liquid and a jellylike tissue.

Microscopic examination of sections of these ovules showed that their development was due entirely to a marked growth of nucellus. The embryo sac was readily recognized, though it had begun to disintegrate at the apical end and the antipodals had moved into the nucellar tissue. Within the sac was found some disintegrated granular material and, in several specimens, the egg and polar nuclei were still visible. No indications of fertilization were found in any of the sections and no remains of the pollen tube could be distinguished, although these might have been present and not have been clearly brought out by the stain used. In no case was there the slightest trace of endosperm or embryo. The nucellus in maize is very distinct from the endosperm and there is little danger of confusing these two tissues. In none of the specimens examined did the nucellus show any indication of starch grain formation. A photomicrograph of one of these ovules is shown in Fig. 4, Plate XXVI.

The natural conclusion is that no fertilization occurred in these partially developed ovules. Either the pollen tubes failed to reach the micropyle, or if they reached it, failed to accomplish fertilization. Apparently, however, the tubes in growing down the styles had in some way transmitted a stimulus to the ovule which resulted in a marked growth of the nucellus and pericarp, even though fertilization did not occur.

Though the nucellus of maize is of minor importance in the mature caryopsis, becoming compressed into a thin integument by the pressure of the growing endosperm, yet these partially developed, though unfertilized, ovules are comparable in certain respects to some of the seedless fruits which are produced in the absence of pollination, and to which the term "parthenocarpic" has been applied.

The term "parthenocarpic" was first used by Noll (1902) to describe the situation in which certain plants, under exclusion of pollen, are able to form fruits outwardly normal, or nearly so, but in which the seeds are absent or aborted. Noll reported this condition in the cucumber and Ewert (1909, 1910) has noted it in other fruits.

Winkler (1908) makes a distinction between "stimulative" parthenocarpic in which the seedless fruits are produced only after pollination with their own or foreign pollen or in consequence of an insect prick or other irritation, and "vegetative" parthenocarpic in which the seedless fruits occur without the action of pollen or other stimuli.

Wellington (1913) found that in *Nicotiana* species, capsules were caused to swell by merely tickling them with a sharp-pointed instrument. Abortive seeds, probably without embryos, were produced by singeing the buds with a hot wire, by exposure of the plants to chloroform gas, and by cutting away a portion of the

pistil and pollinating the stub, or by grafting the stigmatic end of another pistil to the excised one and pollinating the new pistil.

These abortive ovules of *Nicotiana*, as well as those of maize, if they can be legitimately termed parthenocarpic, fall into the category of "stimulative" parthenocarpy since they represent a development brought about by stimuli other than fertilization.

An inflorescence of maize from which pollen has been excluded throughout the season is shown in Fig. 67. Maize never produces seeds of any description in the complete absence of pollen though it may be possible to produce with artificial stimuli, such as used by Wellington in *Nicotiana*, the same development of the ovules which results when pollen tubes enter the style without accomplishing fertilization.

Parthenocarpy is not to be confused with parthenogenesis. In the former no embryo is produced, in the latter normal seed formation may occur. Parthenogenesis occurs regularly in some plants but, so far as is known, has never been found in maize or closely related species. Parthenocarpy, on the other hand, may be widely distributed. In species such as maize, however, where the stimulated parts are of little importance, parthenocarpy is of significance only as it has a bearing on the physiology of pollination, or represents a source of error in genetic experiments.

SOME CONDITIONS WHICH INFLUENCE THE FREQUENCY OF PARTHENOCARPC DEFECTIVES IN MAIZE

Several conditions noted in the experience of making artificial pollinations in maize suggested the possibility that the frequency with which parthenocarpic defectives are produced probably depends to a large extent on the age of the silks. As already mentioned, these seeds were first noted on ears which had received no pollen until several weeks after the silks had emerged. An inbred strain of flint corn in which the husks are extremely long, so that the silks must attain a length of 50 cm. or more before emerging, regularly produces a large proportion of parthenocarpic defectives. This same strain bears normal seeds when the husks are cut back.

These conditions suggest that the silks may become of such a length that the pollen tubes are no longer capable of reaching the micropyle or that the embryo sac, after lying idle for so long a period, becomes disorganized and is no longer capable of entering into fertilization even though the styles still retain their receptivity and furnish a medium for the growth of the pollen tubes.

Parthenocarpic defectives are also frequently produced on ears which have been artificially pollinated several days sooner than the normal time. In making hand pollinations it is sometimes necessary to use ears from which the silks have not yet emerged. The husks are cut back to the tip of the spike, exposing silks which under natural conditions would not have been pollinated until

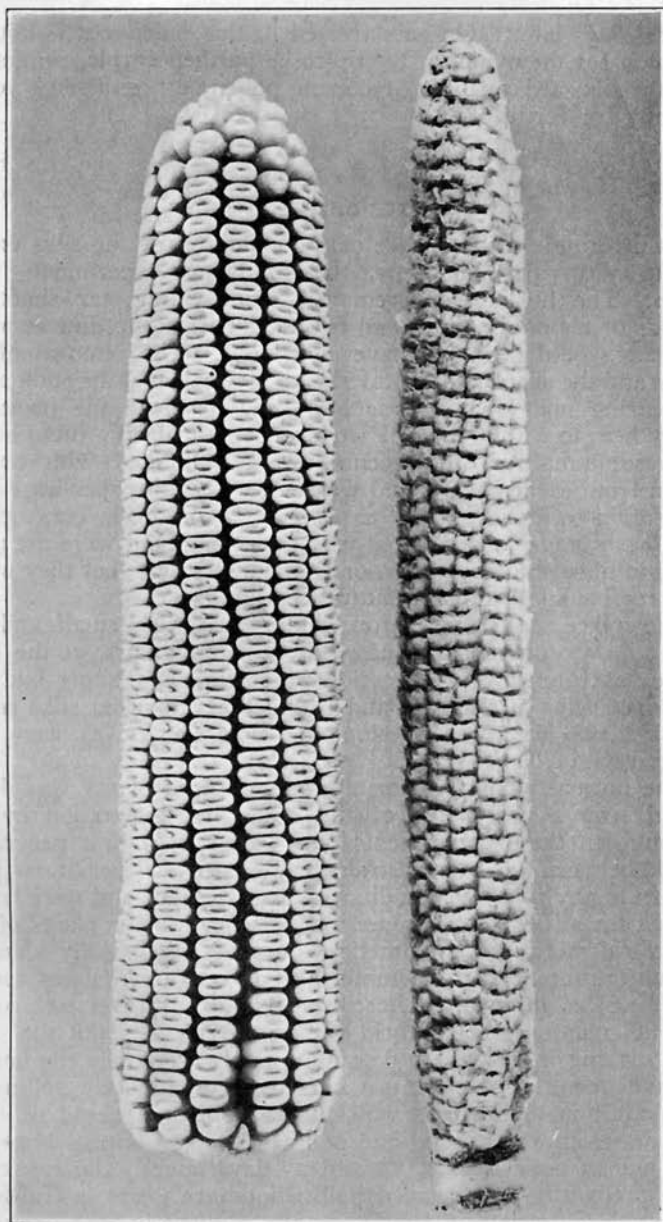


FIG. 67.—An inflorescence from which pollen has been excluded throughout the season, compared to an open-pollinated ear from the same strain. No development of any kind occurs in the complete absence of pollination.

several days later. On ears treated in this manner it is not uncommon for the ovules at the tip to be parthenocarpic, suggesting that the silks are capable of receiving pollen before the embryo sac is ready for fertilization.

INFLUENCE OF AGE OF SILKS

To determine the influence of the condition of the silks on the frequency of parthenocarpy, the following experiments were made: The husks were removed from young ear shoots at periods of about six, four, and two days before the time at which the ears would normally have silked out. The removal of the husks and the surrounding leaf sheath so weakened the stalk at the ear-bearing node that it was necessary to brace the plants by tying them to a thin strip of wood above and below this node.

These immature inflorescences were pollinated with normal pollen from earlier plants and were covered with paper bags after pollination to avoid further exposure. As a check, ears opened at an early stage were covered with paper bags but were not pollinated until several days later, or at about the time that they would have received pollen under natural conditions.

The spikes at six days before silking were very small, and had only a few rudimentary silks, without lateral hairs, at the base. Spikes at four days before flowering time had short but well developed silks at the base and rudimentary hairless silks at the tip. At two days before blooming time the spikes were well covered with silks.

The normal blooming time for all of these plants was determined from sister plants of the same first generation hybrid. Throughout these experiments only plants of a first generation hybrid between two inbred strains were used. These strains were free from any types of hereditary defective seeds and were homozygous for all visible characters. Consequently the plants of the F_1 hybrid were, for all practical purposes, genetically identical and any difference in the number of defective seeds was caused by influences other than heredity. Under uniform soil conditions all plants of this hybrid come into silk at about the same time, making it possible to determine fairly accurately the normal blooming time of the plants which were prematurely pollinated.

In addition to the ears which were pollinated ahead of time, another series was bagged and pollinated at the normal blooming time and at periods of 7, 17 and 25 days later. The results of these premature and delayed pollinations are given in Table 16. The seeds from only a single ear are counted in each case. In the pollinations made at six days before blooming time no seeds were set and in that made at 25 days after silking all the ears

TABLE 16. Influence of the Age of Silks upon the Percentage of Parthenocarpic Defectives.

Days before or after Silking	Total No. of Seeds	No. of Defectives	Percent Defective
6 days before	0	0	0
4 " "	237	85	35.9
2 " "	121	8	6.6
Normal time	783	8	1.0
7 days after	678	11	1.6
17 " "	382	83	21.7
25 " "	60	10	16.7

except one were barren. The one exception produced 60 poorly developed seeds at the tip of the spike of which 50 contained endosperm and embryo and ten were parthenocarpic.

These results indicate that the silks are apparently receptive to pollen for a period greater than that during which fertilization can occur, and that pollinations which are made very early or very late do not accomplish fertilization in many cases but succeed merely in inducing a development of the nucellus and pericarp.

INFLUENCE OF THE AGE OF POLLEN

An experiment was also made to determine whether the condition of the pollen has any influence on the proportion of parthenocarpic defectives which are produced.

This experiment was planned to test the pollen at regular intervals of six hours after collection. In order to avoid the necessity of making some of the pollinations during the night, two collections were made. The first, designated as A in the table, was made at noon, the second, B, from the same plants in the evening, about seven hours later.

The pollen was kept in a cool basement room where the temperature and humidity were relatively constant. Pollinations with the two lots were made every twelve hours on silks which were trimmed off, as nearly as possible, to a uniform length, and a supply of pollen ample to insure full ears under natural conditions was applied. Three ears were pollinated for each six hour period up to 44 hours, which, from previous experience, was considered the maximum period of viability for maize pollen.

When the ears were harvested a marked difference was noted in those resulting from the two lots of pollen. Ears produced from pollination with Lot A were completely filled with the exception of those resulting from the 44 hour pollen. Ears from the B lot were all poorly filled, the earlier pollinations showing a few missing kernels, the later ones, many. Apparently the pollen collected in the evening was injured in some way, perhaps by the higher humidity of the atmosphere at that time.

One ear from each pollination was shelled and the normal and defective kernels counted. The figures are given in Table 17.

TABLE 17. Influence of the Age of Pollen upon the Percentage of Parthenocarpic Defectives.

Hours after Collection	Total No. of Seeds	No. of Defectives	Percent Defective
Lot A			
7	586	23	3.9
19	641	19	3.0
31	718	14	2.0
44	585	46	7.9
Lot B			
0	788	47	6.0
13	554	51	9.2
24	543	57	10.5
37	161	45	28.0

It is noted that in Lot A, where there was apparently an abundance of good pollen at all periods, there is little change in the percentage, of defective seeds, accompanying the increase in age of the pollen. In the ears of Lot B, however, where the number of viable pollen grains was never enough to give completely filled ears, the percentage of parthenocarpic defectives increased progressively with the age of the pollen, beginning with 6.0 per cent at time of collection and ending with 28.0 at 37 hours.

There seems to be no question that the age of pollen has some influence on the frequency with which the parthenocarpic defectives occur. It is probable that in the older pollen many of the grains are capable of germinating but are not vigorous enough to reach the micropyle, and succeed merely in transmitting a stimulus which induces the development of the nucellus and pericarp. Where there is an abundance of pollen these weakened pollen tubes produce no effect since fertilization can be accomplished by more vigorous tubes growing in the same styles. Where the supply of viable pollen is limited, however, so that in many of the styles there is no such competition, the weakened pollen grains germinate but succeed only in producing parthenocarpic defectives.

There are, no doubt, other conditions which influence the appearance of parthenocarpic defectives. Any unfavorable weather conditions, for example, which might prevent the pollen tubes from reaching the micropyle, would probably result in the formation of parthenocarpic defectives. In artificially self-pollinated ears where an abundance of pollen is applied to ears that are fully silked out, the proportion of parthenocarpic seeds which are scattered throughout the ear remains relatively constant. In ears in which it is evident that a large number of these seeds are present

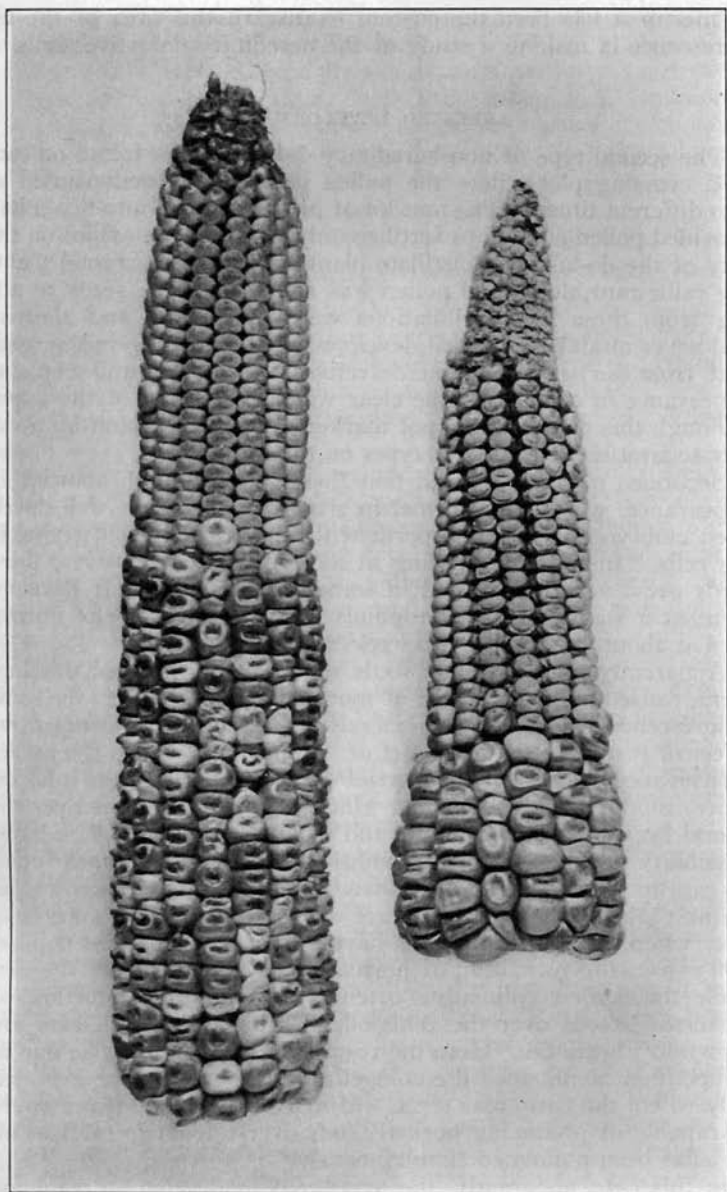


FIG. 68.—Ears which have been pollinated two separate times. The first pollination resulted in normal seeds; the second, made some days later, produced only "arrested" seeds.

at the tip it has been the custom to discard this area of the inflorescence in making a study of the hereditary defective seeds.

ARRESTED DEVELOPMENT

The second type of non-hereditary defective was found on ears in a crossing plot where the pollen parent had been planted at two different times. The first lot of plants to come into tassel had provided pollen enough to fertilize only a few of the ovules on the ears of the de-tasselled pistillate plants. When the second planting came into bloom and pollen was again shed, the seeds resulting from these later pollinations were very small and abortive and never attained a normal development. They differed, in general, from the parthenocarpic defectives, in having a milky opaque appearance in contrast to the clear watery condition of the latter, although this distinction is not marked enough to permit an accurate separation of these two types on the same ear.

Sectioned material showed that these seeds, though abortive in appearance, were quite normal in structure, having a well developed embryo and an endosperm with numerous starch grains in the cells. Instead of growing at a normal rate, however, these seeds grew very slowly and in some cases ceased their development at a stage which corresponds to that found in the normal seed at about ten days or two weeks after fertilization.

Apparently these abortive seeds represent an arrested development, caused by the presence of more advanced seeds on the same inflorescence. This may be merely the result of a competitive effect or it may be due to a sort of "dominance" which the earlier formed seeds have over the physiological processes of the inflorescence, similar to the advantage which a growing tip has over the lateral branches both in plants and in lower animals. The latter possibility is suggested by a condition which is sometimes found on ears in which two separate hand pollinations have been made, the first when only a few silks are out, and the second some days later when the remaining silks have appeared. The first pollination causes the formation of normal seeds at the base of the ear, while the second pollination often results in the production of "arrested" seeds over the remainder of the ear. Such ears are shown in Figure 68. Here the results do not appear to be due to competition alone since the competitive effect would be expected only where the two areas meet. Moreover, the same plant would be capable of producing normal seeds over the entire ear had all the silks been pollinated simultaneously.

In this case, apparently, the first seeds to develop so dominate the physiological processes of the plant, that the later seeds are deprived of normal nourishment. In fact it is not uncommon in such ears for the area containing the arrested seeds to be com-

pletely cut off so that the upper part of the spike disintegrates and is finally pushed off completely.

Seeds of the arrested type are not an important source of error in the defective seed studies, since they occur most commonly when all the silks are not pollinated simultaneously. In some strains, however, they occur regularly even when all the silks are pollinated at once. In such strains due allowance must be made for the disturbance which they cause in the ratios.

IRREGULARITIES IN THE FERTILIZATION MECHANISM

The non-hereditary defectives which are believed to be due to irregularities in the fertilization process are morphologically of two types. The first of these, designated as "germless," contains an endosperm but lacks the embryo, while the second type, termed "miniature," has both structures though these are greatly reduced in size.

In the germless seeds the development of the endosperm varies from a condition in which only a small mass of mealy tissue occurs to one in which well defined floury and corneous regions are distinguishable. In the miniature type the endosperm and embryo are both apparently normal, though the former is greatly compressed by the pressure of the normal seeds on either side and the latter is scarcely half the size of a normal embryo.

These types are found in small numbers on almost any ear of maize. A count of the seeds on fourteen open-pollinated ears of a hybrid between inbred strains known to be free from hereditary defectives, gave total of 10,235 seeds of which 50 or approximately one out of every 205 seeds was germless. The miniature seeds on nine of these ears were also counted and fifteen were found in a total of 6,245 or one in every 416 seeds. The germless seeds apparently occur about twice as frequently as the miniature type.

The suggestion that these two types of non-hereditary defectives, particularly the germless type, are due to irregularities in fertilization, comes from a microscopic examination of sectioned material. In specimens fixed at the early dough stage, it was found that the germless seeds contained normal endosperm tissue and an aleurone layer but that no trace of embryo tissue was present. Opposite the micropyle was a cavity in the endosperm and this appeared to be a remnant of the embryo sac. Within the cavity was found, in two specimens, a single large nucleus, to all appearances the unfertilized or undivided egg, or perhaps the polar nucleus. A photomicrograph of one of these specimens is shown in Figure 3, Plate XXVI.

Apparently this represents a case of single fertilization, in which one of the female nuclei had never been fertilized or, if fertilized,

had not divided. The other nucleus, after fusion, or as the result of some other stimulus, had divided and produced an endosperm, which, though greatly reduced in size, was normal in structural details, the cells being packed with starch grains.

There is, of course, the possibility that fertilization did not occur at all; that the aborted endosperm was produced vegetatively by the division of the polar nuclei. The endosperm of maize is usually the product of a sexual fusion in which one of the sperm from the pollen tube fuses with the two polar nuclei of the embryo sac. In gymnosperms, however, and in some angiosperms, the endosperm is formed independently of any fusion and in some species, where fusion naturally occurs, endosperm formation may be induced by artificial stimuli.

From the experience of artificial pollinations in maize it can be positively stated that no development of the endosperm occurs in the complete absence of pollination. However, the parthenocarpic defectives, already described, show that the mere growth of the pollen tube in the style, transmits a stimulus to the ovule which causes the nucellus to expand and the pericarp to grow. It would, therefore, not seem to be an impossibility for the same stimulus of pollination to occasionally induce endosperm formation even though actual fertilization did not occur. In other words, a condition regularly found in the gymnosperms and some of the angiosperms, might be encountered occasionally as an irregularity in this particular angiosperm.

That the endosperm of the germless seeds is not produced vegetatively by the division of one or both of the unfertilized polar nuclei, is shown by a series of pollinations in which dominant aleurone and endosperm color characters are introduced by the pollen parent. On ears of a variety with white endosperm pollinated by one in which the endosperm is yellow, all of the germless seeds with sufficient tissue to show any color, were yellow. On ears of a strain with colorless aleurone, pollinated by a type with purple aleurone, the most developed of the germless seeds were purple. The appearance, due to xenia, of these dominant characters from the pollen parent, leaves no doubt that the male nucleus has taken part in the formation of the endosperm tissue of these aberrant seeds and that they are not the result of a vegetative division of one or both of the polar nuclei.

On the other hand, if the germless seeds are due to single fertilization, as first suggested, it seems rather strange that seeds containing an embryo, but lacking the endosperm, are not found as frequently as the reciprocal combination. If only one fertilization occurs, the fusion of egg nucleus and sperm might be expected to take place as frequently as that of polar nuclei and sperm, since under normal conditions the two usually occur almost simultaneously. (Weatherwax 1919, Miller 1919.)

There is the possibility that seeds of this type do occur but that the embryo is so dependent upon the endosperm for nourishment that it fails to develop in the absence of the latter, and seeds resulting from a fusion of sperm and egg nucleus alone are classified as parthenocarpic defectives.

Another possibility is, that if single fertilization occurs at all, it always results in formation of an endosperm regardless of which of the nuclei of the embryo sac was fertilized. Such a situation would be in interesting contrast to other species where irregularities of this sort usually result in the production of embryos. In *Naias major*, for example, Guignard found that in some cases the second male nucleus fused with the synergid

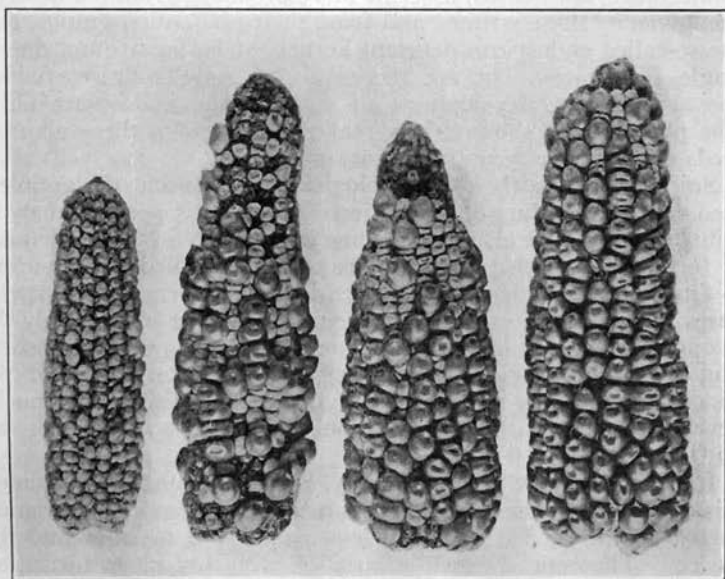


FIG. 69.—Four open-pollinated ears of a homozygous inbred strain showing the varying proportions of non-hereditary defective seeds which may occur under natural conditions.

instead of the primary endosperm nucleus. When this occurred two embryos and no endosperm were produced.

The possibility that single fertilization always results in endosperm formation in this species, is offered only as a suggestion. It seems more probable that seeds with embryos but lacking an endosperm are so poorly developed that they are classed as parthenocarpic and it is expected that such specimens will be encountered when more of the parthenocarpic defectives in early stages of development are examined microscopically.

In this connection it may be mentioned that Harlan and Pope (1925) have recently described a number of defective seeds in barley, in part of which no embryo could be found, in the remainder no endosperm, both types being attributed by them to single fertilization. In the so-called "endosperm deficient" kernels, however, disintegrating masses of tissue whose cells show early stages of starch grain formation are found. These writers believe this tissue to be of nucellar origin.

In the parthenocarpic defectives of maize, in which the nucellus is stimulated to such a marked degree, no evidence of starch grain formation has been found in any stage. Though the possibility of the nucellus assuming the role of an endosperm under some conditions is not denied, it seems more probable, from the description given by these writers, and from their photomicrographs, that the so-called endosperm deficient kernels of barley are not due to single fertilization but are the result of some influence which has arrested the development of embryo and endosperm alike. The photographs show clearly that the embryo in these abortive seeds of barley is by no means normal.

Since there is fairly good cytological evidence that the germless seeds are the product of single fertilization, it is possible that the miniature seeds are also due to some irregularity in the mechanism of fertilization, perhaps to a failure of the antipodal polar nucleus to fuse with the micropylar polar nucleus. Normally the sperm fuses first with the one polar nucleus and almost immediately the two are joined by the second polar nucleus, this process constituting the "triple fusion" characteristic of the formation of the endosperm in many angiosperms. In maize the two polar nuclei never fuse until after fertilization (Miller 1919, Weatherwax 1919).

It is conceivable that occasionally, as often as once in 416 times, this exceedingly precise mechanism might show some variation such that the second polar nucleus would fail to enter into the fusion. Theoretically such a variation would result in formation of embryo and endosperm, though the latter, being the product of the fusion of two haploid entities instead of three would have the diploid instead of the usual triploid number of chromosomes and might be expected to be somewhat reduced in size, as a consequence.

Such a condition is not to be confused with that suggested by Webber (1900) as a possible explanation of mosaic seeds in which part of the endosperm shows maternal characters, the remainder paternal. Webber thought that these seeds might be due to a fusion of the sperm with one polar nucleus while the second polar nucleus divided vegetatively, the two tissues growing side by side. East and Hayes (1911) suggested that these seeds were due to a vegetative segregation, and Emerson (1921) has recently

presented considerable evidence indicating that the mosaic seeds are the result of non-disjunction of single pairs of chromosomes.

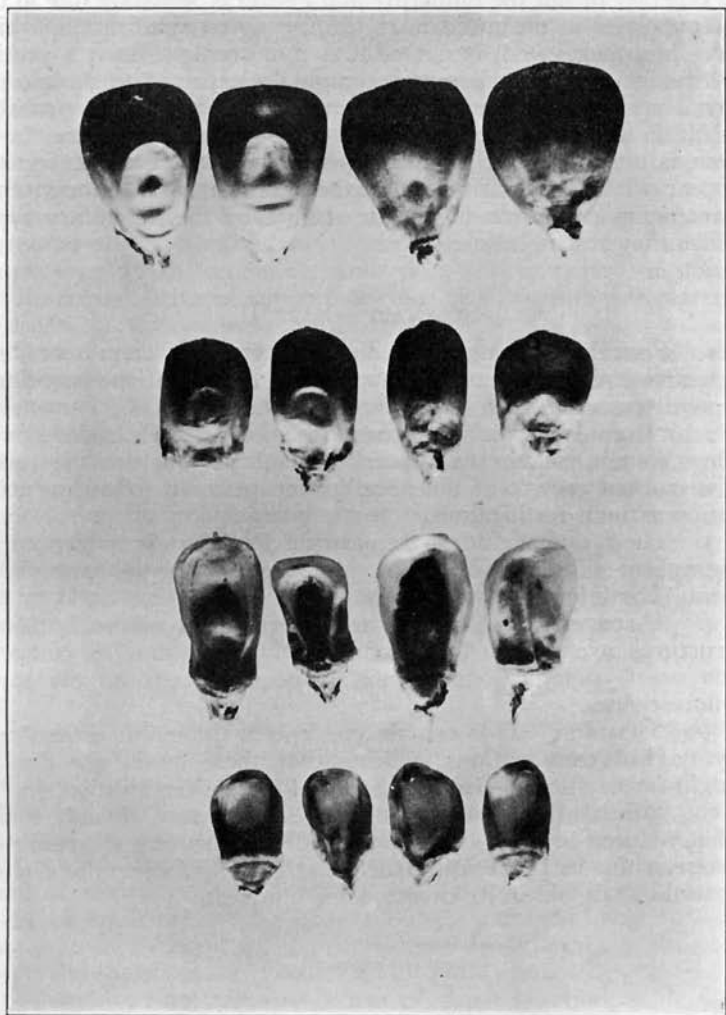


FIG. 70.—From top to bottom: normal, miniature, germless, and parthenocarpic defectives from same ear. The miniature seeds contain both endosperm and embryo, the germless seeds only an endosperm, while the parthenocarpic defectives lack both structures.

The suggested explanation of the miniature seeds, on the other hand, is that one of the polar nuclei is fertilized but that the other,

failing to fuse, does not divide and plays no part in formation of the endosperm.

Whether or not the miniature and germless seeds are due to the irregularities in the mechanism leading up to fertilization, which have been suggested, is not of first importance. Such a precise mechanism as is found in maize might be expected to show occasional accidental variations, and such variations would probably result in defective seeds. The fact of main importance, however, is that these two distinct types of non-hereditary defectives appear with a regularity that makes them a small, though constant, source of error in genetic studies of the hereditary types which they resemble so closely.

SUMMARY—PART II

1. Four morphologically distinct types of non-hereditary defectives, which resemble in appearance certain of the hereditary types discussed in Part I, have been found.

2. "Parthenocarpic defectives resemble complete defectives. They contain neither endosperm or embryo and are the result of a marked growth of the nucellus and pericarp following pollination without fertilization.

3. The frequency of parthenocarpic defectives is influenced by the age of silks, age of pollen, and probably by other environmental conditions.

4. "Arrested" seed contain endosperm and embryo but both structures are retarded in their development due to competition or physiological dominance of normal seeds on the same inflorescences.

5. "Germless" seeds contain endosperm tissue but no embryo. Cytological examinations indicate that these seeds are due to single fertilization instead of the usual double fertilization.

6. "Miniature" seeds are normal in structure though somewhat reduced in size. It is suggested that they are the result of irregularities in fertilization such that their endosperm is diploid instead of triploid in its chromosome number.

PART III

GENETIC FACTORS WHICH INFLUENCE THE TEXTURE OF
THE ENDOSPERM

In addition to the thirteen, and probably many more, genetic factors which primarily affect the *amount* of endosperm produced, there are a number of others which govern the chemical or physical nature of the storage material and result in differences in the *texture* of the endosperm.

Several of these factors are already familiar to geneticists. Three of them, sugary, waxy, and shrunken, are inherited as simple Mendelian recessives and are complementary in their action, crosses between any two of these types resulting in starchy F_1 seeds.

The texture of the endosperm of flint and flour varieties of corn has also been found to show alternative inheritance in some crosses. These characters differ from the other three in that a double dose of one is always dominant to a single dose of the other, with the result that the F_1 seeds of a cross are always maternal in appearance no matter which way the cross is made, while the F_2 progenies show a 1:1 segregation no matter which type of F_1 seeds is planted. (Hayes and East, 1925.)

To these already familiar characters affecting the texture of the endosperm, must be added another one, *brittle*, which has appeared in the course of these investigations.

BRITTLE ENDOSPERM*

In 1922, in a lot of about 100 self-pollinated ears of Sanford White, a typical eight-rowed, New England flint variety, were found two ears which segregated for an endosperm character which had not been noted previously. In appearance the recessive seeds on these two ears were somewhat similar to the familiar sugary seeds, being translucent instead of opaque and wrinkled instead of smooth. They differed from sugary seeds, however, in being generally less wrinkled and having a more shrunken appearance. In fact, except for the translucent condition, they were not unlike the shrunken seeds described by Hutchison.

On examination, the endosperm of these aberrant seeds was found to consist of a mass of amorphous tissue, distinctly sweetish to the taste and very brittle in texture. Because of this latter feature the new character has been given the name *brittle* endosperm and the factor symbol *bt*.

* Prof. J. B. Wentz, Iowa State College, writes that he has recently sent to the Journal of Heredity a manuscript in which he describes this same character as *concave*.

Some of the brittle seeds from one of these ears were planted in 1923. Though the germination was poor and the plants lacked somewhat in vigor, a few self-pollinated pure brittle ears were obtained. The original segregating ear and one of the brittle ears derived from it are shown in Figure 71.

When outcrossed to an unrelated stock the brittle seeds reappeared in the F_2 endosperm generation as simple Mendelian recessives. A count of the normal and brittle seeds from five self-pollinated ears of such a cross is given in Table 18.

TABLE 18. Starchy and Brittle Seeds on Self-pollinated Ears.

Ear No.	Starchy	Brittle
1747	212	61
1748	349	91
1749	273	93
1750	190	57
1751	335	104
Total	1359	406
Ex. 3:1	1324	441
Deviation 35		
P. E., 12.27		
Dev./P. E., 2.9		

Because this new character resembled, in some respects, both sugary and shrunken endosperm, it was crossed with both of these. The F_1 seeds in both series of crosses were starchy, indicating that brittle endosperm is a condition genetically different from either sugary or shrunken.

SUGARY X BRITTLE

Although the brittle seeds on the original segregating ear were similar to sugary seeds in appearance, the two types were distinguishable and could be separated with a fair degree of accuracy when both occurred on the same ears. In addition to the general differences already mentioned, it was noted that on ears of which all the seeds were genetically white, the brittle seeds were characterized by a faint yellowish cast, a sort of discoloration of the endosperm, while the sugary seeds, like the starchy, were a clear white.

Table 19 gives the results of separating the starchy, sugary, and brittle kernels from three ears and Figure 72 illustrates the P_1 , F_1 , and F_2 ears of this cross.

Although the results fit a 9:3:4 ratio the deviation from expectation is rather high, there being an excess of starchy and a deficiency of sugary seeds greater than four times the probable error. The deviation of the brittle seeds is within the limits of random sampling. The marked deviation in the starchy : sugary

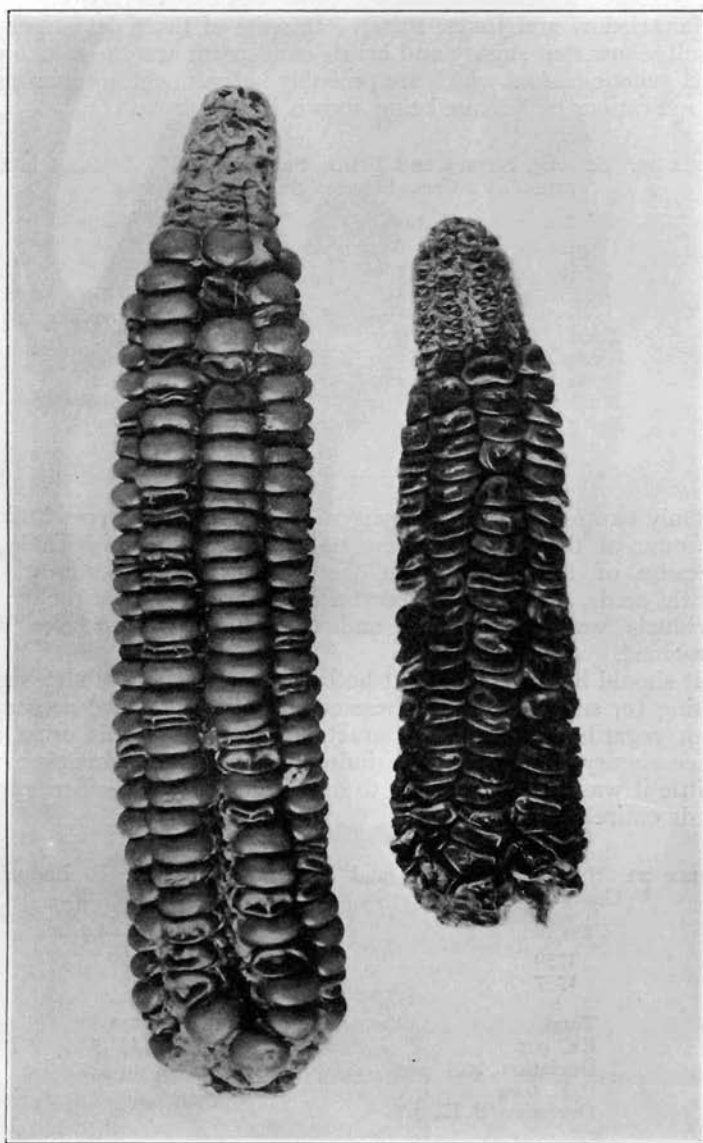


FIG. 71.—Original ear of Sanford White segregating for brittle endosperm and a pure brittle ear derived from it.

ratio may be due to genetic factors affecting the rate of pollen tube growth which are discussed in Part V, published elsewhere

(Mangelsdorf and Jones, 1926). In spite of these deviations the results show that sugary and brittle endosperm are due to two distinct genetic factors which are probably independent in inheritance, no indications of linkage being shown in this cross.

TABLE 19. Starchy, Sugary and Brittle Seeds in the F_2 Endosperm Generation of a Cross between Sugary and Brittle.

Ear No.	Starchy	Sugary	Brittle
422	242	61	78
1754	169	47	63
1755	166	43	72
Total	577	151	213
Ex. 9:3:4	530	176	235
Deviation	47	-25	-22
P. E. 9:7 ratio	10.26		
Dev./P. E.	4.6		

SHRUNKEN X BRITTLE

Only two segregating F_2 progenies of this cross were obtained. A count of the seeds on these two ears is shown in Table 20. Because of the variation in the translucent condition of the brittle seeds, an accurate separation of brittle and shrunken individuals was not possible and these two classes have been combined.

It should be mentioned that both of these ears were also segregating for sugary seeds. These could not be classified accurately with regard to the other characters involved in this cross and since sugary is known to be independent of both shrunken and brittle it was considered safe to disregard the unclassified sugary seeds entirely.

TABLE 20. Starchy, Shrunken, and Brittle Seeds in the F_2 Endosperm Generation of a Cross between Shrunken and Brittle.

Ear No.	Starchy	Shrunken and Brittle
1756	122	90
1757	119	61
Total	241	151
Ex. 9:7	220.5	171.5
Deviation, 20.5		
P. E. 6.62		
Deviation/P. E., 3.1		

Although there is a deviation of 3.1 times the probable error, the results approach a 9:7 ratio. This indicates that brittle and shrunken endosperm are due to two distinct genetic factors which are complementary in their action and are inherited independently. Linkage between the *bt* and *sh* factors would be indicated by an

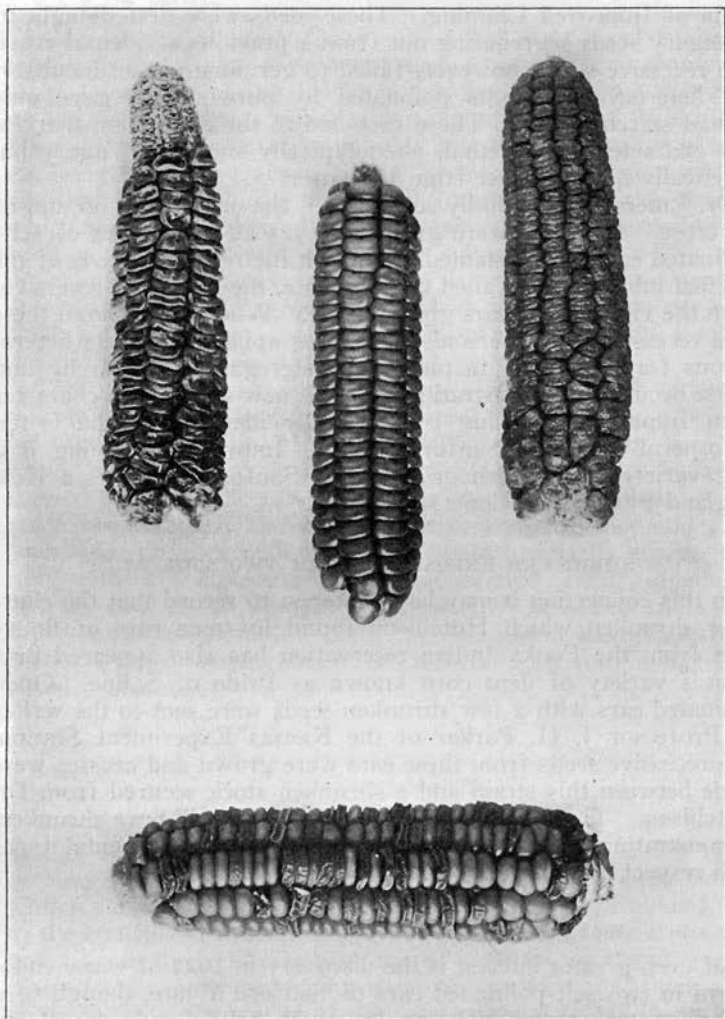


FIG. 72.—Parental, F_1 and F_2 generations of a cross between sugary and brittle endosperm.

excess of the recessive types; actually there is a significant deficiency.

BRITTLE ENDOSPERM FROM TWO VARIETIES

In 1923, Dr. R. A. Emerson, in a letter to Dr. D. F. Jones, wrote that wrinkled, translucent seeds had been found in an inbred

strain of Improved Leaming. These seeds were first thought to be sugary seeds segregating out from a previous accidental cross. The recessive seeds, however, failed to germinate in an incubator and heterozygous plants pollinated by pure sugary gave only normal starchy seeds. These facts led to the conclusion that the new character was a lethal, phenotypically similar to sugary but genetically quite distinct from the latter.

Dr. Emerson very kindly sent seed of the original strain and of the cross. Both lots were grown in 1923 and a number of self-pollinated ears were obtained. Though the recessive seeds of the original inbred strain failed to germinate, the extracted recessives from the vigorous F_1 ears grew readily. When pollen from these pure recessives of Emerson's strain was applied to plants heterozygous for the brittle factor, a 1:1 segregation of starchy and brittle occurred, demonstrating that this new endosperm character from Improved Leaming is genetically identical to the brittle endosperm found in Sanford White. Improved Leaming is a dent variety of Western origin while Sanford White is a New England Flint type of long standing.

SHRUNKEN ENDOSPERM FROM TWO SOURCES

In this connection it may be of interest to record that the character shrunken which Hutchison found in some ears of floury corn from the Ponka Indian reservation has also appeared in a Kansas variety of dent corn known as Pride of Saline. Open pollinated ears with a few shrunken seeds were sent to the writer by Professor J. H. Parker of the Kansas Experiment Station. The recessive seeds from these ears were grown and crosses were made between this strain and a shrunken stock secured from Dr. Hutchison. The F_1 seeds of such crosses were all pure shrunken, demonstrating the genetic similarity of the two parental types with respect to this character.

WAXY ENDOSPERM IN CHINA AND AMERICA

Of even greater interest is the discovery in 1922 of waxy endosperm in two self-pollinated ears of Sanford White, though of a different strain from the one in which brittle endosperm was found.

This peculiar endosperm texture had previously been found only in three isolated localities in China, Burma, and the Philippines. Collins (1920) failed to find a single waxy variety among more than a thousand American varieties which he examined.

The waxy type isolated from Sanford White was crossed with a waxy strain which had come originally from Shanghai, China. This cross gave only pure waxy F_1 seeds, proving that the two strains were genetically alike in their endosperm texture.

So far as is known, the only waxy corn ever grown in Connecticut is the Chinese strain which has been used in genetic investigation at the experiment station farm for a number of years. No corn of any kind has ever been sent from the station to the locality from which the ears of Sanford White were obtained, and it is scarcely possible that the appearance of waxy endosperm in this variety is due to previous crossing with the Chinese waxy. Nor is there any indication that the strain in which waxy has appeared has undergone any recent crossing with such a widely different sort as the Chinese waxy. The strain which carries the waxy endosperm is typical of the variety in every respect, including number of rows of grain on the ear which Collins (in a letter) suggests as the character which would be most affected by crossing.

The origin in an American variety of this peculiar endosperm texture, previously found only in several isolated Asiatic localities, will probably remain a matter for speculation. It may have arisen as a mutation in very recent years or it may have been carried by the stock as a hidden recessive for centuries. Waxy seeds are not particularly conspicuous in appearance and a few such seeds on open pollinated ears would ordinarily escape attention, and the character might be carried along indefinitely. Neither is a recent mutation from starchy to waxy an impossibility, since mutations affecting the endosperm have appeared in homozygous inbred strains of maize in at least four instances, as noted previously.

CONSTANT VARIATION IN THE STORAGE MATERIAL OF THE ENDOSPERM

The finding of brittle endosperm in Leaming and Sanford White, of shrunken endosperm in Pride of Saline and a variety of flour corn from the Ponka Indians, of waxy endosperm in China, Burma, the Philippines, and finally in New England, leads to the conclusion that these recessive characters have a wide distribution in the germplasm of maize varieties, or that the germplasm is constantly producing anew these variations which affect, so profoundly, the nature of the food material stored in the endosperm. Under domestication some of these variations, as for example, sugary, offer economic advantages and are retained. Others may have a survival value under certain climatic conditions and are automatically sorted out. This may have been the origin of waxy varieties in China, Burma, and the Philippines.

It is now generally agreed that maize was confined to the American continent previous to the beginning of the sixteenth century. Why waxy varieties should have been developed in these Asiatic localities and apparently in no other part of the

world is difficult to understand, unless this peculiar type of endosperm has natural advantages under the environmental conditions obtaining in these regions, or unless waxy varieties were isolated because of their economic superiority. Since the waxy corn is generally regarded as inferior to the starchy types for food purposes, where both are known, it seems more likely that the isolation of waxy varieties has been brought about by natural selection. This assumption is substantiated by the discovery of waxy endosperm in varieties of *Coix* and *Andropogon sorghum* from these same regions. (Kempton 1921.)

THE RELATIVE DEVELOPMENT OF ENDOSPERM CHARACTERS

The fact that the brittle seeds show such a low percentage of germination, in some cases none at all, suggests that these various factors which affect the texture of the endosperm are not fundamentally different from the lethal and semi-lethal seeds described in Part I; that all represent "defective" conditions of the endosperm, and that any distinction which is made is one of degree rather than of kind.

It will be recalled that when both normal and defective seed from segregating ears were weighed, the defectives showed a development ranging from 2.4 per cent in the completely lethal types to 59 per cent in the semi-lethal types.

The same method of determining the relative development of these five endosperm characters has been followed. The dominant and recessive seeds from segregating ears were separated, counted, and weighed. The average weight of each type was determined by simple division and the relative development obtained by a comparison of the two quotients.

The average weights of the seeds and the relative development of each type of endosperm texture is shown in Table 21.

TABLE 21. Average and Relative Weights of Dominant and Recessive Seeds from Segregating Ears.

Character Segregating	No. of Ears	Av. Wt. Dominant Seeds in mg.	Av. Wt. Recessive Seeds in mg.	Rel. Wt. of Recessives
Waxy	3	306	295	96.5
Floury	3	208	199	95.6
Shrunken	3	321	295	91.9
Sugary	5	255	226	88.5
Brittle	2	230	143	62.3

It will be noted that in every case the recessive seeds were lower in weight than the dominant seeds from the same ears. Hutchison (1921) weighed a large number of individual starchy and shrunken seeds from segregating ears and though the shrunken seeds

were lower in weight the difference was not significant when the probable error was considered. He concluded, therefore, that the shrunken seeds were equal to the starchy seeds in their development. Although the method used here does not permit the calculation of probable errors it does give a very accurate average weight of the dominant and recessive seeds. Of the many segregating ears which have been used in these and other determinations not one has ever been found in which the recessive seeds were equal in weight to the dominant ones.

Apparently the seed of maize attains its maximum development only when the endosperm is genetically starchy. Genetic factors which cause the formation of other carbohydrates in the endosperm, such as dextrose in the case of sweet corn, possibly some other sugar in the brittle seeds, and perhaps erythrodextrin in the waxy seeds (Weatherwax 1922), do so at the expense of total dry matter laid down. Although the amount of dry matter is not necessarily the sole criterion of endosperm achievement, it nevertheless appears to be the most important one.

The waxy seeds most nearly approach the normal condition in relative development, the floury seeds come next, followed by shrunken, sugary and brittle in the order given. The germination of the seeds and the vigor of the seedlings is closely correlated with the relative development of each type, the waxy and floury seeds being almost equal to starchy in these respects while the shrunken and sugary seeds are somewhat inferior and the brittle seeds are very poor.

DISCUSSION

These five characters which influence the texture of the endosperm and the thirteen factors which govern the amount of tissue can be arranged in a series representing different stages of endosperm formation. All of the factors result in a decreased development of the endosperm as measured by the dry weight of the seed. When the relative development of these six types is represented by points on the normal growth curve of the seed of maize, together with the thirteen defectives, as shown in Figure 73, it is noted that these 18 endosperm characters form a continuous series ranging from the de_{14} type to waxy endosperm.

In other words, the seeds of maize; to attain a relative development of 100 per cent, must pass safely through all the points represented on the growth curve. This means that *in order to attain the normal or "starchy" condition a seed must be dominant for at least 18 genetic factors*, any one of which in the homozygous recessive condition would reduce its development.

The space on the curve between 50 and 90 is not heavily populated and it is possible that characters which fall into this region are still to be found.

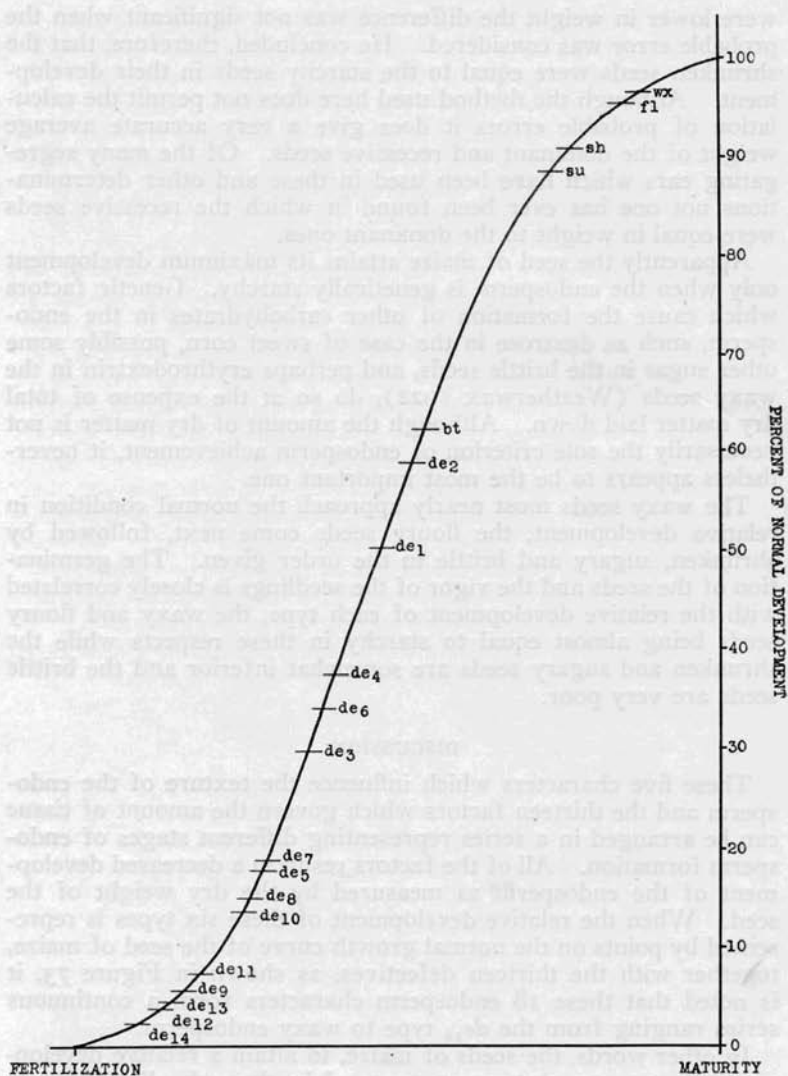


FIG. 73.—Diagram in which the relative development of endosperm characters is represented by points on the growth curve of normal seeds of maize.

The characters which show a development of 50 per cent or more are the most useful to the geneticist because there is enough endosperm development for differences in texture to be apparent

and in most cases these characters can be secured in a homozygous condition. This latter feature constitutes an advantage when linkage relations are studied because it permits the use of backcrosses.

SUMMARY—PART III

1. *Brittle* endosperm, a new character intermediate between sugary and shrunken in appearance, is inherited as a simple recessive.

2. Crosses between brittle and sugary or brittle and shrunken give starchy seeds in F_1 and 9:3:4 or 9:7 ratios in F_2 . No indication of linkage is shown in either series of crosses.

3. Brittle, shrunken, and waxy endosperm have been found in widely separated localities and in unrelated varieties indicating their widespread distribution in the germplasm or constant reappearance by mutation.

4. The endosperm of maize apparently attains its maximum development when genetically starchy. The relative development of waxy, floury, shrunken, sugary and brittle seeds is always less than that of starchy seeds from the same ears.

5. The characters which affect the texture of the endosperm are not fundamentally different from the defective seeds previously described, which influence primarily the amount.

6. The maize seed in order to attain the normal or "starchy" condition must be dominant for at least 18 genetic factors.

PART IV

PREMATURE GERMINATION OF MAIZE SEEDS AND GENETIC FACTORS WHICH GOVERN DORMANCY

In a paper on defective seeds, the writer (1923) mentioned briefly a condition in maize in which the seeds fail to go through a resting period, germinating while still attached to the ear of the growing plant and before the seed is mature. A similar condition was reported simultaneously by Lindstrom (1923) who found among the ears of a strain of Golden Bantam sweet corn, which was being studied for defective endosperm, one ear which also segregated for germinating seeds. Somewhat later Eyster (1924) described a similar character which he calls "primitive sporophyte,"* and more recently, in another paper (Eyster, 1924), he reports a second factor for premature germination.

Since 1921 a number of stocks which segregate for germinating seeds have come under observation of the writer. Self-pollinations and crosses in these stocks indicate that there are at least five complementary factors and two sets of duplicate factors involved in premature germination of maize seeds. A description of these characters and an account of their genetic behavior is given in the following pages.

COMPLEMENTARY FACTORS

 ge_1

The first type of premature germination was found in 1921 in a strain of Gold Nugget flint corn which had been twice self-pollinated. The germinating seeds were not noted in the first generation and either had not appeared, or the germination was so slight as to escape notice. The ratio of dormant to germinating seeds was approximately 3:1 and the character has continued to behave as a simple Mendelian recessive both in the original strain and in crosses. A count of the dormant and germinating seeds from eight ears of this stock is shown in Table 22.

Premature germination induced by the ge_1 factor begins at an

* The term *primitive sporophyte* as used by Eyster in describing premature germination in maize, has not been adopted by the writer because it is thought to be somewhat misleading. Eyster compares the condition in maize to that found in *Selaginella rupestris* in which the sporophyte develops while the egg is still attached to the female gametophyte. The resemblance between this condition and that found in maize is more superficial than real. As a mere term to designate the character, primitive sporophyte would serve as well as any, except for the fact that readers, not familiar with the premature germination in maize, might draw the conclusion that prematurely germinating seeds actually represent a reversion to the primitive condition of the race and that a change in a single gene can carry back the maize plant, phylogenetically, so to speak, to the group in which the club mosses are found.

early stage. The actual sprouting of the seed has usually occurred when the seeds are in the late milk stage but the segregation is

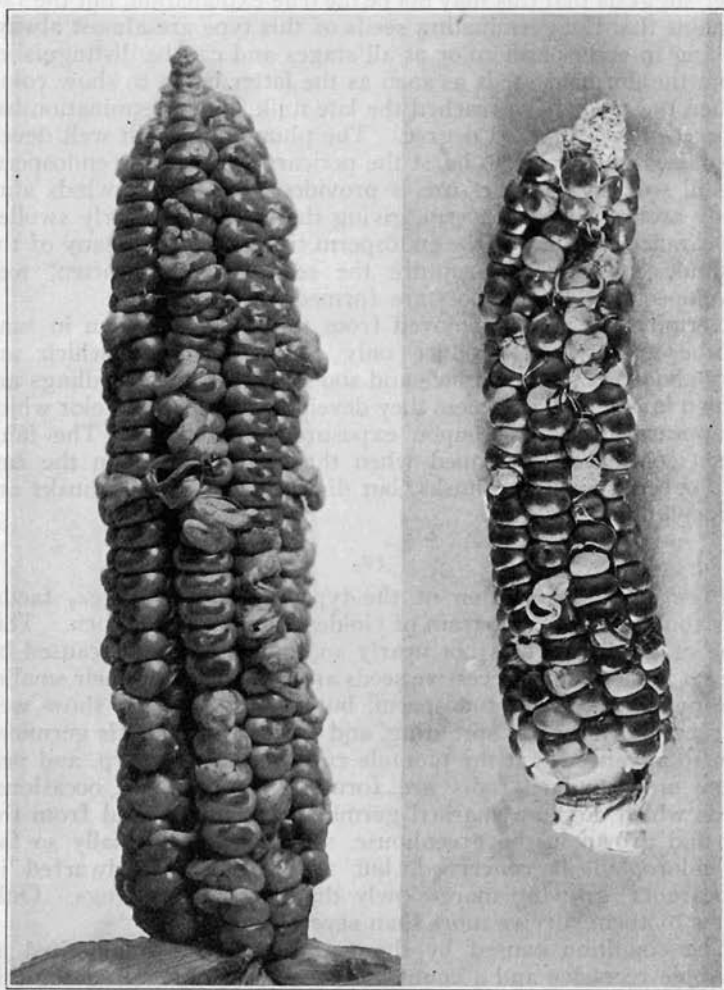


FIG. 74.—Two ears segregating for germinating seeds of the *ge_1* type. Germination begins at an early stage. The ear at the right shows the apparent linkage between endosperm color and premature germination.

apparent at an earlier period because the recessive seeds are, with few exceptions, white, while the dormant seeds on the same ear are yellow. Eyster reports a similar association between pre-

mature germination and the absence of color in the endosperm and explains this on the basis of close linkage. Evidence presented later suggests that this may not be the true explanation, but the fact remains that the germinating seeds of this type are almost always lacking in endosperm color at all stages and can be distinguished from the dormant seeds as soon as the latter begin to show color. When the seeds have reached the late milk stage, germination has proceeded to a marked degree. The plumule, though well developed, is often unable to burst the pericarp because the endosperm is still soft and no pressure is provided. Instead it winds aimlessly around the endosperm, giving the seed a peculiarly swollen appearance. Later, as the endosperm tissues harden, many of the plumules are able to rupture the seed coat and, often, well developed roots and shoots are formed.

Germinating seeds, removed from the ear and grown in sand in the greenhouse, produce only albino seedlings which are very abnormal in appearance and soon die. If such seedlings are grown in complete darkness they develop a faint green color which disappears immediately upon exposure to full light. The faint green color is also formed when the seeds remain on the ear, well covered by heavy husks, but disappears when the husks are stripped down.

ge_2

Premature germination of the type caused by the ge_2 factor was found in an inbred strain of Golden Bantam sweet corn. This type of germination is not nearly so complete as that caused by the ge_1 factor. The recessive seeds are recognized by their smaller size and pale yellow endosperm, but not all of them show well marked indications of sprouting, and only occasionally is germination so advanced that the plumule ruptures the pericarp, and primary or secondary roots are formed. When these occasional seeds which do show marked germination are removed from the ear and grown in the greenhouse, they develop normally so far as chlorophyll is concerned, but are considerably dwarfed in appearance, growing more slowly than normal seedlings. Only a few of them survive more than several weeks.

The condition caused by the ge_2 factor is also inherited as a simple recessive and a count of seven ears segregating for this character is given in Table 22.

TABLE 22. Segregation of Dormant and Germinating Seeds in Stocks ge_1 , ge_2 , ge_3 and ge_4 .

Type	No. of Ears	Total	ge	Ex. 3:1	Dev.	P. E.
ge_1	8	2220	528	555	-27	13.8
ge_2	7	1928	509	482	27	12.8
ge_3	4	446	99	112	-13	6.2
ge_4	1	169	44	42	2	3.8

ge_3

In 1924, Dr. Lindstrom very kindly furnished the writer with seed of his strain which had been segregating for germinating seeds. In some respects this type of premature germination, designated as ge_3 in this series, is almost identical to ge_1 . The germinating seeds are white, with few exceptions, while the dormant seeds have a deep yellow color so characteristic of the Golden Bantam variety. The plumules produced by the germinating seeds are, like those of ge_1 , albinos. They differ from the latter, however, in failing to form the faint tinge of green which characterizes the ge_1 seedlings under certain conditions.

The processes of germination in the ge_3 seeds evidently begin earlier and proceed further than in ge_1 seeds. When the dormant seeds are mature, the endosperm of the germinating seeds is almost completely exhausted and the pericarp is little more than an empty shell containing the withered remains of the partly grown seedling. This character is inherited as a simple recessive as indicated by the counts of the seeds from four ears shown in Table 22.

 ge_4

Germinating seeds were noted on an ear of an eight-rowed, yellow flint variety of the Longfellow type, received from Mr. T. B. Macaulay of Montreal, Canada. No count of the seeds was made on the original ear which was thought to be open-pollinated. When the dormant seeds from this ear were grown and the plants self-pollinated, segregating ears were again obtained. The proportion of dormant to germinating seeds on one of these ears is given in Table 22.

Germination in this stock does not begin until the seeds are fairly hard. The premature germination is not associated with absence of color in the endosperm as in the three stocks already described, nor are the seedlings albinos as in the case of ge_1 and ge_3 . Germinating seeds of this type, when removed from the ear and planted in the greenhouse, produce seedlings which are almost normal in appearance. By growing a large number of such seedlings it might be possible to mature a few homozygous plants in the greenhouse during the winter months. This stock, however, is the only one in which there seems to be any possibility of ever attaining a homozygous condition.

 ge_5

This factor evidently arose as a mutation in either the ge_1 or ge_3 stocks. A cross between these two stocks, in addition to giving the expected 9:7 and 3:1 ratios, also produced a few progenies in which the ratio was clearly 27:37, as the figures in Table 24

indicate. Evidently three factors for germinating seeds instead of two were involved in this cross. It is not known which of the parental stocks contributed the third factor but it seems fairly certain that it arose in one or the other by recent mutation, since both strains had previously given only mono-hybrid ratios and a later generation has done the same.

This new factor is almost identical to ge_3 in its effect. The recessive seeds are completely lacking in endosperm color and the sprouts are entirely without chlorophyll. The two types cannot be distinguished from each other but both are separable from ge_1 for a brief period during which the faint green color is visible in the sprouts of the latter.

DESCRIPTION SUMMARIZED

The chief characteristics of these five types of germinating seeds may be briefly summarized as follows:

Type	Approximate Time Germination Begins	Color of Endosperm	Color of Plumule
ge_1	late milk	white	white (green tinge)
ge_2	dough	pale yellow	green
ge_3	early milk	white	white
ge_4	hard dough	yellow	green
ge_5	early milk	white	white

It should be emphasized that all these stocks are genetically yellow in their factors for endosperm color and genetically green with regard to seedling color. The absence of endosperm and chlorophyll color is apparently due to the physiological effects of premature germination.

All these characters are fully as lethal in their effect as the defective seeds described in Part I. Under field conditions the growing seedling dies as soon as the plant matures and the moisture supply is cut off.

PHENOTYPICAL AND GENETIC DIFFERENCES

The breeding program with these five types of premature germination involves crossing them in all combinations. Only seven of the ten possible crosses have so far been made, but the distinct phenotypical differences between several of the types almost precludes the possibility that they are genetically alike, and a tentative conclusion has been reached that all five are probably genetically distinct.

It is noted that the types fall into two distinct classes with regard to plumule color, ge_1 , ge_3 , and ge_5 producing white sprouts, ge_2 and ge_4 producing only green plumules. That the first three

are genetically distinct is shown by the 27:37 ratios given in Table 24. The ge_2 and ge_4 types are considered to be distinct from the remaining three because of the marked phenotypical differences between the two groups, and crosses which have so far been made confirm this assumption. The cross between ge_2 and ge_4 has not yet been made and though these two differ in the amount of endosperm color, the time at which germination begins, and the vigor of the seedlings, these differences are all of degree and might result from the action of modifying factors. There

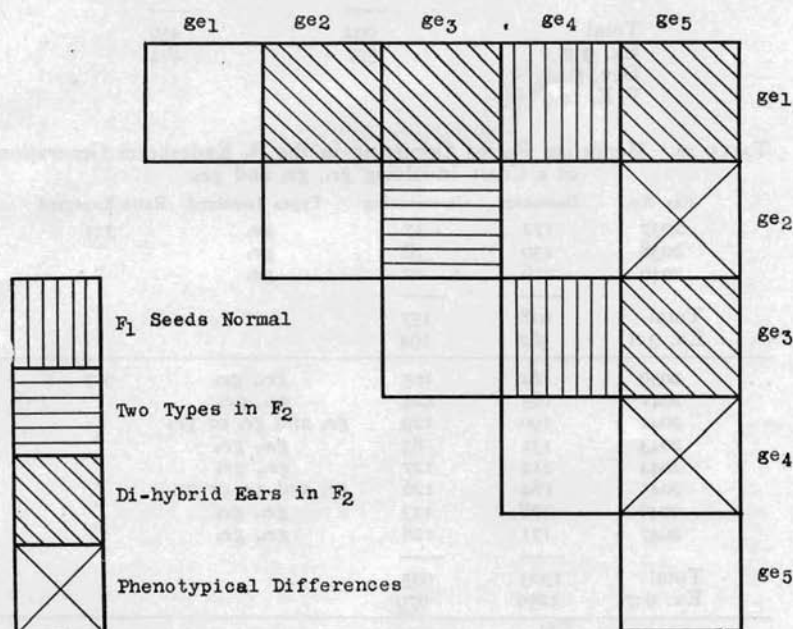


FIG. 75.—Diagram showing the crosses which have been made among the five stocks in which complimentary factors governing premature germination are involved.

remains the possibility, therefore, that ge_2 and ge_4 are genetically identical.

The situation with regard to these five types is shown in Figure 75. Squares with vertical cross hatching represent crosses in which the F_1 seeds are dormant and the F_2 has not yet been grown. Those with horizontal cross hatching indicate the combinations in which both types were recovered in F_2 although no di-hybrid ears were obtained. Diagonal cross hatching represents crosses in which di-hybrid ratios were found in F_2 , while two intersecting diagonal lines indicate marked phenotypical differ-

ences between the types. The crosses in which di-hybrid ears were obtained are given in detail in Tables 23 and 24.

TABLE 23. Ears Segregating for Both Factors in the F₂ Endosperm Generation of a Cross of *ge*₁ and *ge*₂.

Ear No.	Dormant	Germinating
711	149	99
712	139	112
713	146	118
714	168	130
Total	602	459
Ex. 9:7	597	404
Dev. 5		
P. E. 10.9		

TABLE 24. Mendelian Ratios Occurring in the F₂ Endosperm Generation of a Cross Involving *ge*₁, *ge*₂ and *ge*₃.

Ear No.	Dormant	Germinating	Types Involved	Ratio Expected
2037	172	57	<i>ge</i> ₁	3:1
2038	230	78	<i>ge</i> ₁	"
2040	216	22	<i>ge</i> ₁	"
Total	618	157		
Ex. 3:1	582	194		
2039	122	105	<i>ge</i> ₂ , <i>ge</i> ₃	9:7
2041	165	124	<i>ge</i> ₂ , <i>ge</i> ₃	"
2042	190	129	<i>ge</i> ₁ and <i>ge</i> ₂ or <i>ge</i> ₃	"
2043	131	83	<i>ge</i> ₂ , <i>ge</i> ₃	"
2044	212	127	<i>ge</i> ₂ , <i>ge</i> ₃	"
2045	184	126	<i>ge</i> ₁ and <i>ge</i> ₂ or <i>ge</i> ₃	"
2046	128	113	<i>ge</i> ₂ , <i>ge</i> ₃	"
2047	171	128	<i>ge</i> ₂ , <i>ge</i> ₃	"
Total	1303	935		
Ex. 9:7	1259	979		
2048	91	178	<i>ge</i> ₁ , <i>ge</i> ₂ , <i>ge</i> ₃	27:37
2049	137	184	<i>ge</i> ₁ , <i>ge</i> ₂ , <i>ge</i> ₃	"
2050	94	125	<i>ge</i> ₁ , <i>ge</i> ₂ , <i>ge</i> ₃	"
Total	322	487		
Ex. 27:37	341	468		

The evidence so far as it goes indicates that these five types are all different and that the plant must be homozygous for the dominant allelomorphs of all five factors in order for all of its seeds to remain dormant until maturity.

DUPLICATE FACTORS

Premature germination due to the action of duplicate factors was first found on the F₁ ears of a cross between two strains of

Canada Flint which had been inbred for four generations. Germinating seeds had never been noted in either of these inbred strains nor did they appear among the F_1 crossed seeds. When the F_1 plants were grown, however, a number of the ears bore germinating seeds, scattered at random over the inflorescence. These ears were open-pollinated, and no attempt was made to determine the ratio in which the segregation occurred. Dormant seeds from one of these ears were grown and five self-pollinated ears were obtained. Three of these were clearly segregating in a 3:1 fashion. The fourth deviated from a 3:1 ratio by an amount equal to ten times the probable error, but exactly fitted a 15:1 ratio. The counts on these ears are arranged in Table 25.

TABLE 25. Segregation of Dormant and Germinating Seeds when Duplicate Factors ge_6 and ge_7 are Involved.

Ear No.	Dormant	Germinating	Ratio	Nearest Mendelian Ratio	Deviation from Nearest Ratio
2171	123	46	2.7:1	3:1	1.1 times P. E.
2172	104	31	3.4:1	3:1	.9 " "
2173	121	43	2.8:1	3:1	.5 " "
2174	230	15	15.3:1	15:1	.1 " "

The appearance of germinating seeds in the second generation of a cross between strains which had never previously shown these characters and the 3:1 and 15:1 ratios obtained in F_3 can be explained by assuming a pair of duplicate factors, one of which was contributed by each of the inbred strains. These factors are given the symbols ge_6 and ge_7 and the following genetic constitution of the parents and F_1 plants is suggested:

Parents	F_1 Plants
$Ge_6Ge_6ge_7ge_7$	$Ge_6ge_6Ge_7ge_7$
$ge_6ge_6Ge_7Ge_7$	

The F_1 plants when selfed should give 15:1 ratios. Whether or not they did could not be determined because the ears were all wind-pollinated. The dormant seeds from these plants should, when grown, give three types of progenies; (1), non segregating, (2), segregating 3:1, (3), segregating 15:1. Had the F_1 plants been selfed these three types of progenies would be expected in the ratio of 7:4:4 respectively. The non-segregating ears would be expected when one or both of the recessive factors is lacking; 3:1 ratios should occur when the plant is homozygous for one and heterozygous for the other, while the 15:1 ratios are expected on plants which are heterozygous for both factors.

It is noted that all three types of progenies have been obtained although the number of selfed ears examined is too small to determine in what proportions the various types are appearing.

Apparently the two factors involved in this stock are independent. Linkage would be indicated by a distortion of the 15:1 ratio, an excess of recessives being expected in the coupling phase, a deficiency in the repulsion phase.

Premature germination in this stock is somewhat similar to that caused by ge_2 . The plumules are green but the endosperm color, instead of being pale yellow throughout, is affected only in the regions adjacent to the embryo. The endosperm color of the dorsal sides of the seed is, in most cases, full yellow.

RATIOS OF 8:1 AND 41:1

Another stock in which the action of duplicate, or perhaps triplicate, factors is suggested is the result of pollinating a chimera plant which appeared in the third generation of inbreeding in a strain of Sanford White, with pollen from an unknown yellow stock. The chimera had produced a pure white ear shoot and it was desired to obtain both selfed and crossed seeds from this plant. Accordingly pollen from an F_1 hybrid of two inbred strains of yellow flint corn was applied, but unfortunately no record was made of the row from which the pollen was taken and it is not known whether this particular hybrid had ever produced germinating seeds. It is known, however, that the inbred strain of Sanford White in which the chimera appeared had never shown germinating seeds.

TABLE 26. Segregation of Dormant and Germinating Seeds when Duplicate Factors ge_5 and ge_6 are Involved.

Ear No.	Dormant	Germinating	Ratio	Nearest Mendelian Ratio	Deviation from Nearest Ratio
2178	165	4	41.3:1	15:1	3.1 times P. E.
2179	163	22	7.4:1	15:1	4.6 " "
2180	187	21	8.9:1	15:1	3.3 " "

Four self-pollinated ears were obtained from the yellow seeds produced by cross-pollination with the unknown pollen parent. Three of these segregated for germinating seeds and the counts on these ears are set forth in Table 26. Two of the ears gave ratios approximately alike, the proportion of dormant to germinating seeds being about 8:1. This ratio deviates from 3:1 by more than nine times the probable error and from 15:1 by 5.6 times the error. The third ear, No. 2178, gave a ratio of 41:1 which differs from a 15:1 by 3.1 times the probable error and from 63:1 by an amount equal to 3.2 times the error.

These results may be explained on the basis of linkage between the two members of a pair of duplicate factors. A ratio of 8:1 would be expected if the two genes were linked with crossing over of approximately 34 per cent. The same two factors in the

repulsion phase should give a ratio of 17:1. Ear No. 2178 may represent the repulsion phase since it deviates from the expected 17:1 ratio by less than three times the probable error.

It is also possible that three factors instead of two are involved. Plants heterozygous for two of these and homozygous for the third would be expected to give 8:1 ratios if the two heterozygous factors were linked with crossing over of 34 per cent. Plants heterozygous for all three, two of which are linked, should give 35:1 instead of 63:1 ratios. Ear No. 2178 fits such a ratio very closely. The data, however, are not sufficiently comprehensive to substantiate such an interpretation. If three factors are involved with 34 per cent crossing over between two of them, the following ratios are expected when dormant seeds from ear No. 2178 are grown; 3:1, 8:1, 15:1, 17:1, 35:1, and 71:1. Another season's results should show definitely whether duplicate or triuplicate factors are involved.

Premature germination in this stock is similar to that caused by ge_4 . Germination begins at a very late stage and the endosperm color is not greatly affected.

SUMMARY OF BREEDING BEHAVIOR

Tentatively, then, the genetic situation with regard to the stocks which have been studied may be outlined as follows:

- | | |
|---|--|
| $\left. \begin{array}{l} ge_1 \\ ge_2 \\ ge_3 \\ ge_4 \\ ge_5 \end{array} \right\}$ | Five single factors, any one of which in a homozygous recessive condition causes premature germination. |
| $\left. \begin{array}{l} ge_6 \\ ge_7 \end{array} \right\}$ | A pair of independent duplicate factors which cause premature germination when both are present in the recessive condition. |
| $\left. \begin{array}{l} ge_8 \\ ge_9 \end{array} \right\}$ | A pair of linked duplicate factors causing premature germination when both are present. Crossing over is about 34 per cent. A third factor in this set, ge_{10} , is also indicated. |

LINKAGE RELATIONS

A detailed study of the linkage relations of these factors with other well known characters has not yet been undertaken. Data are available, however, to show the relation of the ge_1 , ge_2 , ge_3 , and ge_5 genes with the factor for sugary endosperm. In all these cases the germinating seeds cannot be accurately classified with regard to endosperm texture and linkage must be detected by the distortion in the starchy : sugary ratio among the dormant seeds. In the repulsion phase the percentage of sugary seeds in

the dormant class should vary between 25 and $33\frac{1}{3}$, depending on the intensity of the linkage. This is shown diagrammatically in Figure 63, Part I. In the coupling phase, linkage would be expected to cause a deficiency of sugary seeds in the dormant class, the percentage ranging from 25 to 0.

$$ge_1 \times Su$$

The linkage relations of ge_1 with Su are determined from the cross between the ge_1 and ge_2 stocks. The former is a yellow flint variety, the latter a yellow sugary. Eight ears which were segregating for ge_1 were obtained from this cross and the proportion of sugary seeds in the dormant class on these ears is shown in Table 27.

TABLE 27. Starchy:Sugary Segregation in Dormant Seeds from Ears Segregating ge_1 .

Ear No.	Starchy	Sugary	Percent Sugary
710	145	60	29.3
715	159	61	27.7
721	179	73	29.0
722	146	69	32.1
723	162	44	21.4
725	110	43	28.1
726	164	71	30.2
727	153	53	25.7
Total	1218	474	28.0
Ex. 3:1	1269	423	25.0
Dev. 51			
P. E. 12.0			

It is noted that there is an excess of sugary seeds, amounting to 4.25 times the probable error. Such a deviation would be expected by chance alone only once in about 250 trials. The excess, though it occurs in all but one of the eight ears, would not be regarded as significant if seeds from a smaller number of ears were counted. When all the ears are combined, however, the accumulation of small deviations in one direction results in a total deviation which can scarcely be attributed to chance. The excess of sugary seeds in this class can be explained by assuming linkage between the genes ge_1 and Su with approximately 40 per cent crossing over. It is realized, of course, that linkage values determined by the distortion in a single class necessarily have a large probable error and the value given must not be regarded as more than an approximation.

That the excess of sugary seeds in the dormant class is due to some relation with the ge_1 factor and not to errors in classification or to genetic factors affecting the rate of pollen tube growth, is further indicated by a count of the sugary seeds on eight ears

from the same cross which are not segregating for ge_1 . On these ears the percentage of sugary seeds is very close to expectation and deviations are minus almost as frequently as plus, as is indicated in Table 28.

TABLE 28. Starchy:Sugary Segregation in Ears Not Segregating ge_1 from Same Cross as Ears in Table 27.

Ear No.	Starchy	Sugary	Percent Sugary
728	192	48	20.0
729	221	80	26.6
730	211	65	23.6
731	204	69	25.3
733	163	57	25.9
734	185	82	30.7
735	227	68	23.0
736	175	79	31.1
Total	1578	548	25.8
Ex. 3:1	1594	532	25.0
Dev. 16			
P. E. 13.5			

$ge_2 \times su$

The relation of ge_2 and su is shown by ears from this same cross which are segregating for ge_2 and sugary but not for ge_1 . The counts from seven such ears appear in Table 29. Linkage in this case would be indicated by a deficiency of sugary seeds since the two factors entered the cross in the coupling phase. Actually there is a slight but not significant excess of sugary seeds in the dormant class and it appears safe to conclude that ge_2 and su are independent.

TABLE 29. Starchy:Sugary Segregation in Dormant Seeds of Ears Segregating ge_2 from Same Cross as Ears in Tables 27 and 28.

Ear No.	Starchy	Sugary	Percent Sugary
705	121	44	26.7
706	172	59	25.5
707	146	67	31.5
708	158	52	24.8
716	134	41	23.4
717	155	54	25.8
718	152	64	29.6
Total	1038	381	26.8
Ex. 3:1	1064	355	25.0
Dev. 26			
P. E. 11.0			

ge_3 and $ge_3 \times su$

Five of the ears represented in Table 24 are segregating for su , ge_3 and ge_3 . The two types of germinating seeds cannot be distinguished but if either one is linked with sugary a distortion

of the starchy : sugary ratio in the dormant seeds would be expected. A count of the two types of seed in the dormant class on these five ears appears in Table 30. The agreement with expectation on the basis of independent inheritance is very good and it seems certain that both factors are independent of sugary.

TABLE 30. Starchy:Sugary Segregation in Dormant Seeds of Ears Segregating ge_3 and ge_5 .

Ear No.	Starchy	Sugary	Percent Sugary
2039	99	23	18.9
2041	121	44	26.7
2043	96	35	26.7
2044	154	58	27.4
2046	99	29	22.7
2047	124	47	27.5
Total	693	236	25.4
Ex. 3:1	697	232	25.0
Dev. 4			
P. E. 8.9			

APPARENT LINKAGE WITH ENDOSPERM COLOR FACTORS

As has already been mentioned, there is a strong association between germinating seeds and absence of color in the endosperm. In the case of ge_1 , ge_3 and ge_5 the recessive seeds are, with few exceptions, completely white, while in the ge_2 strain the germinating seeds are a pale yellow.

TABLE 31. Apparent Linkage between ge_1 and White Endosperm.

Ear No.	Yellow		White	
	Ge	ge	Ge	ge
710	204	1	1	66
715	219	0	1	56
721	252	1	0	67
722	213	1	2	74
723	205	0	1	62
726	234	0	1	61
754	205	0	1	74
Total	1532	3	7	460

Occasionally, however, germinating seeds with yellow endosperm are found as well as white seeds which have not sprouted. The frequency of these exceptions in stocks 1 and 3 is shown in Tables 31 and 32. At first glance the situation represents a clear cut case of close linkage with less than 1 per cent of crossing over. Eyster (1924) has assumed this to be the situation in his stock and has calculated the amount of crossing over as 1.26 per cent. The writer (1923) had previously suggested physiological correlation as an explanation of these results and the evidence

indicates that this is probably the correct interpretation in the stocks reported here.

There are at least three series of facts which are not compatible with a linkage hypothesis:

1. All of the stocks in which the association between premature germination and color of endosperm appears have originated from varieties which were homozygous for yellow endosperm. White seeds might be expected to arise occasionally by mutation, but the appearance of four genetically distinct factors for white seeds, each one closely linked with a factor for germinating seeds, cannot reasonably be assumed.

2. When pollen from plants which are segregating for germinating seeds is applied to silks of a white variety, only yellow seeds are produced. If the segregating plants were heterozygous for endosperm color, as they appear to be, such crosses should produce 1:1 ratios, providing that the white endosperm of the germinating seeds has the same genetic basis as the white endosperm of common white varieties.

3. The apparent cross overs, white seeds which fail to sprout, should breed true if it is assumed that they are homozygous for a recessive endosperm color factor. Only a small number of these seeds have been available but all those which were grown produced only plants segregating for white seeds which germinated, with few exceptions.

TABLE 32. Apparent Linkage between ge_3 and White Endosperm.

Ear No.	Yellow		White	
	Ge	ge	Ge	ge
758	70	1	0	22
760	60	0	0	17
761	101	0	0	27
Total	231	1	0	66

If the association between germinating seeds and endosperm color is not due to linkage, to what may it be attributed? A histological study of the germinating seeds of the ge_1 stock has given evidence which seems to have some bearing on this question.

PREMATURE DIGESTION AND PIGMENT FORMATION

Some of the white seeds were removed from the ear at an early stage, killed and fixed, imbedded in paraffin, sectioned, and stained. It was found that even at this early stage the processes of germination had already begun. The cells in the epithelial layer of the scutellum had elongated and the invaginations of this layer, so characteristic of mature seeds of maize, were already apparent. Sargent and Robertson (1905) have made a thorough

study of the scutellum of maize and are of the opinion that these invaginations are glandular in nature and that their function is the secretion of diastase. There is some appearance of digestion in the cells of the endosperm of germinating seeds even at the early milk stage, and in material gathered from the same ears a week later, the digestion is quite marked.

It is possible that the normal production of color in the cells of the endosperm cannot proceed while digestion is occurring in these cells. The yellow color in the endosperm is found in the matrix which surround the starch grains, and if this matrix is being digested as rapidly, or more rapidly, than new material is being supplied by the plant, it is hardly to be expected that pigment formation would proceed in the normal fashion.

The apparent cross overs, the yellow seeds which germinate and the white seeds which remain dormant, may be merely variations of this condition.

Some of the germinating seeds probably remain yellow because the digestion does not begin soon enough or is not rapid enough to inhibit the formation of endosperm color. This would appear to be the case in the stock where the duplicate factors ge_6 and ge_7 are involved. In this stock the pale yellow is usually confined to an area adjacent to the embryo and the dorsal side of the seed retains the full yellow color.

The other class of apparent cross overs, the white dormant seeds, are more difficult to explain. The fact that all of these seeds which have ever been grown have given ears segregating for germinating seeds might suggest that this character occasionally manifests itself in the heterozygous condition.

THE RELATION OF PREMATURE GERMINATION TO CHLOROPHYLL DEVELOPMENT

Evidently there is also a physiological relation of some sort between premature germination and chlorophyll development. Types which begin germination at a very early stage, such as ge_3 and ge_5 , always produce pure white sprouts. The ge_1 type, in which germination begins somewhat later, ordinarily produces white plumules but occasionally these show a tinge of green. The remaining types in which germination begins only after the kernels are well developed, produce only normal green sprouts. Apparently the premature germination, if it begins early enough, completely inhibits the formation of chlorophyll just as it prevents the laying down of yellow pigment in the endosperm.

This association, too, is characterized by occasional exceptions. Germinating seed of ge_1 , ge_3 and ge_5 are sometimes found which produce sprouts of normal green color, but dormant seeds which give albino seedlings when germinated have never been observed.

PHYSIOLOGY OF PREMATURE GERMINATION

Oppenheimer (1922) has found that in seeds of tomato, gourd, cucumber and *Nicotiana rustica*, germination can be suppressed by surrounding the seeds with crushed tissue of the receptacles of the mother plants or by growing them on filter paper saturated with an extract from these tissues.

The degree of suppression is approximately proportional to the amount of tissue present or the concentration of the extract. This suppression can be overcome by heating the tissue or extract to 100° C. Apparently the mother plants of these species normally supply the growing seed with inhibiting substances which prevent germination while the seeds are still attached to the plant. Mazé (1910) is of the opinion, and presents some evidence in favor of his view, that dormancy in seeds, buds, bulbs, and tubers is due, in some cases, to the action of volatile esters which prevent growth until they are eliminated.

Oppenheimer did not include seeds of maize in his experiments but a test made by the writer, in an effort to determine at what stage of development germination in the normal seed still attached to the plant could be induced, may have some bearing on the problem. An ear in the early milk stage was stripped down and wrapped with cotton. Around this were wrapped several layers of cloth. The ends of the cloth were submerged in a vessel of water and served as a wick, keeping the cotton surrounding the ear constantly saturated. The grains swelled considerably, indicating that water was being absorbed, but no germination occurred. A number of seeds which had been removed from this ear and placed in an ordinary germinator at approximately the same temperature and with the same moisture supply, began to sprout after about ten days. This is much longer than the time required by immature, dry seeds to germinate under the same conditions and indicates that inhibiting substances were first eliminated before germination could begin.

THE EFFECTS OF PREMATURE GERMINATION ON THE
GROWTH OF THE SEED

An attempt was made to determine whether the germinating seeds receive the normal amount of nourishment from the plant while germination is going on or whether these seeds cease their development after germination begins. Ears which were segregating for germinating seeds (ge_1) were harvested at three weeks after pollination, and at intervals of one week thereafter, until maturity. These ears were dried on a rack until thoroughly dry, at which time the kernels were shelled off, the dormant and germinating seeds separated, counted and weighed, and the average weight of each class determined by simple division. The results

are shown in two curves in Fig. 76. It will be seen that already in the early milk stage there was a noticeable difference in the relative development of the two types as represented by their dry weights. This difference increased in the second week under observation and thereafter the germinating seeds no longer increased in weight and actually fell off somewhat during the last

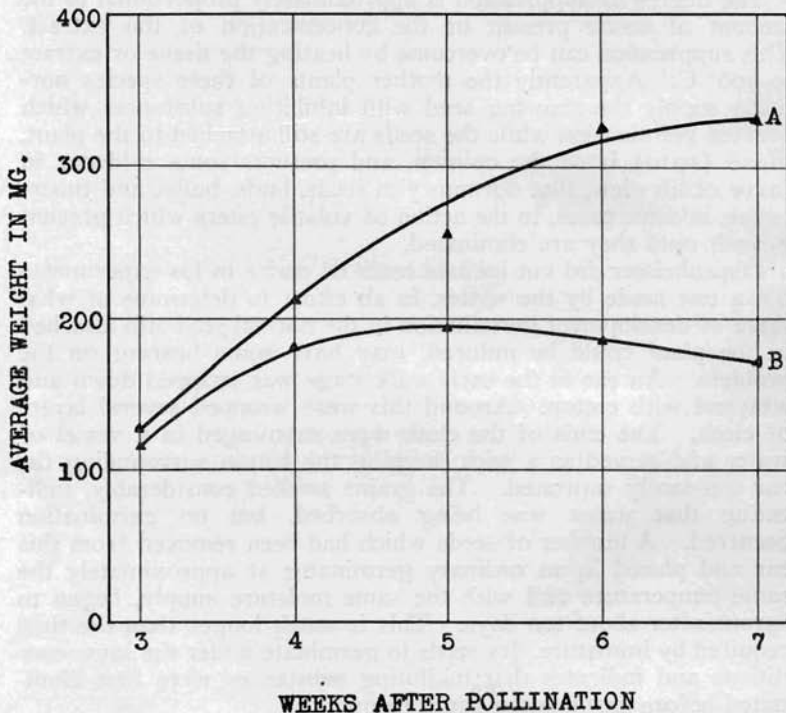


FIG. 76.—Growth curves of dormant and germinating (*ger*) seeds from the same segregating ears. The sprouting seeds do not receive enough nourishment from the plant to replace the material lost in germination.

three weeks. The normal seeds on the same ears showed an increase in dry matter during every week of the test. It is evident from these two growth curves that the amount of nourishment supplied to the aberrant seeds by the plant is not sufficient to replace that consumed in germination. In fact, it is quite likely that the germinating seeds become partially or wholly "physiologically isolated" from the plant during the later periods. At maturity the germinated seeds weighed only 51 per cent as much as the dormant seeds from the same ears.

DISCUSSION

For a period of several weeks, while the seed is in the milk or dough stage, natural conditions for germination are almost at an optimum. The temperature is fairly high and the moisture supply is abundant. The embryos are sufficiently developed to produce plants capable of surviving and the endosperm contains enough food material to nourish the seedling until it begins to manufacture food for itself. That this is true is shown by the behavior of immature seeds harvested at these early stages when many of them are capable not only of germinating but of producing almost a normal yield of grain. (See Part I.)

Why is it, then, that the partially developed seed ordinarily never germinates while still attached to the plant? Apparently the mother plant, though it provides conditions suitable for germination, at the same time supplies inhibiting substances which prevent germination from beginning.

The physiological processes involved in maintaining a period of dormancy, which permits the embryo to attain a maximum development and the endosperm to accumulate a mass of food material, are probably very complicated. It is not at all surprising, therefore, to find a number of distinct genetic factors operating during this period. Every stage in the ontogeny of the sporophyte is evidently controlled by many genetic factors and the maintenance of a normal period of dormancy which prevents premature germination with its disastrous effects, and permits the sporophyte to pass safely through unfavorable seasons, is no exception. Nine Mendelian factors which govern this stage have already been identified. Many others will undoubtedly be found as maize is investigated more extensively.

SUMMARY—PART IV

1. Nine Mendelian factors involved in the maintenance of a normal period of dormancy in maize seeds have been identified.
2. Five of these are complementary factors. When any one of these is lacking the seed germinates prematurely. Plants heterozygous for one, two or three factors give 3:1, 9:7, and 27:37 ratios respectively.
3. A pair of independent duplicate factors results in ratios of 15 dormant: 1 germinating when plants are heterozygous for both.
4. A pair of linked duplicate factors gives 8:1 ratios when plants are heterozygous for both. Crossing over is about 34 per cent.
5. The ge_1 factor appears to be linked with the gene for sugary endosperm. Crossing over is about 40 per cent. ge_2 , ge_3 and ge_4 are found to be independent of sugary.

6. An apparent case of close linkage between endosperm color and several types of germinating seeds is probably due to the physiological effects of premature germination upon the accumulation of pigment in the cells of the endosperm.

7. A similar association between germinating seeds and white seedlings may also be due to physiological complications. Seeds which germinate at early stage produce only white plumules; those which germinate later have normal green sprouts.

8. Premature germination is apparently caused by the lack or loss of inhibiting substances normally supplied by the plant to the growing seeds.

9. It is suggested that many genetic factors are involved in the maintenance of a normal period of dormancy in maize seeds.

CONCLUSION

The mature, dormant seed of maize with its well developed embryo and the cells of its endosperm packed with starch grains, represents a real organic achievement.

Each ovule has its separate style; each style, in order for a seed to develop, must receive a pollen grain capable of germinating and producing a tube sufficiently vigorous to reach the micropyle. Failure of the growing tube to attain its goal results in the production of "parthenocarpic" defectives without endosperm or embryo.

After the pollen tube has entered the micropyle, a very precise mechanism of fertilization begins to function. Failure of this intricate mechanism in any detail may cause the formation of "germless" seeds, lacking an embryo, "miniature" seeds in which the endosperm is greatly reduced in size or perhaps aborted seeds of several other types.

The fertilization mechanism having functioned properly, the growing seed begins to receive the influence of various genetic factors. Thirteen distinct factors have been found which arrest the development of the seed and cause it to be defective and incapable of normal growth and germination. Five additional factors may affect the nature of the stored food material to such a degree that the seed is handicapped and cannot attain a maximum development.

In addition to the 18 genetic factors so far found which *retard* development to a greater or lesser degree, nine other factors have appeared which *stimulate* certain functions prematurely, with equally disastrous consequences. The seed, in order to reach maturity and pass safely through unfavorable seasons must remain dormant while still attached to the plant, even though it is capable of germination at this stage and the conditions favoring germination are almost optimum. Five complementary factors

and two pairs of duplicate factors are involved in the maintenance of dormancy during development. The loss of any one or pair of these causes the seed to germinate prematurely with fatal results.

A fully mature, normally developed, dormant, white, starchy seed, then, represents the cumulative action of 27 Mendelian factors of which we know the mode of inheritance. How many additional factors are involved would be difficult to estimate, but since all these permanent departures from the normal condition of the germplasm have been found in a limited amount of material, it is certain that many more hereditary factors of a similar nature will appear. This gives some clue as to the infinitely large number of genes always working to produce a normal seed. The majority of these can not be known because they do not vary.

All hereditary units here studied concern only the seed which comprises a brief period between fertilization and the resting stage of the embryo. What, therefore, must be involved in the ontogeny of the entire plant? The young seedling, the growing plant, the chlorophyll processes, reproductive machinery and even the gametophyte generation are all controlled by genetic factors the tabulation of which has only been started.

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EXPLANATION OF PLATES.

PLATE XXI. Longitudinal sections of normal seed and three successive stages of development of defective seeds of *de₁₁* stock. x 11.

1. Normal seed at early milk stage.
2. Defective seed, early milk.*
3. Defective seed, late milk.
4. Defective seed, dough.

PLATE XXII. Three successive stages in development of defective seeds of the *de₅* stock. x 11.

1. Defective seed, early milk.
2. Defective seed, late milk.
3. Defective seed, dough.

PLATE XXIII. Three successive stages in development of defective seeds of the *de₈* stock. x 11.

1. Defective seed, early milk.
2. Defective seed, late milk.
3. Defective seed, early dough.

PLATE XXIV. Successive stages in the development of the normal embryo. x 15.

1. Blister stage.
2. Early milk.
3. Milk.
4. Late milk.
5. Dough.

PLATE XXV. 1. Ears of a uniform first generation hybrid harvested at successive stages of maturity. From left to right the ears represent stages of 14, 21, 28, 35, 41, 51 and 75 days after pollination.

2. Fifty seeds from each of the ears. In appearance and dry weight these normal seeds harvested at successive stages of development resemble various types of hereditary defectives.

3. The results of planting the fifty seeds shown in 2. In ability to germinate the immature normal seeds are superior to hereditary defectives of the same relative development.

PLATE XXVI. Four morphologically distinct types of seeds which may occur on any ear of maize. x 11.

1. Normal seed with well developed endosperm and embryo.
2. Hereditary defective with aborted endosperm and embryo.
3. Germless seed containing endosperm but no embryo. This type is probably due to single fertilization.
4. Parthenocarpic defective with neither endosperm nor embryo. This type is caused by pollination which fails to accomplish fertilization.

* The relative stage of development, in every case, is that of normal seeds from the same ear, and not of the defectives themselves.

PLATE XXI.

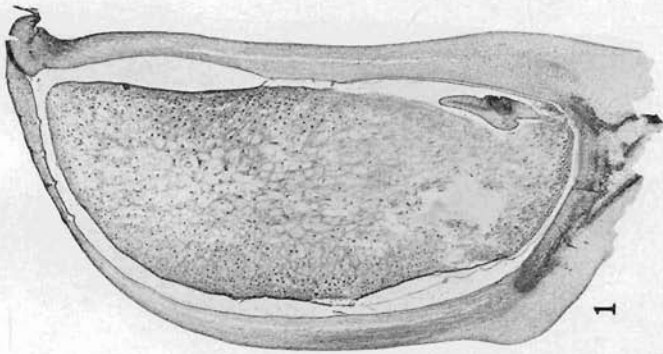
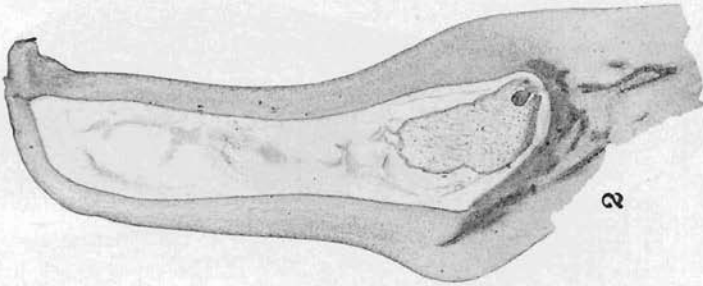
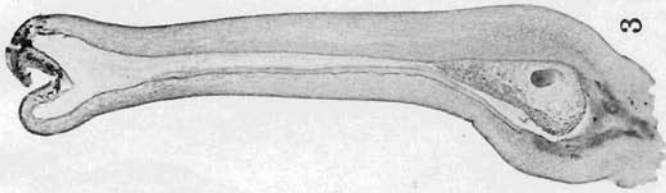
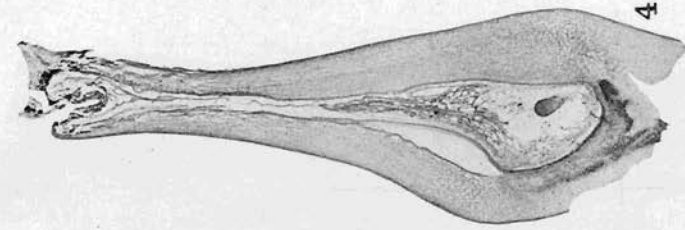
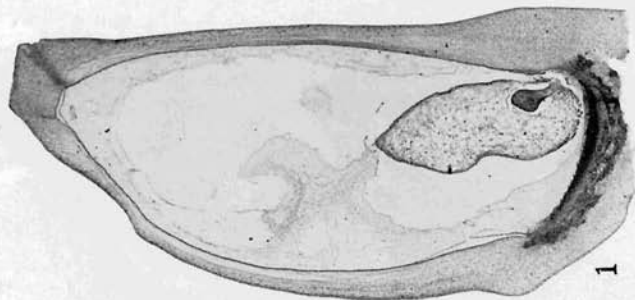
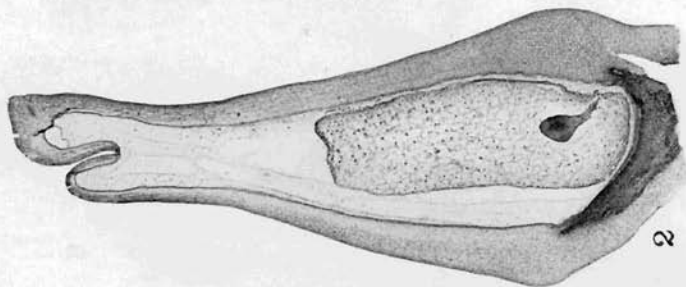
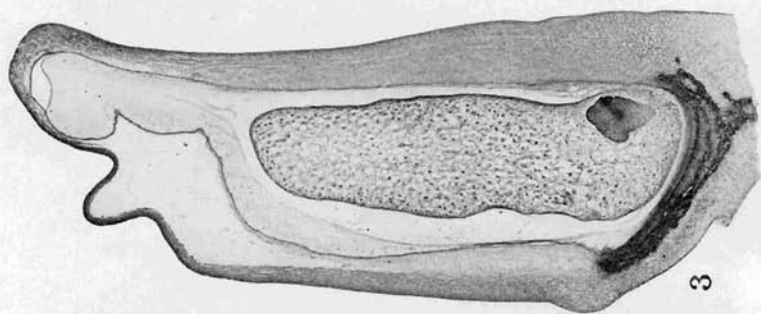


PLATE.XXII.



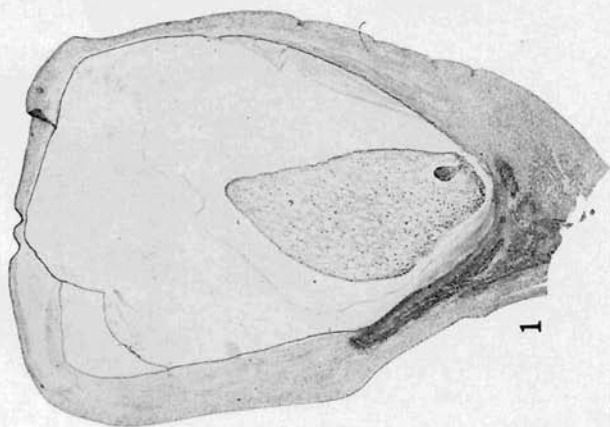
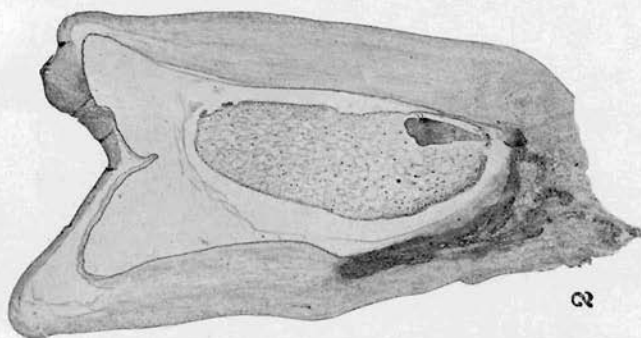
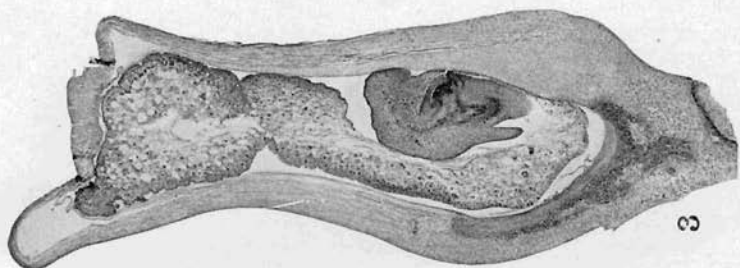
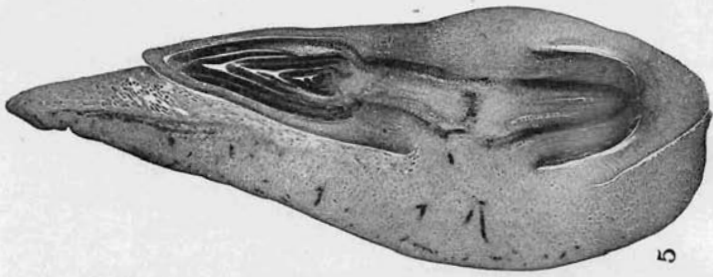


PLATE XXIV.



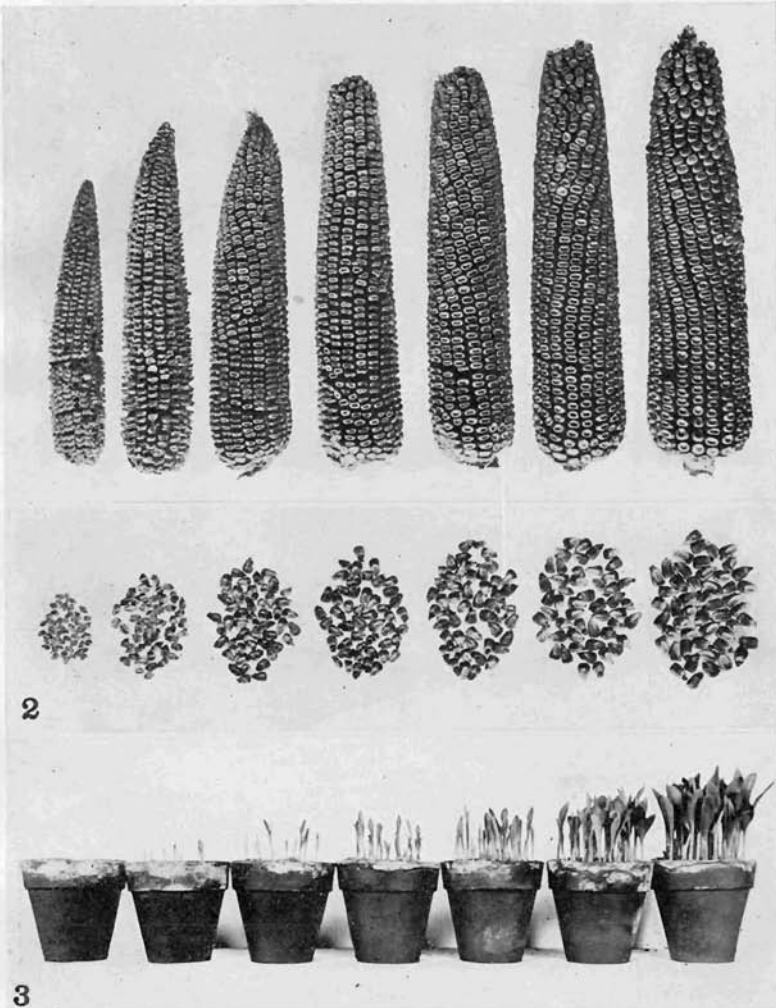


PLATE.XXVI.

