SELECTION OF PSEUDO-STARCHY ENDOSPERM IN MAIZE

D. F. JONES

Connecticut Agricultural Experiment Station, New Haven, Connecticut

[Received March 24, 1919]

TABLE OF CONTENTS

PAGE

INTRODUCTION 364
Occurrence of anomalous endosperm in other material 372
Behavior of pseudo-starchiness in crosses 374
Theoretical consideration of the results 382
CONCLUSION 390
SUMMARY 392
LITERATURE CITED 393

INTRODUCTION

Endosperm differences in maize were among the first utilized in testing the generality of MENDEL'S doctrine of the independent segregation of unmodified determiners. They served as material for the early investigations of DE VRIES, CORRENS, LOCK and WEBBER. Among the characters employed, the starchy and sweet types of seed formed a useful contrasting pair, as the difference was marked, segregation was distinct and in practically every case the inheritance clearly followed the rule of the monohybrid. More recently conditions have been described and pictured which seem to indicate that this simple state of affairs in the endosperm of maize does not always exist. Semi-starchy endosperms more or less intermediate in appearance between true starchy and true sweet types have been noted and where the inheritance of such conditions has been followed no clear-cut segregation has been observed.

Some of this anomalous material appeared after a cross between a starchy and a sweet variety of maize. A possible inference from this has been that a contrasting pair of factors, which usually segregate distinctly, may sometimes affect each other, while in hybrid union, in such a way that segregation is not complete and a re-arrangement of hereditary substances is thereby brought about resulting in new and unstable effects. This state of affairs has been spoken of as contamination of genes. Cases of this kind have been supposed to occur in other material and the whole matter has been a subject for some dispute which has considerable importance, since incomplete segregation or contamination of factors, if it does exist, would seriously limit the usefulness of Mendelian formulas. The investigation to be reported here was undertaken in the endeavor to throw some light on this problem by determining the relation of this new endosperm condition to the ordinary starchy type of seed and to find out the response of this semi-starchy character to selection.

EAST and HAYES (1911) in a publication dealing with inheritance of many characters in maize, reported a large number of crosses involving the starchy and sweet type of endosperm, and found in practically every case that segregation was precise and that no unusual deviations from a monohybrid ratio were obtained. The recessive continued true to type when recovered and tested, except in one instance. This occurred in the progeny of extracted sweet seeds from a cross of dent (EAST and HAYES No. 8, Illinois High Protein strain) by sweet (No. 54, Black Mexican sweet). All but three ears came true to the type of typical recessive sweet seeds. These three unusual ears are described as semi-starchy. One of them is shown in the publication referred to (plate III b, p. 40), and is compared with a pure extracted sweet ear from a sister plant, and with a pure extracted starchy ear. As illustrated the seeds of this semi-starchy ear appear intermediate in the amount of opaqueness and shrinking of the endosperm as compared with the other two ears which may be taken to represent the parental types which went into the cross, as far as the endosperm texture is concerned.

A typical sweet ear, such as the one pictured in the illustration of EAST and HAYES, has the seeds deeply wrinkled and their surfaces strongly contorted with rough, angular projections caused by a pronounced shrinking of the contents of the seeds on drying. In addition to the large depressions, fine wavy markings on the surface of the seeds may also be observed. Sweet seeds are more or less translucent, allowing the outlines of the embryo to be seen, and have a hard, brittle texture of a glassy nature accentuated by lines of fracture which frequently occur. The appearance of sweet seeds is modified by the size and shape of the seeds. Varieties of latent dent type differ considerably from those of latent flint type. Lying next to the embryo a thin layer of opaque white material can be seen, when the seed is broken, which resembles the floury starch of dent varieties. When examined under the microscope, however, the starch grains are unequal in size, irregular in outline and are accompanied by a large amount of amorphous substances making the separation of the grains difficult.

Starchy varieties of maize include several different types with respect to the texture and shape of the seeds. They range from the hard translucent seeds of the pop and flint varieties (*Z. mays everta* and *indurata*) to the soft, opaque seeds of the floury type (*amylacea*). Both soft and hard starch occur together in dent seeds (*indentata*) where the typical indented tip is caused by unequal contractions of the two kinds of starch. The surface of the seeds of all starchy varieties is smooth without the wrinkling and folding of the outer layers and in this respect the two types of maize are generally quite distinct. All classes of starchy maize differ from sweet varieties in the fact that the starch grains, particularly from the white opaque portions of the seed, are large, plump, nearly round in outline and show characteristic markings. It is this ability to complete the development of the starch grains that forms the principal hereditary difference between starchiness and sweetness. This is shown noticeably in chemical composition. Sweet seeds, because they have a lower starch content, have proportionately higher percentages of other ingredients—sugar, protein, fat, ash and fiber. Their high sugar content gives them their name, and is the reason why they are preferred as a vegetable. Their ability to produce sugar has undoubtedly been selected for until the total production in amount of this substance has been increased to a greater extent than in field varieties.

Somewhat recently a type of endosperm coming from China, differing from both sweet and starchy, has been described. This is called waxy and has a peculiar tough consistency distinguishing it from other kinds of maize. Sweet and waxy are complementary in their action in inheritance so that when the two are crossed starchy endosperm is produced. They can be considered as endosperm deficiencies—one lacks one thing; the other lacks something else—both of which are necessary for normal starch production.

The semi-starchy seeds obtained by EAST and HAYES (1911) differed from the pure sweet segregates in having solid areas of opaque white varying in amount and in location in various parts and in different seeds on an ear. But this substance was most noticeable at the center of the seeds, surrounding the embryo, and at the tips of the seeds, at the point of attachment of the stigmas. The seeds were distinctly wrinkled but the surface was not so rough as in the case of the typical sweet seeds. The diameter of the starch grains was compared to the conditions of the two normal segregates (EAST and HAYES's table 10, p. 45) and in this respect the semi-starchy type was found to be intermediate. As men-

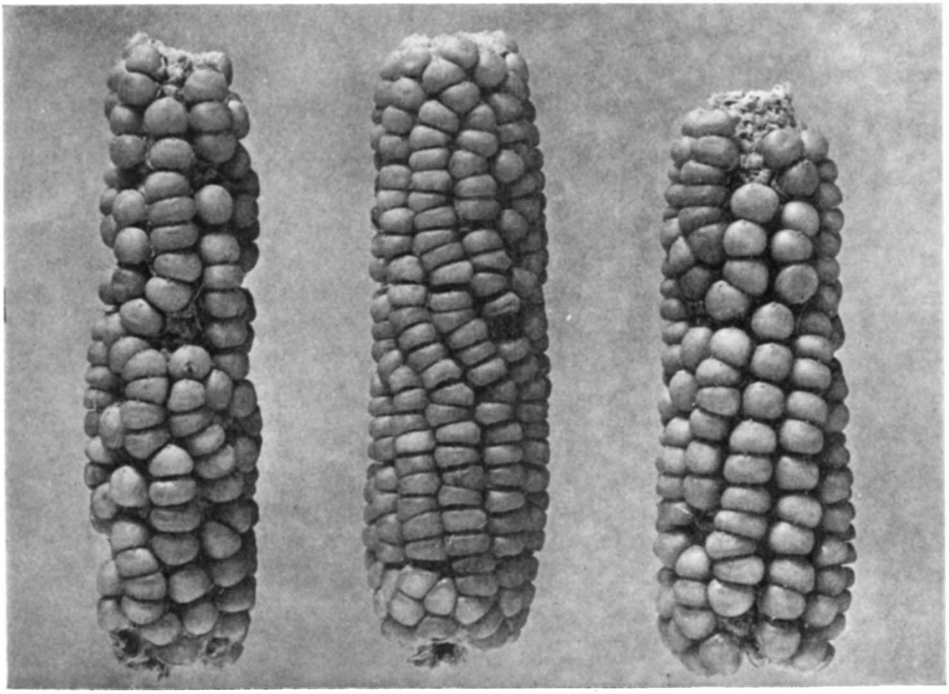

FIGURE 1.—Result of selecting for the most starchy-appearing individual during ten generations of self-fertilization in pseudo-starchy material originating from an extracted sweet seed out of a cross of starchy and sweet.

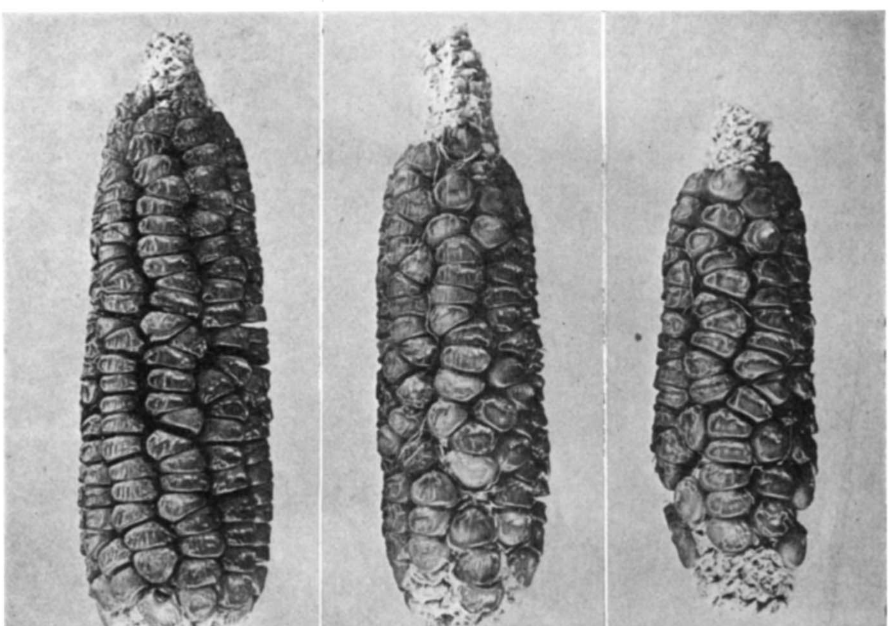

FIGURE 2.—Result of selecting for the most sweet-appearing individual during nine generations of self-fertilization from the same source as in figure 1.

tioned before there was some variation on the ear in the amount of opaque substances in the semi-starchy seeds. The most starchy- and the least starchy-appearing seeds from one of these ears were separated and planted. Some of the resulting ears from these two selected lots of seed are shown in the same publication (plate IV). No great differences are to be observed between these two progenies. Apparently selection in one generation had very little visible effect, although the starchy selection shows slightly more of the opaque white areas than the sweet selection. From these two lines the most starchy ear and the most sweet ear were selected and grown each year thereafter, the plants in practically every case being self-fertilized.

The original cross of dent by sweet (8 × 54) was made in 1907. The semi-starchy ear appeared in the F_2 plant generation in 1909 grown from a sweet seed out of an F_2 segregating starchy-sweet seed population. The first selection was made from the one ear that year and the selection continued to the present time (1918) so that the material on hand represents the result of selecting for two extremes, starchy and sweet, during 10 generations and, in all, 11 generations of self-fertilization after the original cross. This material is not well suited for a selection experiment as hand-pollinated ears only can be used. From 10 to 15 such ears were produced every year in each of the two lines. All of the 1915 seed of the sweet line failed to germinate in 1916 so that seed of the previous year had to be planted and one generation was lost in this way.

The selection and growing of the material was done at the start under the direction of Dr. E. M. EAST and later of Professor H. K. HAYES up to the year 1915 when the experiment came into the hands of the writer who wishes to acknowledge his indebtedness for the material and the outline of the methods of carrying on the experiment. The writer is particularly under obligation to Dr. EAST for his examination of the material for the past several years, for his help with the interpretation of the results and criticism of this report.

After 10 years of selection in the starchy line and 9 years in the sweet line the two resulting types are shown in figures 1 and 2. These ears were grown in 1917 but are like the ones grown the past year so may be used to represent the conditions at the close of the period of selection. It will be seen in these reproductions that the ears of both lines are as different in appearance as any sweet and flint varieties. The ears of the sweet selection vary somewhat in the amount of opaque substance, but on the whole are as clear and wrinkled as most varieties of the latent flint type and more so than many. On the other hand the seeds of the most starchy-appearing ear of the other selection are perfectly smooth, plump and wholly opaque without patches of wrinkling or translucence. Other ears of this starchy line show the effect of some shrinking but for the most part are quite smooth and opaque. The depressions where they occur are in the form of dimples rather than angular distortions on the surface although there are areas on a few seeds which closely resemble the wrinkled condition of sweet seeds.

The three ears of both lots represent fairly well the range of variation. These two selections have come true to these types during the four years that they have been under my observation. Just when they first appeared as they now are and as constant, the records do not show. A few ears of both lines grown in 1913 after 5 years of selection are available and show as great differences as at the present time. Whether or not all the ears were as different as these, that is, whether the variation was any greater at that time, is not known. The differences between individual ears are so slight that no statistical way of presenting the fluctuations within the lines or the distinction between the two has been attempted. Since there was little response in the first generation apparently selection brought about the maximum differences in four generations. After the first five generations changes have been slight until during the last four or five years no visible alterations have taken place.

It is apparent, therefore, that selection operating with this semi-starchy character which originated in a rather questionable manner, has had a profound effect on the end results. Types visibly as different as the two parents which were crossed have been recovered from an intermediate condition suggesting incomplete segregation of the determining factors. Without other evidence to judge from one might be justified in saying that here is a case of a blending of heredity factors. The blend has been variable and from it by selection parental types have been gradually reestablished and stabilized. Nowhere is there evidence of definite segregation. Fluctuation is the rule until constancy is obtained only after several years of selection. Even the usual interpretation of quantitatively variable characters as due to many determiners meets with a hindrance here as in such cases selection, after crossing, is followed by an immediate response and the effect is greatest right at the first.

When these two types, which are visibly so different, are crossed with any true starchy variety the beginning of an understanding of the true situation is made. The selected sweet strain fertilized with starchy-carrying pollen shows perfect dominance of starchiness and segregation in the next generation, as expected, in the 3 to 1 starchy-sweet ratio. The selected starchy strain pollinated from the same source shows no dominance of starchiness because it is already starchy *in appearance,* but in the next generation it segregates 3 to 1, *starchy* and *sweet,* just like the first cross. This is enough to make us suspect the nature of the starchy-appearing strain. Upon examination, the starch grains of this pretending amylaceous selection are still far from being the same as those of true starchy seeds. In this respect dominance is shown in the cross just mentioned. From its behavior in heredity, and because of the difference of the starch grains this condition of the endosperm of maize that we are dealing with is called pseudo-starchy. This character is considered to be distinct genetically from true starchiness and no particular significance is attached to the fact that it appeared after a cross of garden and field types. In its inheritance it is independent of true starchiness. Moreover it occurs in other types of sweet maize which so far as known have not been crossed with the dominant endosperm. Thus selection is as far from reproducing true starchiness at the close of the experiment as it was at the start and segregation of the two principal allelomorphs in the original cross was exact and not accompanied by any unusual processes. This pseudo-starchy character will now be described more in detail as well as its behavior when crossed with true sweet and true starchy endosperms.

One of the principal points of difference between the three types of endosperm is to be found in the starch grains. In figures 3, 4, and 5 are shown the magnified particles obtained from starchy seeds, from seeds of the pseudo-starchy selection and from the sweet selection. The seeds were soaked in weak alcohol for about ten days, then cut in half and a smear obtained by pressing the cut surfaces against a glass slide. This material was stained with iodine, dried and mounted in balsam. The size and condition of the grains is comparative. The starchy seeds used were of the dent type, large, well developed with considerable areas of white, floury starch. Most of the starch grains in the illustration came from these areas as such grains separated the most easily when the smears were made. As can be seen these grains are large and nearly round in outline. Those from the wrinkled sweet seeds are small, indistinct, irregular in outline and most of them are many-sided, being pentagonal or hexagonal. They are accompanied by a large amount of amorphous substances which causes the grains to aggregate. Large particles are shown in the photograph which are probably not single grains but groups. The smear from the pseudo-starchy selection resembles more nearly that from the sweet seeds than from the starchy seeds, but the grains are more distinct and larger. They are quite angular, giving the impression that they have been tightly packed together.

When typical seeds of the pseudo-starchy selection are cut into they are found to be brittle and in this respect very much like typical sweet seeds. At the center, above the embryo, there is usually a cavity surrounded by a small area of opaque white substance resembling floury starch, but there is a marked difference in consistency. In true starchy seeds the floury portions can be easily scraped out and are much like chalk. In the pseudo-starchy seeds the white areas are tough and cannot be easily separated from the other parts of the seed. When examined under the microscope the differences are as shown in figures 3 and 4. Around the white area and making up the bulk of the endosperm is a hard layer having much the same texture as sugary endosperm but more opaque.

The principal visible difference between pseudo-starchy and sweet seeds is that the former on drying shrink in the center and contract towards an outer hull which retains its shape, whereas in sweet seeds all parts contract towards the center on drying leaving the surface contorted and irregular. In behavior the sweet and pseudo-starchy seeds are considerably alike. Both are more subject to decay while maturing than starchy seeds although they differ in this respect, the sweet seeds being more susceptible.

In chemical composition pseudo-starchiness and true starchiness are more nearly alike as shown by the results given in table 1. These figures were obtained under the direction of Dr. E. M. BAILEY in the analytical laboratory of the CONNECTICUT EXPERIMENT STATION. The determinations are averages of closely agreeing duplicates and have been made according to the official methods of analysis. The results for the starchy and sweet types agree fairly well with other published data (PEARL and BARTLETT 1912) in everything but the soluble and insoluble carbohydrates. It is thought that these differences are largely due to the methods of analysis and that the three determinations given in the table are comparative. It should be remembered that the results represent only a gross chemical analysis. Great differences may exist and not be shown by similarity in percentage content of soluble and insoluble carbohy-

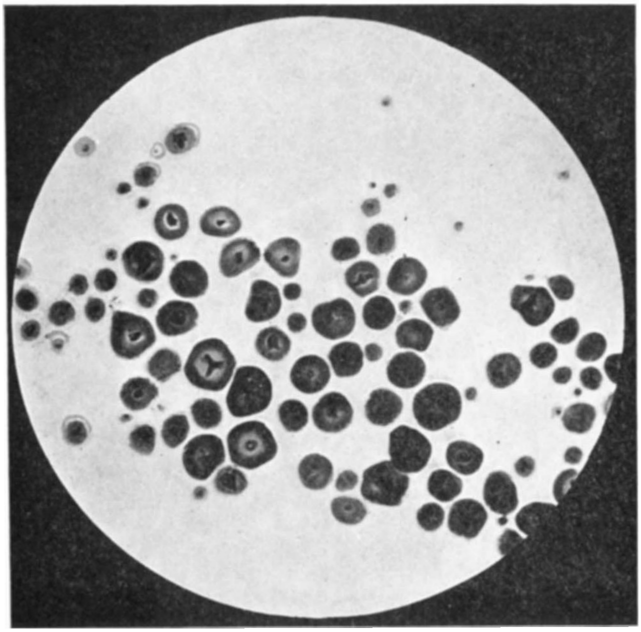

FIGURE 3.—Starch grains from true starchy seeds.

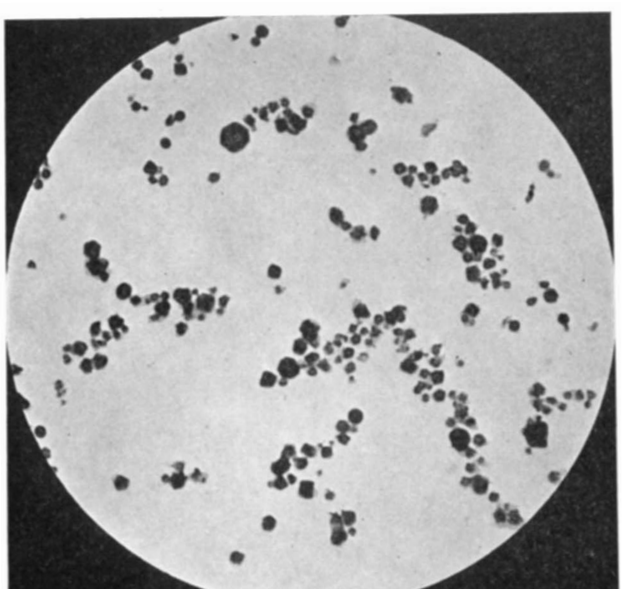

FIGURE 4.—Starch grains from pseudo-starchy seeds.

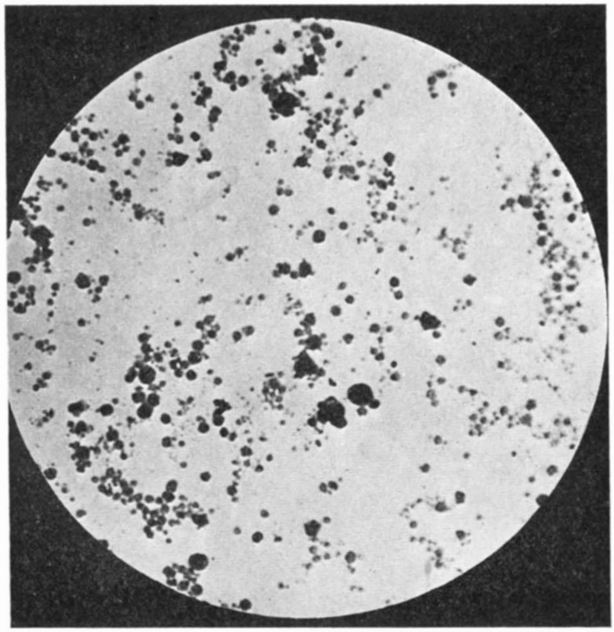

FIGURE 5.—Starch grains from sweet seeds.

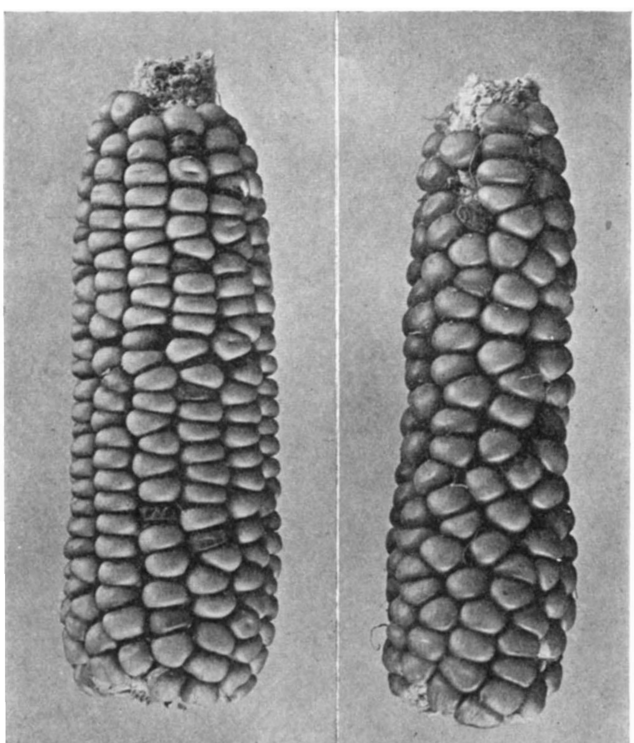

FIGURE 6.—Pseudo-starchy ears showing a few anomalous translucent seeds.

TABLE I
Chemical composition of pseudo-starchy seeds compared to true starchy and sweet seeds.

Ingredients	Sweet	Pseudo-starchy	Starchy
Moisture	10.60	11.09	11.41
Ash	2.07	1.65	1.46
Protein	14.51	12.69	11.60
Fiber	1.89	1.73	1.31
Fat (crude)	7.52	5.94	4.07
Total soluble carbohydrates as dextrose	26.15	7.19	4.45
Insoluble carbohydrates, starch	29.00	51.50	58.73
Undetermined nitrogen-free extract by difference	8.26	8.21	6.97
	100.00	100.00	100.00

drates. As far as the figures are significant the pseudo-starchy maize is intermediate in moisture content, somewhat more like sweet maize in percent of fiber, crude fat and undetermined nitrogen-free extract. In ash, protein and particularly soluble and insoluble carbohydrates the pseudo-starchy type approaches very closely to the condition of true starchy maize.

Summing up these statements it can be said that in external appearance and in gross chemical analysis the pseudo-starchy type is much like starchy maize. In the nature of the starch grains and texture of the endosperm it is more like sweet maize. In its behavior in crosses it is wholly unlike the true starchy character and in this respect differs also from pure sweet endosperm as will be shown later.

This pseudo-starchy endosperm seems to be entirely unlike the waxy endosperm described by COLLINS and KEMPTON (1913). No crosses have been made between the two types as yet, but the brittle, glassy texture is quite unlike the tough consistency of waxy seeds. This pseudo-starchy is apparently a new kind of maize to be added to the long list of types based on endosperm constitution. STURTEVANT (1899) described semi-starchy ears for which he proposed the specific name of *Zea amyleasaccharata*. He had only three specimens upon which to base his description. They showed an intermediate condition probably similar to the original ear from which selection was started in this experiment. There is hardly a doubt but that STURTEVANT was dealing with the same character, although not so highly intensified, as in the selected material described here.

OCCURRENCE OF ANOMALOUS ENDOSPERM IN OTHER MATERIAL

This character in a partially developed condition occurs quite commonly; more or less of it can be found in nearly every variety of sweet corn. It is found sometimes rather highly developed and causes the seedsmen and sweet corn growers some concern. Just what effect this character has upon the quality of the product for table purposes is undecided. Chemical analysis indicates that it is undesirable. The percent of soluble carbohydrates (sugars) is reduced from 26.15 in the sweet selection to 7.19 in the pseudo-starchy material. But many sweet varieties, highly esteemed for their "quality" (which usually means high sugar content) show considerable amounts of this pseudo-starchiness, notably Golden Bantam and Black Mexican. The latter variety often has large quantities of this substance which is obscured by the colored aleurone.

The starchy-appearing character is most pronouncedly developed in the extremely early varieties of sweet corn. Early Dawn, Malakhov and other early ripening sorts have some seeds which are almost entirely opaque but still show some wrinkling. Apparently this character increases the resistance of the seeds to decay, helps the germination of the seeds and the hardiness of the seedling plants grown from them. There is a notable difference in this respect between sweet and starchy maize. There is also a difference between the sweet and pseudo-starchy seeds in this respect but not so great. As stated before, all the seed of the sweet selection failed to germinate one year although the pseudo-starchy seeds germinated well. In the production of early, hardy varieties of sweet corn this pseudo-starchy character undoubtedly has value. This earliness and hardiness, however, are obtained with some sacrifice of sugar content.

Halsted (1909) has observed the same character and has practiced selection in open-pollinated cultures. The starchiness first appeared in a cross of two sweet varieties, Malakhov and Premo. The material resulting from this cross he called "Malamo." After selecting for starchy-appearing ears without hand pollination for several years, he obtained one ear which was practically solid flinty in appearance. From this ear he grew 66 ears of which 34 were flinty and 32 generally sweet. The sweet extremes were nearly like pure sweet. Flint extremes were found with a few slightly wrinkled kernels as were ears showing all gradations between the two extremes. His conclusions in regard to the matter are as follows:

"A study of the whole set of ears leads one to feel that in some instances the flintiness or its absence seems to indicate a plant character, for in some ears the sweet grains are the rare exceptions and in others the flinty ones are scarce. Sometimes an ear will have all its grains in a semi-flinty condition. Again, one may be led to the opinion that the grain in its starch unum acts quite independently of its neighbors, for among a large number of smooth ones will be a strongly wrinkled one."

Although HALSTED was not working with controlled cultures there is a close agreement between his results and our own. The character is quantitatively highly variable and responds to selection. It is controlled by heredity determiners which seem to be partly plant factors and partly seed factors.

A point to be noted in regard to HALSTED's results is that this character arose in a cross of two sweet varieties, not with a starchy variety as was the case in the present experiment. Yet, if he had practiced selection with controlled pollinations he would undoubtedly have obtained as highly developed and as pure a starchy strain as we obtained, because many of his individual seeds and ears showed this character fully developed. Of course, all varieties of sweet corn are subject to more or less crossing with field varieties, and it is possible that HALSTED's material was so crossed previous to his taking up selection with it, but the impression is reinforced from his observations that there is no necessary relation between the pseudo-starchy character and true starchiness.

The last statement of HALSTED's referring to the occurrence of a few strongly wrinkled seeds among many seeds which are smooth, brings up another matter the discussion of which has been reserved to this point. In our starchy selection ears were obtained which were perfectly smooth and opaque with exception of one, two, or several translucent and slightly wrinkled seeds. The difference between the two kinds of seed was abrupt, there being no transitional seeds upon the ears. Figure 6 shows two such ears, one with five seeds and the other with one seed of this kind. Not all of the ears obtained each year showed these seeds but usually several did. During the last four years such ears have been avoided in selecting the individual for continuing the starchy line. In 1917 one ear was obtained which had several translucent seeds. These were planted and from them one selfed ear was secured. This is like its parent ear. All the seeds are smooth, plump and perfectly opaque with the exception of two translucent seeds one of which is slightly wrinkled. These two seeds are perfectly distinct from the others. No other ears which contained seeds of this kind were found in the starchy selection that year. Apparently this phenomenon is the same or similar to what sometimes occurs in true starchy maize. The writer has obtained one self-pollinated ear of true flint type with three translucent wrinkled seeds resembling sweet seeds in a total of some three or four hundred seeds. Such a proportion could hardly be a chance deviation from a 3 : 1 segregation on the supposition that the plant which produced the ear was accidentally crossed by sweet-carrying pollen in the year previous. Neither can it be a segregation distorted by lethal factors, unless such factors act upon the pollen alone, as the ear is fully developed with no missing seeds.

Various hypotheses have been advanced to shed light on the occurrence of anomalous-endosperm seeds in maize. Whether they are due to somatic mutation, cytological aberrations or unknown causes still remains in doubt. The subject has been reviewed by EMERSON (1918). It is found that most of the anomalies of this kind occur in hybrid seeds. In this pseudo-starchy material and the flint ear just mentioned, we are dealing with a homozygous type or very nearly such. The translucent condition of the endosperm does not correspond with the embryo because such seeds reproduce the normal type. Yet the *tendency to produce the aberrancy* seems to be inherited in some fashion since the number of the seeds has become less in the later generations of selection until in the last year no ears with off-type seeds were obtained except in one case in which the parent seed was itself off-type. The material under consideration has not been sufficiently investigated in this connection to warrant any further treatment of the matter. The occurrence of these peculiar seeds will be considered as a problem in itself and as unrelated to the variability of the ears as a whole with respect to the degree of development of the pseudo-starchy character.

BEHAVIOR OF PSEUDO-STARCHINESS IN CROSSES

Since from six to ten generations of self-fertilization is ordinarily sufficient to bring about nearly complete homozygosis, confidence can be placed in the belief that the plants which were used in the crosses represent fairly pure types. The crosses were made in 1915 after the parents had been self-fertilized eight years. The plants were quite uniform and considerably reduced in size and vigor. This fact furnished a very reliable check on the accuracy of the results since whenever foreign pollen gains access to the silks by accident such out-crossed plants the next year show a great increase in size and vigor so that they cannot be possibly mistaken. Moreover the leaf characters and plant habit are so characteristic that unintentional pollinations can be detected with a high

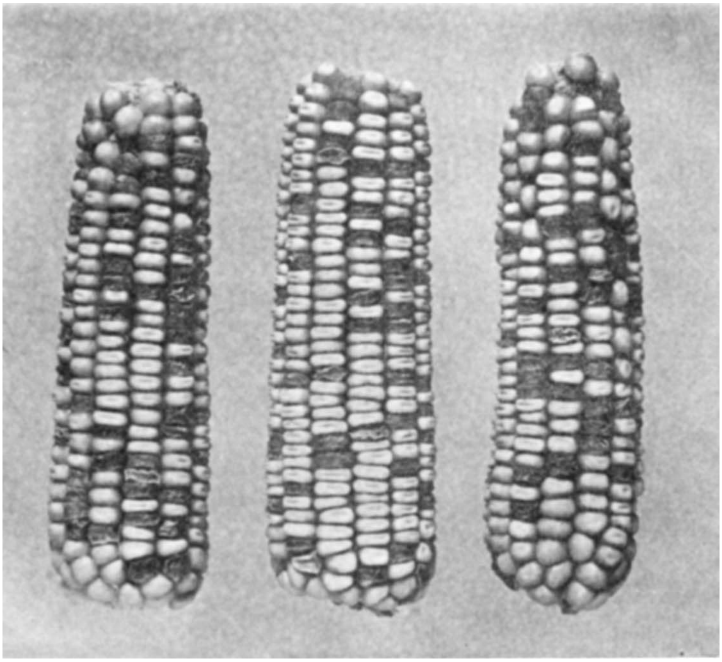

FIGURE 7.—F_2 seed populations from a cross of true starchy by sweet.

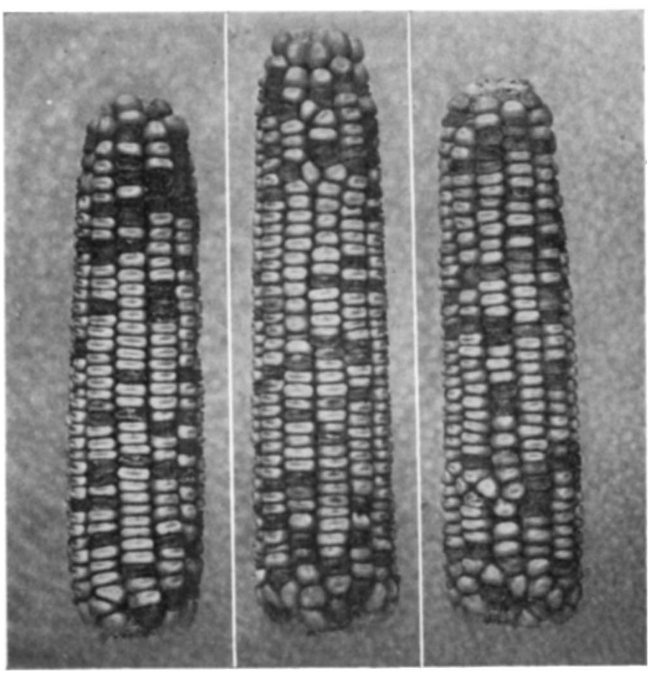

FIGURE 8.—F_2 seed populations from a cross of true starchy by pseudo-starchy.

degree of surety. Not many of such illegitimate plants were found in the course of this experiment but a few seem to be unavoidable.

When pseudo-starchy and true starchy plants are crossed the result in the second generation is, at first sight, exactly the same as when sweet and starchy are crossed as has been stated before. The numbers obtained from reciprocal crosses and back-crosses are given in table 2 and show

TABLE 2

The number and ratio of starchy and sweet seeds obtained in F_2 and in back-crosses from reciprocal crosses of pseudo-starchy and true starchy endosperm.

Second generation selfed	Starchy	Sweet	Total	Ratio per 4	Deviation	Probable error
Starchy × pseudo-starchy, F_2......	1832	550	2382	3.0764: .9236	0.0764	0.0239
Pseudo-starchy × starchy, F_2......	1918	761	2679	2.8638: 1.1362	0.1362	0.0226
Total	3750	1311	5061	2.9638: 1.0362	0.0362	0.0164
Back-crosses with sweet				Ratio per 2		
Starchy × pseudo-starchy) × sweet	832	779	1611	1.0329: .9671	0.0329	0.0168
Sweet × (starchy × pseudo-starchy)	494	468	962	1.0270: .9730	0.0270	0.0218
Pseudo-starchy × starchy) × sweet	1280	1401	2681	.9549: 1.0451	0.0451	0.0130
Sweet × (pseudo-starchy × starchy)	241	310	551	.8748: 1.1252	0.1252	0.0287
Total	2847	2958	5805	.9809: 1.0191	0.0191	0.0124

no marked deviations from the expected ratios. Segregation is definite and the ears look like those obtained from the other cross. Samples of these two lots of ears are shown in figures 7 and 8. Instead of the pseudo-starchy endosperm coming out in F_2 as it went into the cross it appears as sweet endosperm. When these wrinkled segregates are examined closely, however, they are seen to contain more of the opaque substances than similar seeds of the other cross. Apparently true starchiness and pseudo-starchiness are genetically independent of each other and the former has such a pronounced effect that it overwhelms, for the time being, whatever differences there may be between pseudo-starchy and sweet endosperms. It is important to note that the factors brought in by the starchy parent greatly alter the size and shape of the seeds and for this reason cause the new endosperm character to be very much reduced in expression and in fact almost obliterated.

When the two selected lines, sweet and pseudo-starchy, are crossed reciprocally, very little immediate effect can be seen. In this respect also pseudo-starchiness differs from true starchiness. When the sweet selection is used as the female parent the seeds show faint traces of opaqueness, usually at the tips of the seeds, but the whole effect is far from an intermediate condition. The reciprocally crossed seeds show slightly more shrinking and approach the sweet condition about as far as in the reverse case. The self-pollinated ears grown on F_1 plants, either way the cross is made, are distinctly intermediate with a large range of variation in the seed populations on an individual ear as well as among the ears themselves. Three F_1 ears selected to show the range of variation between different ears and in the seeds on an ear, are reproduced in figure 9. It can be seen that one ear is almost entirely sweet, one is nearly starchy with some appearance of segregation and one is distinctly segregating but no sharp differences between individual seeds can be seen. All gradations from smooth opaque to wrinkled translucent seeds can be found on this ear. The dark-colored seeds on these ears have been attacked by mold. The differences between the individual seeds, it can be assumed, are due to genetic differences in endosperm factors coupled with some environmental variation. The differences between individual ears cannot be so easily considered to be due to genetic differences since they are produced on F_1 plants and the parents have been assumed to be homozygous. Of course, the fact that the parental lines were self-pollinated for a number of generations does not insure homozygosity. MULLER (1918) has demonstrated ways by which enforced heterozygosity may be maintained by various systems of lethal factors, particularly when two different lethal factors balance each other by being in homologous chromosomes. When this condition occurs together with factors which reduce or prevent crossing over, almost every conceivable kind of unusual results can be produced. While some such state of affairs may exist in this material no definite evidence of this has been found. Moreover maize is particularly favorable material in which to detect lethal factors, as the whole progeny of a plant is produced in an inflorescence where commonly all the seeds are placed in regular order. All lethals which destroy the female gametes or stop development immediately after the zygote is formed, show up at once in the missing seeds whose places are distributed throughout the ear. Self-pollinated ears showing regular vacancies are sometimes found and warrant further study but as far as known nothing of this kind occurs in this material under investigation. Lethal factors which destroy male gametes or which prevent the germination of seeds could not be easily detected. It seems more logical, however, to attribute the variations in F_1 ears to

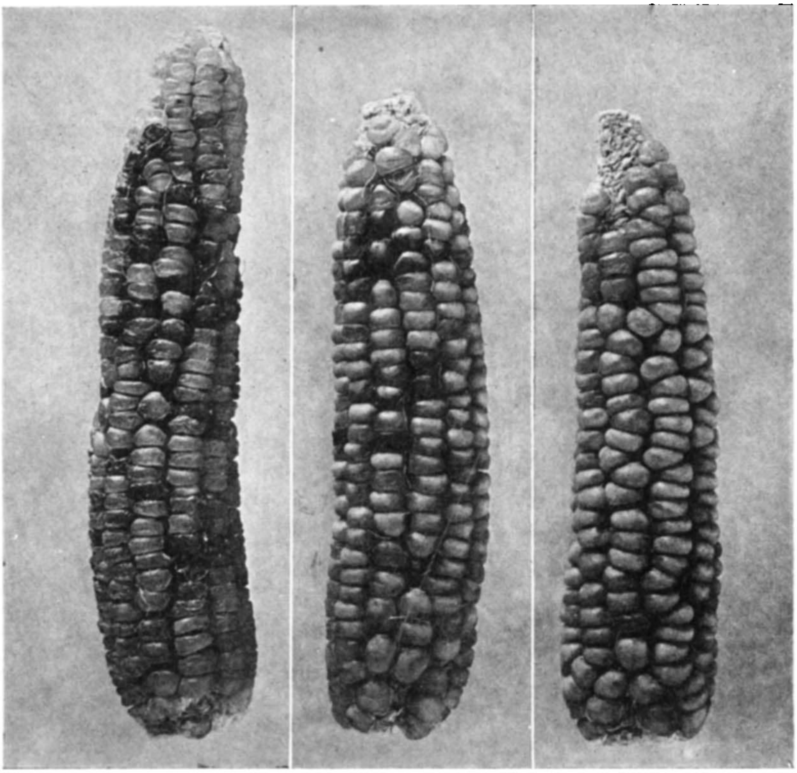

FIGURE 9.—F_2 seed populations from a cross of sweet by pseudo-starchy (dark seeds are discolored by mold).

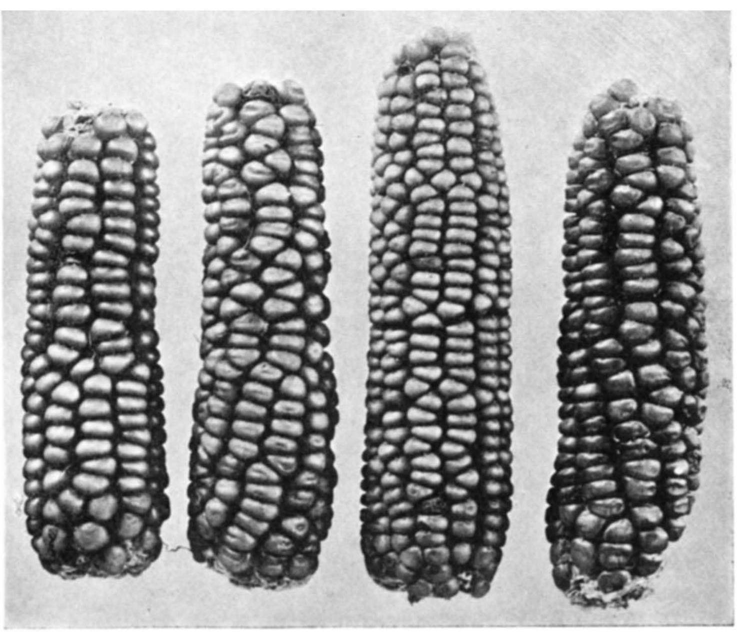

FIGURE 10.—Result of selecting the most starchy-appearing seeds from an F_2 seed population from a cross of sweet by pseudo-starchy.

external effects. Since there is some variation in the parental types, variability in F_1 plants is also permissible, but to the eye the amount of variation is somewhat greater. Differences in time of ripening affect this endosperm condition which is rather unstable. Individual ears are found with the tip seeds more wrinkled and translucent than those at the center of the ears. This non-genetic variability may be greater in the heterozygous than in the homozygous condition.

From F_1 ears like the one in the center of figure 9, which gave the greatest indication of segregation, seeds were selected which showed the most development of opaqueness and seeds which showed this least developed. Selection was made from two such ears. The self-pollinated ears grown from one of these individuals are shown in the accompanying illustrations: the progeny from the starchy selected seeds in figure 10 and from the sweet selected seeds in figure 11. The results from the other ear were similar to those two lots. Selection of the extremes in F_2 seed populations grown on F_1 plants, brings about small visible differences in the next generation. On the whole, however, the most translucent ear of the sweet selection differs considerably from the most opaque ear of the starchy selection. Apparently endosperm factors have something to do with the development of the character under investigation.

None of these ears grown on F_2 plants gave any more evidence of distinct segregation than did the F_1 ears. Some showed segregation more than others, however. This fact is not clearly brought out in the reproductions. The seeds from two segregating ears—one from the sweet, one from the starchy selection—were again separated into most starchy- and most sweet-appearing and grown the following year. Also from the two F_2 plant progenies which had been selected for sweet one ear from each was chosen for seed which most nearly approached the original sweet parent. Likewise from the two F_2 plant progenies selected for starchiness one ear from each was taken for planting which most nearly approached the original starchy parent. This procedure and the results obtained may be outlined as shown in diagram 1.

Since the gradations between individual seeds and between different ears are so small and indefinite there is no good statistical method of presenting the data. In the diagram the progenies resulting from the selections are lettered so that they can be referred to. The results obtained can be briefly stated.

The immediate reciprocal crosses are without any marked effect in

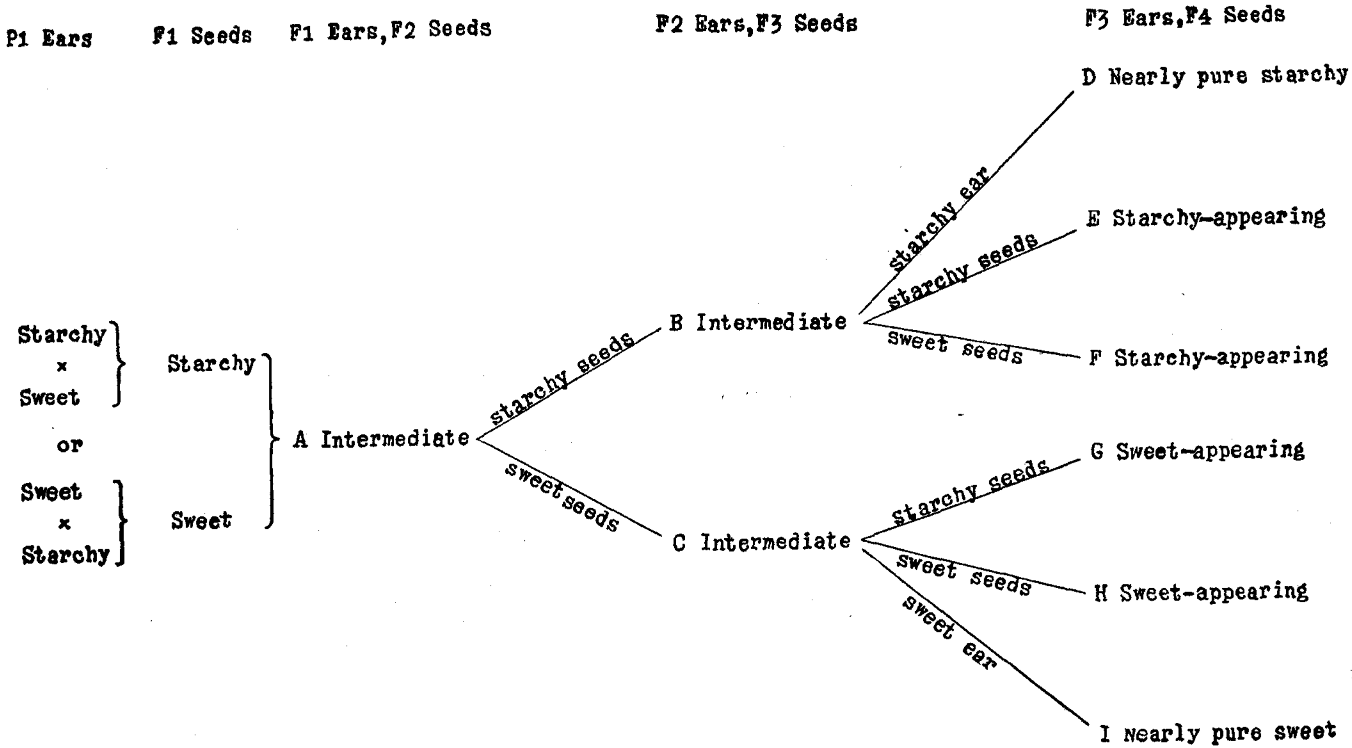

DIAGRAM 1.—To show the method and progress of selection after crossing pseudo-starchy and sweet. The letters designate the selected progenies referred to in the text.

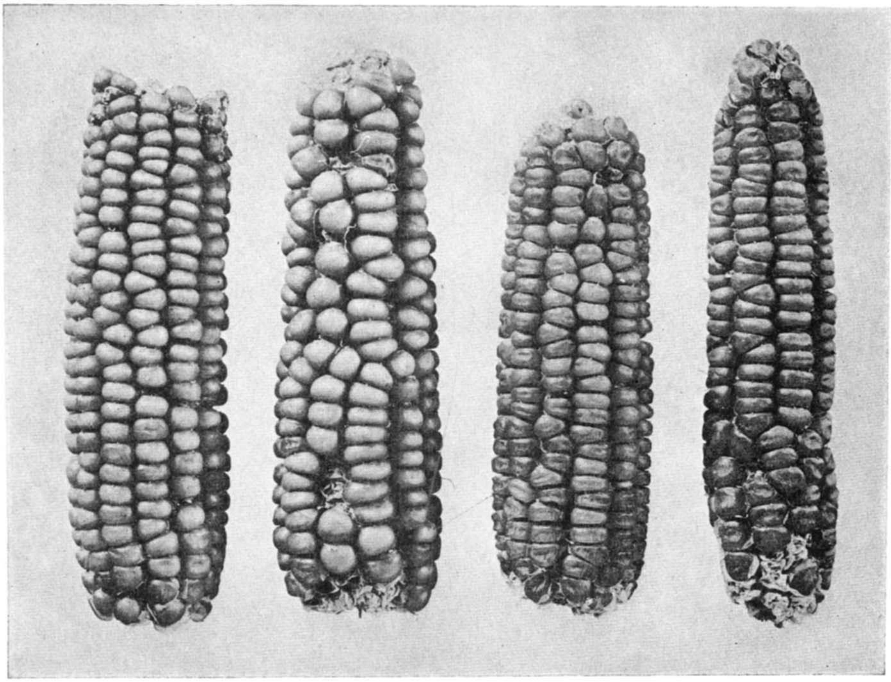

FIGURE 11.—Result of selecting the most sweet-appearing seeds from the same ear as in figure 10.

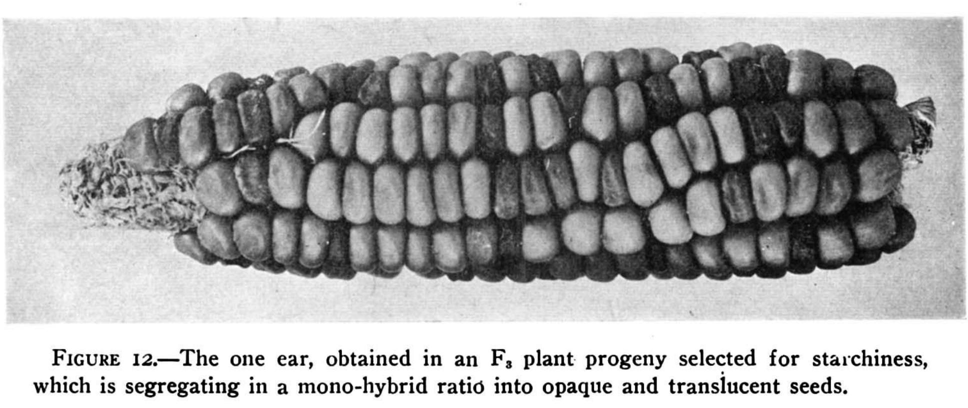

FIGURE 12.—The one ear, obtained in an F_3 plant progeny selected for starchiness, which is segregating in a mono-hybrid ratio into opaque and translucent seeds.

altering the appearance of the seeds. The F_1 ears, A, are intermediate with some variability between different ears, which is assumed to be non-genetic, and show indefinite segregation in the seed populations. Selection of extreme seeds on one of these ears gives F_2 progenies, B and C, which are still intermediate and show some response in the direction of selection although the differences between the two progenies are small. Individual ear selections from these two F_2 progenies give F_3 progenies, D and I, which show a marked response to selection. In the starchy progeny, D, ten self-pollinated ears were obtained which altogether resemble the parent type closely, five of these ears being apparently as pure for the character as any ears of the parental line. No ears were obtained which indicated any marked segregation among the seeds. In the sweet progeny, I, eight ears were selfed all of which show some traces of opaqueness. One or two of these ears, nevertheless, might pass as pure sweet. Some show considerable evidence of segregation. Most of them are uniform and nearly pure sweet. The differences between the two lots, D and I, are almost as great as between the two original types which went into the cross.

The two oppositely selected progenies, E and F, from the starchy selection of the previous year are nearly alike but both together are considerably more starchy than the F_2 ear from which they both came. A similar result was secured from the other two selections, G and H. coming from one ear of the sweet line. Both are almost exactly alike and together are more sweet than the F_2 ear which produced them. Nine selfed ears in each of the E and F progenies are on hand and some of each of these are visibly pure starchy. Eight and eleven ears from the two progenies, G and H, were self-pollinated and in each of these a few ears are visibly pure sweet.

Summing up these results there is proof that segregation of genetic factors has taken place in producing the F_2 seed populations grown on F_1 plants. Selection here, however, is not followed by an immediate response in the next generation although there is a slight effect, but in the following generation there is a marked response whether in plant-selected or seed-selected progenies and whether selection was in the same or in the reverse direction. In general selection is followed by a mass action which is as different from the usual types of Mendelian segregation as could be conceived. The behavior resembles that of quantitative characters governed by several factors with overlapping variability because variation is continuous. It differs from the usual cases of this kind in that the changes initiated by selection in the F_2 seed generation do not make their appearance until two generations later. Moreover these changes cannot be checked in one succeeding generation by selecting from seed populations in the reverse direction. In this respect the behavior is unique.

The experiment outlined in the preceding diagram was duplicated with a reciprocal cross with the exception that selections from F_2 seed populations were not made, therefore the F_3 progenies, E, F, G, and H, were not grown. The two selected progenies, D and I, were grown with the same result as in the previous experiment with one notable exception in the starchy line.

In the sweet line eight ears were self-pollinated. A few are nearly pure sweet while some show slight amounts of opaqueness, some of these having indications of segregation. In the starchy line twelve ears were obtained. Eleven of these duplicate the similar ears in the first experiment. All of the ears are uniform and very nearly pure starchy. Some are entirely opaque and smooth with no indications of segregation. The remaining ear, however, shows clear-cut segregation into opaque and translucent seeds with a ratio of 3 to 1 (actual numbers 159 to 52). No other ear like this has been found. Contrary to the usual behavior segregation in this one instance is quite distinct. This ear is shown in figure 12. The first supposition is, naturally, that it resulted from an accidental out-cross with a true starchy plant the year previous. A number of facts make this possibility highly improbable. In size and conformation this exceptional ear closely resembles the eleven other ears of the same lot. Also in color of cob and seeds and in shape, size and texture of the opaque seeds there are no differences. Since the type of plant in this material is quite different from any other grown at the same time it is inconceivable that this individual could have been crossed without marked changes. Moreover the plants are reduced by inbreeding, this particular one in question being the third generation of a union of two related types, so that any out-crossing with a true starchy plant would noticeably increase the size of the plant and the ear. One such crossed plant was found in another progeny. This plant was nearly twice as tall and the ear three times as large as others in the same line and striking changes in plant and ear characters served to distinguish this individual at once. The exceptional ear under consideration was not noticed in the field at time of harvesting as starchy-sweet segregations are not clearly brought out until the seeds are dry. If the plant had shown any striking differences, however, this would have been noted. There is no doubt in the mind of the writer but that this singular ear is a legitimate member of the family and must be considered at its face value. Moreover, the recessive segregates on this inflorescence are not strongly wrinkled as can be seen. In fact there is a considerable difference between these seeds and those of the sweet selection. The feature that makes them stand out is their translucence as contrasted with the opaqueness of the other seeds. It is therefore reasonable to assume that these sweet-appearing individuals are the result of a different factorial situation than is characteristic for other sweet varieties.

Before taking up a theoretical discussion of these results one more phase of the experiment must be considered. The cross of pseudo-starchy and true starchy maize has already been mentioned as giving clear segregation of starchy and sweet seeds in the F_2 seed populations. The sweet segregates were stated to have somewhat more opaque substances than similar segregates of the sweet-starchy cross. The two lots of sweet segregated seeds from one ear of each of the two crosses were grown with the result that the ears coming from the cross of which pseudo-starchy was one parent, were considerably more opaque than those from the other cross. No completely pseudo-starchy ears were obtained but one selfed ear was found which showed more evidences of definite segregation than any secured previously. The seeds ranged from those almost entirely smooth and opaque to those which were quite wrinkled and translucent with gradations in between. The numbers of the two kinds of seeds seemed about equal. Selection of extremes was made and the two resulting lots grown from these seeds and self-pollinated are shown—the starchy selection in figure 13 and the sweet selection in figure 14. From the selected wrinkled, translucent seeds nine selfed ears altogether were self-pollinated (three of these are not shown in the illustration as they were nearly destroyed by mold). Of these two are completely wrinkled and translucent, five show some opaqueness but are uniform and two are clearly segregating. One of these latter two (the ear at the right in figure 14) reproduces very well the parent ear from which the selections were made.

From the selected smooth, opaque seeds six selfed ears were harvested. Of these one is quite smooth and opaque, three are intermediate and uniform and two are intermediate with considerable evidence of segregation.

These results can be summed up briefly. In one selection the ears range from the selected parent type to a half-way point. In the opposite selection the variation goes from the half-way point to the other parent. In this respect they differ from the preceding effects of selection in the pseudo-starchy by sweet material. Segregation is more pronounced and selection is followed by an immediate response. How all these facts can be most simply interpreted will be taken up in the next section.

A large number of crosses and back-crosses between the pseudo-starchy type and commercial varieties of sweet maize were made, but since all of these varieties carried more or less of the pseudo-starchy character and were in a heterozygous condition for many factors, no satisfactory analysis of the results can be made. In general the pseudo-starchy character behaved in these crosses similarly to the crosses already described.

THEORETICAL CONSIDERATION OF THE RESULTS

As far as the chemical nature of this visibly starchy endosperm is concerned the use of the term pseudo-starchy is perhaps not justified. The term was suggested by its genetic behavior largely for want of a better word which would emphasize the distinction between characters which are visibly alike but genetically so different. The term semi-starchy is undesirable because it implies a relaton between the new endosperm condition and the well known starchy endosperm where no relationship apparently exists.

Since reciprocal pollinations between pseudo-starchy and sweet have no marked effect the character differs in its mode of inheritance from ordinary endosperm characters where dominance is displayed and distinct segregation takes place in the next generation. On the other hand it is not entirely a plant character since segregation does occur in F_2, because selection made here is somewhat effective. It was thought at first that the character was similar to the corneous-floury endosperm character of starchy maize where (as worked out by HAYES and EAST 1915) the double condition of the female endosperm nucleus outweighs the effect of the second male nucleus and reciprocal crosses do not produce any immediate effect. In the next generation segregation is in the ratio of 1 to 1 which is merely the female gametic ratio, the second male nucleus, whatever it carries, having no power of expressing itself. The hypothesis of one such factor alone cannot be used to account for these results, particularly since it would offer no explanation for the one ear which was finally obtained where segregation is well defined and in the ratio of 3 to 1.

The simplest hypothesis that brings all the facts into line assumes both

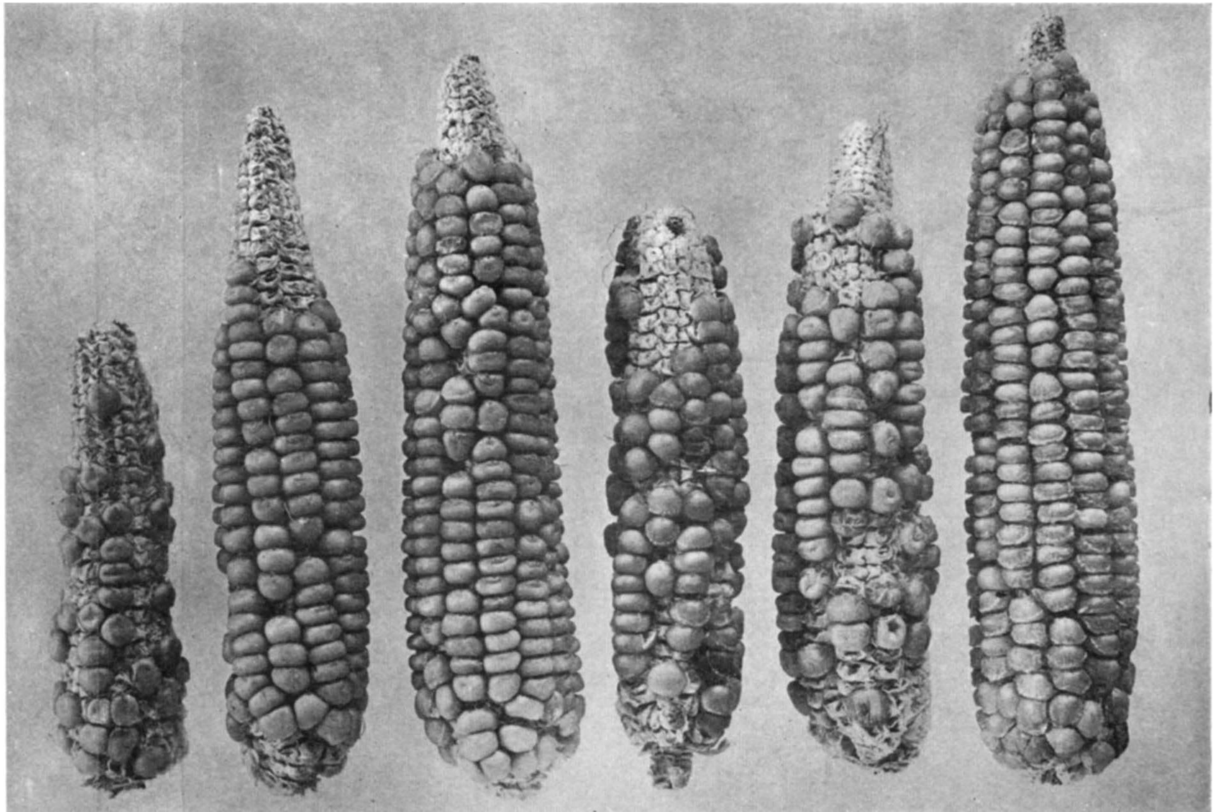

FIGURE 13.—The result of selecting the most starchy-appearing seeds from a segregating ear obtained in an extracted sweet F_2 plant progeny from a cross of pseudo-starchy by true starchy.

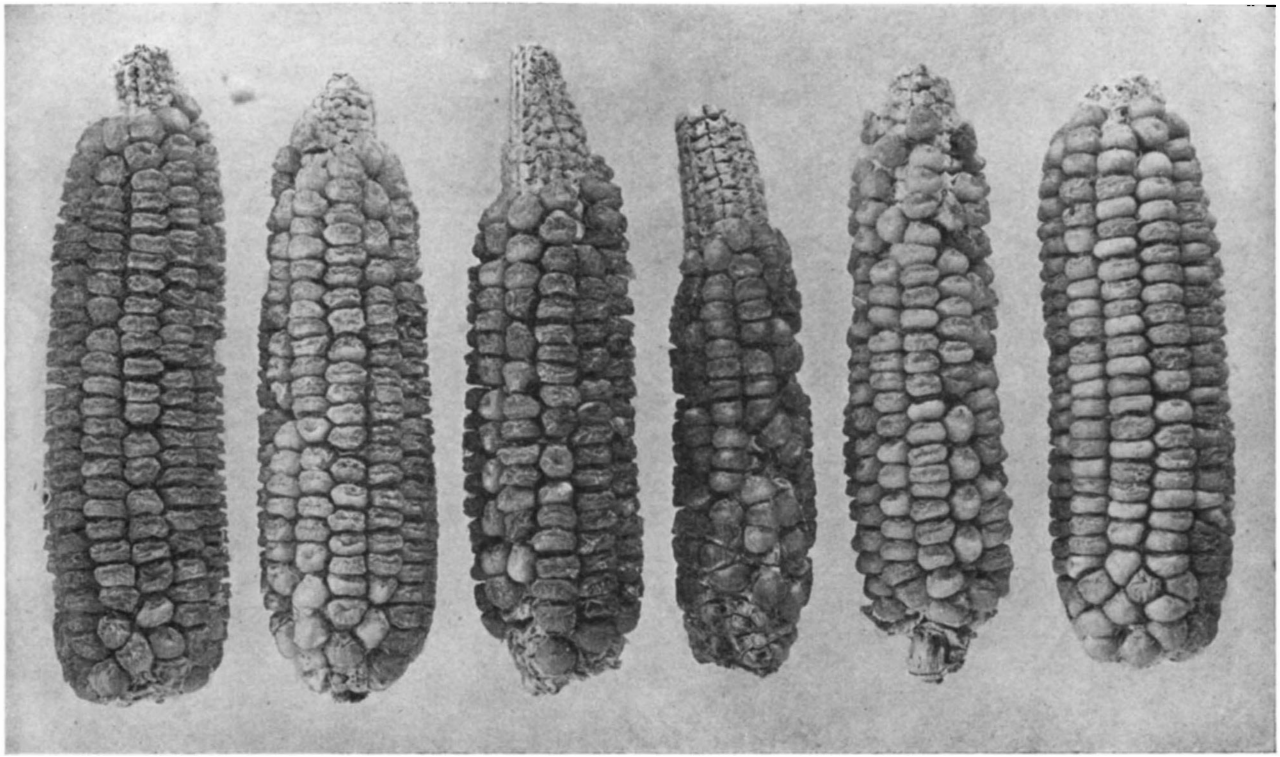

Figure 14.—The result of selecting the most sweet-appearing seeds from the same source as in figure 13. This parent ear is closely reproduced in the one shown at the right.

seed factors and plant factors which govern the development of this endosperm character called pseudo-starchy. The employment of plant factors which determine endosperm characters has not previously been necessary. It seems perfectly plausible, however, for an endosperm character to be dependent in some degree upon plant conditions. For example, a certain chemical substance may be needed to bring about a given result but if the plant does not supply the material the effect cannot be produced whether the immediate factor determining its production is there or not. Furthermore, we have seen that when pseudo-starchy endosperm is out-crossed to a large-seeded dent variety the resulting alteration of the shape and size of the seed and other heterotic effects governed by plant factors almost completely obliterates the expression of the pseudo-starchy character. Many factors in the maize plant determine the size and shape of the seeds; the arrangement of the corneous and floury starch, the thickness and consistency of the pericarp, the arrangement and size of the ears, so that it is quite permissible to assume that the character under investigation is modified by many plant factors which have no direct relation to endosperm factors themselves.

In addition to plant determiners there must be at least one endosperm factor difference which, with a suitable basic factorial composition, determines the production of opaqueness or translucence in the seeds. The evidence for this is based on only one segregating ear but it is difficult to avoid this assumption. The visible effect of this gene, in a proper medium, is much the same as the one governing the well known difference between starchy and sweet varieties of maize. In both cases dominance is complete and segregation is distinct in a mono-hybrid ratio.

Besides this one endosperm factor which brings about usual Mendelian segregation, there is evidence for another of the same nature as the one concerned in the corneous-floury condition of maize, perhaps the very same factor, only here acting in the absence of true starchiness. The part which this gene plays follows the female gamete in its visible effect, i.e., the double dose in the fusion nucleus almost entirely overcomes the single dose coming from the second male nucleus and segregation is in an equal ratio. The evidence for this assumption is based on the segregating ear and the two selected progenies obtained from it shown in figures 13 and 14.

As to the exact number of plant factors involved in the material worked with the rather scanty data and the indefinite nature of the character do not permit any reliable analysis. As an illustration it will be sufficient to assume one such factor of such a nature that without it the two endosperm determiners cannot produce the full expression of the pseudo-starchy character. These three factors, each different in mode of operation, but all working towards the same end, may be used arbitrarily to show what can be expected from such a scheme and I believe they will satisfy all reasonable demands as they are used to show merely how recombination of such Mendelian units *may* account for the results. This can be done without a thorough analysis of all the factors concerned. The three factors assumed are as follows:

A—a plant factor necessary for the full expression of pseudo-starchiness in the endosperm, which part of the organism is one generation beyond the plant in which this factor operates.

B—an endosperm factor which prevents the characteristic shrinking of sweet seeds; this factor has a greater effect when coming from the female side than from the male, i.e., *BBb* and *bbB* appear different but behave genetically exactly alike.

C—an endosperm factor determining opaqueness, which shows dominance, i.e., *CC* and *Cc* are visibly alike. *C* and *c* have their greatest differentiating effect only when *A* and *B* are present in the homozygous state.

It is also assumed that *A* and *B* are cumulative in effect, whereas *C* is not. According to this scheme:

Plant	Seed
AA	*BBB CC* = pseudo-starchy endosperm;
aa	*bbb cc* = sweet endosperm.

The reciprocal pollinations are without effect because of the following factorial situation:

Plant	Seed
AA	*BBb Cc* = pseudo-starchy × sweet;
aa	*bbB cC* = sweet × pseudo-starchy.

In the first cross the double dose of *B* and the dominating nature of *C* prevent any noticeable wrinkled or translucent effects appearing. In the reciprocal pollination the single doses of *B* and *C* are non-operative, or feebly so, in the absence of *A*. (*A*, of course, is brought into the seed, but has nothing to do with the plant.) In the following generation which is the F_1 for the plant and F_2 for the seed, the situation set forth in table 3 can be expected:

TABLE 3

The theoretical composition and the appearance of F_2 seeds with respect to the endosperm factors B and C and their behavior in the following generation according to the plant factor A.

Composition of F_1 plant	Composition and number of F_2 seeds		Appearance of F_2 seeds	Appearance of F_2 ears grown from F_2 seeds according to the composition of the plant with respect to *AA, Aa* or *aa*
Aa	1 *BBB CC*	4	Smooth, opaque	Intermediate, uniform except with *AA* which gives pure pseudo-starchy.
	2 *BBB Cc*	8	Smooth, opaque	Intermediate, variable except with *AA* which segregates 3 to 1.
	1 *BBb CC*	4	Smooth, opaque	Intermediate, variable
	2 *BBb Cc*	8	Smooth, opaque	Intermediate, variable
	1 *bbB CC*	4	Wrinkled, opaque	Intermediate, variable
	2 *bbB Cc*	8	Wrinkled, opaque	Intermediate, variable
	1 *BBB cc*	4	Smooth, translucent	Intermediate, uniform
	1 *BBb cc*	4	Smooth, translucent	Intermediate, variable
	1 *bbB cc*	4	Wrinkled, translucent	Intermediate, variable
	1 *bbb CC*	4	Wrinkled, translucent	Intermediate, uniform except with *aa* which gives sweet appearance.
	2 *bbb Cc*	8	Wrinkled, translucent	Intermediate, variable except with *aa* which gives sweet appearance.
	1 *bbb cc*	4	Wrinkled, translucent	Intermediate, uniform except with *aa* which gives pure sweet.
	16	64		

The F_2 seeds fall into four theoretical groups:

Smooth, opaque	6
Wrinkled, opaque	3
Smooth, translucent	2
Wrinkled, translucent	5
	16

But none of these groups is sharply expressed because of the heterozygous nature of the plant having *Aa*. Moreover there will be variability according to whether the seed has the composition *BBB* or *BBb* and *bbB* or *bbb* as the hypothesis states that *B* is cumulative in effect. These causes for an intermediate position with hereditary variability together with some non-genetic fluctuation are sufficient to account for almost any amount of indefinite segregation in F_2 seed populations. Moreover there may easily be a physiological correlation between smoothness and opaqueness on the one hand and wrinkledness and translucence on the other such that the other two combinations are not perfectly developed.

The behavior of the F_2 seeds in the following generation depends not only on their composition with respect to *B* and *C*, but also upon their carrying *AA*, *Aa* or *aa*. None of these three combinations of this factor have anything to do with the appearances of the seeds that bear them, but they will have a marked effect upon the ears grown from these seeds. The extreme complexity of the F_2 plant population with only these three factors is readily apparent. Moreover, selection of F_2 seeds will have little efficacy in the following generation because of the difference in appearance, but similarity in behavior, of *BBb* and *bbB* together with the fact that the *A* or *a* factors do not make their presence known. Nearly all of the F_2 ears bearing F_3 seeds will be intermediate, some tending towards one type and some towards the other, but part of them will be uniform with respect to the seed population and part will be variable, i.e., will show more or less evidence of segregation. This is an important point as it is the best means of reconciling the theory with the actual results obtained.

Summing up the expectation for the F_2 plants the following numbers are obtained:

1 Pure pseudo-starchy, like parent, breeds true (viz., *AA BBB CC*);
2 Nearly pure pseudo-starchy, do not breed true (viz., *Aa BBB CC*);
11 Intermediate and uniform;
44 Intermediate and variable;
3 Nearly pure sweet like parent, breed true (viz., *aa bbb CC* or *Cc*);
1 Pure sweet like parent, breeds true (viz., *aa bbb cc*);
2 Segregate 3 to 1 opaque-translucent (viz., *AA BBB Cc*).

64

Without going into all the possibilities of this factorial arrangement, the actual results fit in reasonably well although it is granted that the theoretical expectations are sufficiently complicated to cover almost any variable character like this one. Several agreements of facts with theory justify this interpretation tentatively without analyzing this situation completely. Only about ten self-fertilized ears were obtained in each generation in a progeny. Selection of F_2 seeds was followed by very slight response in the next generation. According to the theory 55 out of 64 are expected to be intermediate in various degrees, but the expression of the pseudo-starchy character in the F_2 ear is not closely correlated with the appearance of the F_2 seed which produced it. Selection of F_2 plants however is expected to have a marked effect on the following generation because the extreme ears are homozygous for one or more factors for starchiness or sweetness as the case may be. Moreover the appearance of the ears as a whole in this generation is more closely associated with the genetical composition of the plant so that plant selection is effective.

The segregating F_3 seed populations are like segregating F_2 populations with respect to endosperm factors and seed selection is followed by as little response in this generation as in the preceding. The fact that the two reversely selected progenies in F_3 (F and G in the diagram page 378) differ greatly, can be attributed to the possibility that in the starchy-selected line the segregating ear was homozygous for *AA* and that in the sweet-selected line a similar appearing ear was homozygous for *aa*. Such an occurrence would be somewhat of a coincidence, it is true, but one that could easily happen.

Some of the F_2 ears obtained looked like pseudo-starchy, but two which were tested further did not prove to be a complete return to the pseudo-starchy parent. Nor is this remarkable in view of the small numbers, since only one out of 64 is expected. According to theory it is somewhat easier to recover a true-breeding sweet. None of the F_2 ears approached the sweet parent as closely as the extremes in the opposite direction resembled the other parent, and in F_3 neither of the two sweet-selected progenies duplicated the sweet parent but several individual ears look like pure sweet. By far the majority of the ears obtained are intermediate. Some of these are uniform and some are variable, but it is impossible to classify them numerically as one grades into the other so indistinctly.

No definitely segregating ear appeared in F_2 and only one in F_3 in a total of 22 ears in two separate lines selected for starchiness. In an unselected population such ears would be expected once in 32 times. According to the hypothesis these segregating ears occur only when *A* and *B* are present and homozygous. Perhaps it would be more reasonable to expect them when *A* is heterozygous as well as homozygous. In that case such an ear would be looked for 6 in 64 times in an unselected population and more frequently in a starchy selection. On this assumption, only one ear was found where several should have appeared.

Let us now consider the other selection experiment, starting with a variable ear which, it will be remembered, came from the extracted sweet seeds of a cross of pseudo-starchy by true starchy. Since segregation is more distinct on this ear than upon any ear in the F_2 seed generation of the other cross, the logical supposition is that there are less genetical differences involved in this case. In other words, let us assume that the true starchy variety used in making the cross also possessed some of the factors for pseudo-starchiness as well as the main factor for true starchiness. There is evidence to support this assumption from the fact that other crosses, not specifically mentioned previously, of sweet $\times$ true starchy, showed traces of pseudo-starchiness in the sweet-segregating seeds.

From our scanty data there is no way of knowing how many factors were brought in by the starchy parent. To fit the interpretation to the preceding discussion it will be assumed that there has been a simplification of the genetical composition such that the segregating ear in question is homozygous for the C factor and heterozygous for only A and B. The C factor difference is the one ruled out because no evidence was obtained in this material of a clean-cut segregation approximating 3 to 1 as in the previous case.

The ear used in this second selection experiment, then, supposedly has been produced on a plant homozygous for the C factor and heterozygous for A, and having four visibly different kinds of seeds according to their composition with respect to B, as follows:

$$\begin{array}{c|l} \text{Plant} & \text{Seeds} \\ Aa & \left\{\begin{array}{l} \left.\begin{array}{l}BBB\\BBb\end{array}\right\}\text{selected starchy;} \\ \left.\begin{array}{l}bbB\\bbb\end{array}\right\}\text{selected sweet.}\end{array}\right. \end{array}$$

With the assumption as before, that B does not differentiate sharply, the seeds gradate from nearly pure starchy to nearly pure sweet (see figure 14, ear on the right) and the variation is equally balanced between the two extremes. C is present in all seeds but has little effect with bb and heterozygous Aa. Selection of the most starchy- and most sweet-appearing seeds consists of choosing the BBB and BBb seeds on the one hand, and the bbB and bbb seeds on the other. All four types carry indiscriminately AA, Aa or aa, so that the numerical possibilities in the next generation are as shown in table 4.

TABLE 4

The theoretical result, in the following generation, of selecting starchy- and sweet-appearing seeds from a segregating ear borne on a plant homozygous for C and heterozygous for A and B.

Type of seeds selected	Composition and number of F_3 seed populations and F_2 plants which produce them				Appearance of F_3 seed populations
		Plant	Seeds		
Starchy seeds *BBB*	8	*AA*	*BBB*	2	Pure pseudo-starchy
		Aa	*BBB*	4	Intermediate, uniform
		aa	*BBB*	2	Intermediate, uniform
Starchy seeds *BBb* and sweet seeds *bbB*	16	*AA*	*BBB*	1	Pure pseudo-starchy
		AA	*BBB*, *BBb*, *bbB*, *bbb*	2	Intermediate, variable
		AA	*bbb*	1	Intermediate, uniform
		Aa	*BBB*	2	Intermediate, uniform
		Aa	*BBB*, *BBb*, *bbB*, *bbb*	4	Intermediate, variable
		Aa	*bbb*	2	Intermediate, uniform
		aa	*BBB*	1	Intermediate, uniform
		aa	*BBB*, *BBb*, *bbB*, *bbb*	2	Intermediate, variable
		aa	*bbb*	1	Pure sweet-appearing
Sweet seeds *bbb*	8	*AA*	*bbb*	2	Intermediate, uniform
		Aa	*bbb*	4	Intermediate, uniform
		aa	*bbb*	2	Pure sweet-appearing
	32			32	

Summing up the theoretical situation and comparing this with the results actually obtained, the agreement is close.

TABLE 5

Numbers found	Classification of ears in both selected progenies	Proportion	
		Expected	Found
1	Pure starchy	3	2
8	Intermediate and uniform	18	16
4	Intermediate and variable	8	8
2	Sweet-appearing	3	4
15		32	30

None of the apparently pure types have been grown as yet to test the propriety of their classification. The intermediate ears, both uniform and variable, differ in the degree in which they approach the one or the other extreme as shown in figures 13 and 14. The distinction between intermediate variable and intermediate uniform is somewhat arbitrary although it can be easily seen in the illustration that some of the ears are segregating and others are not.

It should be noted that, according to theory, the sweet ears of this last material have a different constitution than the sweet segregated seeds of the one notable ear of the other material. In that segregating ear the translucent seeds are given the formula *AA BBB cc* while in this case the sweet seeds are *aa bbb CC*. The seeds of both are translucent. In the latter they are strongly wrinkled while in the former they are not. Whether or not these assumptions are correct can be easily tested by crossing the two different types of sweet seeds. The pollination made one way should give pseudo-starchy seeds,—made the other way should have very little effect, as the reciprocally crossed seeds differ markedly in composition. In the first instance they are *AA BBb cC* pseudo-starchy; in the other *aa bbB Cc* sweet or only slightly modified. In the next generation both crosses should give intermediate, indefinitely segregating ears similar to the ones shown in figure 9, with the possibility of recovering complete pseudo-starchiness in later generations.

CONCLUSION

The reader may question the desirability of presenting such an elaborate hypothesis with so few data to support it. The inheritance of pseudo-starchy endosperm is a task in itself and is not primarily the problem we undertook to solve in determining the effects of selection. The inheritance of this character will be followed up. It will be surprising if the interpretation given here will be entirely adequate to cover all the possibilities in this material. The one presented shows how a complicated situation may be due to relatively simple genetical differences. And whether or not the interpretation given is wholly correct is aside from the main point at issue since it seems clear to the writer that some similar factorial analysis will bring the results into alignment if the one given is not altogether satisfactory.

What we undertook to find out was:

(1) Whether or not this original semi-starchy condition was an exception to the complete segregation of the two allelomorphs represented in starchy and sweet endosperms.

(2) Whether or not the effect of selection upon this apparent blend was the result of a progressive change of a single unstabilized Mendelian unit.

Both of these questions can be answered decidedly in the negative. It has been shown beyond doubt that pseudo-starchiness, as described here, and true starchiness, as commonly understood, in maize endosperm, are genetically dissimilar. Therefore, segregation of the one principal allelomorphic pair involved in the original starchy and sweet endosperm types was complete. It has also been clearly shown that several Mendelian units are concerned with this new character and that therefore the effect of selection upon this material, in all probability, is due to the sorting-out and re-arrangement of various of these units. The evidence for this lies in the facts that (1) selection was most effective in the early generations of self-fertilization and alterations ceased as homozygosis in other factors was brought about; (2) when the extremes of selection were crossed no clear-cut segregations occurred in F_2 but in later generations, as the germplasm was automatically simplified by self-fertilization, different types of segregation of a more definite nature appeared, with which selection was more effective.

To believe in the truth of these assertions it is not necessary to have the inheritance of all factors having to do with this rather complex endosperm situation worked out in all detail. Such a line of investigation is interesting but less important than the main points already covered. This material is not favorable for making an exact and minute genetical analysis. For this purpose we already have at hand a number of beautiful illustrations which are apparently far more complicated than this situation in maize but have been put on a satisfactory factorial basis. One does not hope to offer anything from this endosperm character, equal, for example, to the rigorous analysis of the beaded wing character in Drosophila by MULLER (1918). The recent work of EMERSON (1918) with aleurone color and of LINDSTROM (1918) with chlorophyll color in maize ought also to be noted in this connection. Our meager results considered with such investigations as these and with other experiments dealing directly with the problem of selection, particularly those of MAC DOWELL (1915), PEARL (1917), MORGAN (1917), STURTEVANT (1918) and PAYNE (1918) fit in with the general conclusion to be derived from present-day genetical research along this line, which is, that the hypothesis of stable hereditary units, while similarly subject to limitations, is as useful in the field of genetics as the atomic theory is in the realm of chemistry.

SUMMARY

1. Amylaceous seeds in an extracted recessive progeny from a cross of starchy and sweet varieties of maize suggested that an imperfect segregation or contamination of the two allelomorphs might have occurred. This material was the basis for a selection experiment attempting to recover both parental types.

2. After five generations of selection in opposite directions in self-fertilized lines apparently pure sweet and pure starchy strains were obtained which remained constant during from four to five additional years of self-fertilization and selection.

3. When crossed with an ordinary variety of starchy maize both the sweet- and starchy-appearing selections behave the same, segregating 3 to 1 in the second generation into starchy and sweet seeds.

4. Examination of the starch grains of the two selections shows them to be much alike and both lots together are not fully developed as compared to starchy varieties.

5. In chemical composition the starchy selection is more like other starchy types, particularly in soluble and insoluble carbohydrates.

6. Nevertheless, on account of its behavior in inheritance, from the nature of its starch grains and the qualities of the seeds this starchy-appearing selection is considered to be independent and genetically distinct from true starchiness. This new form of endosperm is called pseudo-starchy.

7. Crosses of pseudo-starchy and sweet give indistinctly segregating seed populations in F_2 which show little response to selection in the next generation but in later filial periods selection is able to recover the parental patterns.

8. The pseudo-starchy type is also recovered from the extracted sweet seeds of the cross of pseudo-starchy and true starchy.

9. In both these crosses segregation becomes more distinct in later generations after the plants have been inbred. Two different kinds of segregation are observed; one giving a sharp mono-hybrid ratio of opaque and translucent seeds; the other giving a less definite splitting in a 1 to 1 fashion which responds immediately to selection.

10. Endosperm and plant factors working together are assumed in the interpretation of these results such that reciprocal crosses have slight immediate effect and in the F_2 seed populations the appearance of the seeds is not closely correlated with their genetic composition and hence their behavior in later generations. Tentative factorial schemes are used to illustrate ways by which these conditions may be brought about.

11. No evidence is found that imperfect segregation or contamination of factors occurred in the separation of the determiners of starchy and sweet endosperms in the original cross. The effects of selection with this character can be most logically attributed to the sorting-out and re-arrangement of hereditary factors.

LITERATURE CITED

COLLINS, G. N., and KEMPTON, J. H., 1913 Inheritance of waxy endosperm in hybrids with sweet corn. U. S. Dept. of Agric., Bur. Plant Ind. Cir. 120, pp. 21-27.

EAST, E. M., and HAYES, H. K., 1911 Inheritance in maize. Connecticut Agric. Exp. Sta. Bull. 167, pp. 142.

EMERSON, R. A., 1918 A fifth pair of factors, *Aa*, for aleurone color in maize and its relation to the *Cc* and *Rr* pairs. Cornell Univ. Agric. Exp. Sta. Memoir 16, pp. 225-289.

HALSTED, B. D., 1909 Report of the Botanist. New Jersey Agric. Exp. Sta. Rep. pp. 269-281, *plate III.*

HAYES, H. K., and EAST, E. M., 1915 Further experiments on inheritance in maize. Connecticut Agric. Exp. Sta. Bull. 188, pp. 31, *plates I-VII.*

LINDSTROM, E. W., 1918 Chlorophyll inheritance in maize. Cornell Univ. Agric. Exper. Sta. Memoir 13, pp. 68.

MACDOWELL, E. C., 1915 Bristle inheritance in Drosophila. Jour. Exp. Zoöl. **19**: 61-97.

MORGAN, T. H., 1917 The theory of the gene. Amer. Nat. **51**: 513-544.

MULLER, H. J., 1918 Genetic variability, twin hybrids and constant hybrids, in a case of balanced lethal factors. Genetics **3**: 442-499.

PAYNE, F., 1918 An experiment to test the nature of the variations on which selection acts. Indiana Univ. Studies, No. 36, 45 pp.

PEARL, R., 1917 The selection problem. Amer. Nat. **51**: 65-91.

PEARL, R., and BARTLETT, J. M., 1912 The Mendelian inheritance of certain chemical characters in maize. Zeitschr. f. ind. Abstamm. u. Vererb. **6**: 1-28.

STURTEVANT, E. L., 1899 Varieties of corn. U. S. Dept. Agric., Office of Exp. Sta. Bull. 57, pp. 7-103.

STURTEVANT, A. H., 1918 An analysis of the effect of selection. Carnegie Inst. of Washington, Publ. 264, 68 pp.