# GENETIC STUDIES ON THE PROTEIN CONTENT OF MAIZE

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[Received May 4 1920

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## INTRODUCTION

Agricultural crops owe their popularity to a variety of qualities. If they will keep for long periods, if they can be stored economically and shipped easily, if they suit all palates, their usefulness is naturally enhanced. But, other things being equal, their content of particular

desirable constituents determines not only their true worth, but their commercial value. Such a statement is no more than a platitude when it concerns plants grown for fibers, drugs, dyes, tannins, rubbers, or essential oils. In the great food plants, the matter is not so obvious; yet data collected some months ago by one of us, indicate a rather striking class difference and noteworthy uniformity within each class in the prices people have come to pay for carbohydrates, fats, proteids and vitamine carriers, when correction is made for the variables noted above.

Naturally, appreciation of differential values in plant constituents has led to many plant-breeding projects in which the chief aim has been to develop the desirable quality rather than to increase the yield of the crop. One cannot criticize efforts to raise the quantity and quality of fiber in cotton and flax, or the yield of rare oils and drugs. In other cases one cannot be so certain that such efforts are advisable. It may be true, for example, as maintained by HOPKINS and SMITH, that there is demand for maize varieties rich in high or low protein or in high or low oil. It is possible, however, that the best economic practice is to grow other plants for these particular purposes. If the difficulties in the way of breeding wheat and maize for high protein content or maize for high oil content are extreme, it is probably wiser to obtain proteid concentrates or commercial oils from plants naturally rich in these substances. Nevertheless facts regarding the inheritance of such chemical constituents as protein are always valuable, and the writers submit this paper hoping that the results set forth have some present value to genetic theory and the possibility of future value to agricultural practice.

The work is based upon an experience of nearly twenty years. From 1900 to 1905, familiarity with the problems involved was gained by contact with the maize-breeding projects of the ILLINOIS AGRICULTURAL EXPERIMENT STATION. In 1906 mass-selection experiments were started at the CONNECTICUT AGRICULTURAL EXPERIMENT STATION. It was soon apparent, however, that information additional to that secured by the ILLINOIS AGRICULTURAL EXPERIMENT STATION was not likely to be obtained until more was known about the inheritance of simpler characters. Accordingly the investigations on protein inheritance were held in abeyance for several years. Since 1909, however, the work has been prosecuted with some vigor by Professor H. K. HAYES now of the UNIVERSITY OF MINNESOTA and by the authors, as a coöperative project between the CONNECTICUT AGRICULTURAL EXPERIMENT STATION and HARVARD UNIVERSITY. The funds for the plat work were furnished wholly by the first-named institution, the pedigree cultures were grown

upon its experimental farm, and the chemical determinations were made within its laboratories,—in earlier years by the authors, in later years by the staff of the Chemical laboratory. Current analyses of the results and plans for each year from 1909 to 1917, were made first by H. K. HAYES and E. M. EAST and later by D. F. JONES and E. M. EAST during the winters in the laboratories of the BUSSEY INSTITUTION of HARVARD UNIVERSITY.

## RESULTS OF PREVIOUS WORK

It would serve no useful purpose to describe in detail the early plant-breeding work wherein the chief object was to enhance the value of a plant by increasing the production of a particular chemical constituent. The classical example, and one of the earliest projects for breeding plants on a large scale, is the work of the French chemists and agriculturists on the sugar beet, inaugurated in the early part of the nineteenth century by the great Corsican, and carried on continuously since that time both in France and in Germany. Similar work on the sugar cane has been carried on in Java and to a limited extent in Cuba. No other comparable work of such magnitude or of such commercial importance exists, yet each year sees the initiation of some plan of this kind on a small scale. There are schemes for increasing and decreasing protein in wheat and maize, for increasing oil in maize, peanuts, castor beans and soy beans, for obtaining greater yields of the essential oils used in perfumes and of the valuable ingredients in certain drug plants. And recently both English and American companies have undertaken work designed to augment the yield of rubber latex in *Hevea brasiliensis*.

Few of these projects have resulted in any supposed or actual increase in genetic knowledge. In nearly every case mass selection has been practised with no more powerful tool of knowledge than the empiric formula "like tends to produce like." Even the numerous German contributions to literature on the sugar beet have had no effect on current genetic thought. They have resulted in a better knowledge of the varying composition of different parts of the root and in the effects of factors of environment on the elaboration of sugar, but even today one cannot say with certainty whether new variations have occurred which aid in the production of higher sugar content. Elimination of lines with a low sugar content, and accumulation of favorable genetic factors by segregation and recombination may account for everything in the sugar beet, though one hears much about the *change* in the ability of the plant to produce sugar. It may be doubted whether the extreme individual

beet is higher in sugar today than in the year 1800, but there are more of these high individuals.

An exception to these remarks is the work carried on since 1896 at the ILLINOIS AGRICULTURAL EXPERIMENT STATION, where the primary object has been to establish commercial strains of maize characterized by high or low protein content, or by high or low oil content, but where the idea of contributing to genetic knowledge has never been allowed to lapse. It is natural, therefore, that these experiments should be described in some detail in this paper.

In 1892, JENKINS and WINTON compiled the published analyses of American feeding stuffs. Among them were analyses of maize seeds produced by plants of different varieties grown in various parts of the country. The range and average of protein content calculated to water-free material are shown in table 1. These reports of protein are in

TABLE 1

*Percentage protein content of maize seeds in water-free material.*

| Type | Number of samples | Maximum | Minimum | Average |
|---|---|---|---|---|
| Dent | 86 | 13.8 | 8.2 | 11.5 |
| Flint | 68 | 14.0 | 7.7 | 11.8 |
| Sweet | 27 | 17.0 | 10.3 | 12.8 |
| Pop | 6 | 14.4 | 11.0 | 12.5 |
| Soft starch | 5 | 15.5 | 9.5 | 12.5 |

reality determinations of total nitrogen multiplied by the factor 6.25. That this factor is sufficiently correct for all practical purposes is demonstrated by the work of CHITTENDEN and OSBORNE (1892) where the weighted average percent of nitrogen in the different proteid bodies was found to be 16.00.

There are various proteids in maize as these investigations show:

| | *Percent* |
|---|---|
| 1. Proteose soluble in water | 0.06 |
| 2. Very soluble globulin | 0.04 |
| 3. Maysin soluble in dilute salt solution | 0.25 |
| 4. Edestin soluble in concentrated salt solution | 0.10 |
| 5. Zein soluble in alcohol | 5.00 |
| 6. Proteids soluble in dilute alkalies | 3.15 |
| 7. Proteids insoluble in these solvents | 1.05 |

Thus zein is the important proteid, comprising over 50 percent of the total. Unfortunately zein lacks the essential amino acids, glycocoll, lysine and tryptophane, and contains relatively small amounts of arginine

and histidine; hence it cannot be used as the sole proteid food in building up animal tissues, as has been shown by OSBORNE and his co-workers in numerous investigations.

Variations comparable to those of table 1 were found in all the constituents of maize, and gave HOPKINS the basis for starting the ILLINOIS AGRICULTURAL STATION experiment. Admittedly, a considerable portion of these deviations were due to varying environmental conditions, but he was satisfied that there were heritable differences in composition which could be made the basis of selection. He was not able to prove this at the time, but offered some presumptive evidence in the shape of individual analyses of 50 selected ears of Burr's White maize grown on a field having particularly uniform soil conditions. The frequency distribution of proteins and of oil contents (table 2), showing, as they did, deviations as great as the compiled analyses of maize grown under extremely varied conditions, were accepted as indicating differences due to heredity.

TABLE 2

*Frequency distribution of percents of protein and of oil in 50 ears of Burr's White maize grown under uniform soil conditions.*

| Classes | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distribution of protein | 2 | 3 | 2 | 6 | 8 | 8 | 6 | 9 | 2 | 3 | 0 | 1 |

| Classes | | | 4.0 | 4.3 | 4.6 | 4.9 | 5.2 | 5.5 | 5.8 | 6.1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distribution of oil | | | 5 | 9 | 11 | 16 | 5 | 3 | 0 | 1 | | |

HOPKINS assumed, with little biological, but with considerable pragmatical justification, that the ear of the maize plant may be taken as a unit. Marked variations were found when samples of seed were taken from top, middle or butt seeds, but several samples of three rows of seeds taken from the whole length of the ear showed very small deviations (table 3). Similarly the comparative uniformity of the protein content of single seeds from the same ear—after 3 years of selection for protein—was later taken as warranting the conclusion that the composition of the ear is approximately uniform throughout (table 4).

Starting with these assumptions, 163 ears of Burr's White maize were analyzed (HOPKINS 1899). Considering only the protein and the oil contents, the distribution of the analyses was as follows (table 5). From selected ears of this lot, breeding in four directions was begun,—(1) high protein, (2) low protein, (3) high oil, (4) low oil. In general the method

was to select twenty-four ears of each type by chemical analysis every year, and to continue each line in an isolated breeding-plot by the ear-row method. The main criterion of selection was the chemical composition, but naturally some attention was paid to the appearance of the ear and to its yield as measured by the resulting progeny.

TABLE 3

*Deviation in percentage of protein in random samples from single ears.*

| Ear number | Sample from | | |
|---|---|---|---|
| | Tip | Middle | Butt |
| 1 | 11.77 | 12.24 | 12.39 |
| 2 | 11.98 | 12.49 | 13.06 |
| 3 | 9.70 | 10.08 | 10.48 |
| 4 | 10.60 | 11.04 | 11.00 |
| 5 | 10.82 | 11.33 | 11.30 |
| | 1st 3 rows | 2nd 3 rows | 3rd 3 rows |
| 1 | 10.77 | 10.96 | 10.69 |
| 2 | 11.99 | 12.03 | 12.14 |
| 3 | 10.10 | 10.16 | 10.18 |
| 4 | 10.46 | 10.26 | 10.08 |
| 5 | 11.20 | 10.64 | 10.86 |

TABLE 4

*The percentage protein content of individual seeds on the same ear.*

| Seed number | Ear number | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 1 | 12.46 | 12.17 | 11.53 | 7.45 | 7.72 |
| 2 | 12.54 | 12.94 | 12.32 | 7.54 | 8.41 |
| 3 | 12.44 | 12.51 | 12.19 | 7.69 | 8.37 |
| 4 | 12.50 | 13.42 | 12.54 | 7.47 | 8.31 |
| 5 | 12.30 | 13.12 | 12.14 | 7.74 | 8.02 |
| 6 | 12.49 | 14.49 | 12.95 | 8.70 | 8.76 |
| 7 | 12.50 | 13.21 | 12.84 | 8.46 | 8.89 |
| 8 | 12.14 | 13.43 | 12.04 | 8.69 | 9.02 |
| 9 | 12.14 | 13.16 | 12.75 | 8.86 | 8.96 |
| 10 | 12.71 | 14.05 | — | 8.10 | 8.89 |

The desire to keep up the yield and to preserve a good physical type, as well as the fact that selection was made only through the mother, obviously prevented a rapid shift of type; yet the results were rather remarkable. Through the kindness of Dr. L. H. SMITH we are enabled to quote the gross averages up to the year 1919.

In table 6, the crop averages for the high-protein and the low-protein plots are presented. There is an increasing difference in the protein content of the two strains which continues until the high-protein type contains 8.17 percent more protein than the low-protein type. When the data are shown graphically (figure 1), two facts stand out impressively. The shift in the average is rapid at first; but becomes slower and slower, until the shape of the fitted curves are changed from concave to convex at about the sixth or seventh generation. Nevertheless there is a continuous shift of the average, and apparently the end is not in sight. Second, the influence of environment is very marked. The protein

TABLE 5

*Distribution of protein and oil in the 163 ears of maize with which the Illinois Agricultural Experiment Station maize-breeding investigations were started.*

| Oil, percent | Protein, percent | | | | | | | | | | | | | Total number of ears |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 | |
| 3.8 | | | | | | | | 1 | | | | | | 1 |
| 4.0 | | | 2 | 2 | | | 2 | 3 | 1 | 1 | 1 | | | 12 |
| 4.2 | | | | 3 | 2 | 3 | 3 | 2 | 3 | | 2 | | | 18 |
| 4.4 | | | | 3 | 4 | 2 | 7 | 3 | 2 | | | | | 21 |
| 4.6 | | | 2 | 1 | 1 | 8 | 5 | 8 | 4 | 3 | | | | 32 |
| 4.8 | 1 | 1 | | 5 | 6 | 3 | 7 | 2 | 4 | 5 | | | | 34 |
| 5.0 | | 1 | | 2 | 1 | 3 | 3 | 5 | 4 | | 1 | | | 20 |
| 5.2 | | | | 2 | 3 | 2 | 2 | 1 | | 3 | 1 | | | 14 |
| 5.4 | | | | | | 2 | | | | | | | | 2 |
| 5.6 | | | | 1 | 1 | 1 | 1 | | 1 | | 1 | | | 6 |
| 5.8 | | | | | | | 1 | | | | | | 1 | 2 |
| 6.0 | | | | | 1 | | | | | | | | | 1 |
| Total number of ears | 1 | 2 | 4 | 19 | 19 | 24 | 31 | 25 | 19 | 12 | 6 | 0 | 1 | 163 |

content of the two plots goes up or down according to the season, but always the difference between the two increases.

Table 7 shows the figures for the high-oil and the low-oil plots in the same way. The high-oil strain in 1918 has finally reached the remarkable figure of 9.35 percent, while the low-oil type contains only 1.87 percent. Graphically the curves (figure 2) for the changes in oil contents show rather more regularity than is the case with the protein. The rate of change is comparatively constant, seasonal differences apparently having little to do with the matter.

The remarkable results obtained in these experiments have been the object of much comment; and, as BABCOCK and CLAUSEN (1918) point

out, the theoretical interpretations of the rôle which selection played, have been varied. The conclusions of HOPKINS and SMITH (1903–1917), of DAVENPORT (1908) and of CASTLE (1916), have been similar in that they seem to attribute a peculiar creative power to selection which meets with a certain response on the part of the plant. The reason for these

TABLE 6

*Results of selecting maize for high and for low protein content at the Illinois Agricultural Experiment Station. Average percent protein in crop each generation.*

| Year | High strain | Average for period | Low strain | Average for period | Difference | Difference for period |
|---|---|---|---|---|---|---|
| 1896 | 10.92 | | 10.92 | | | |
| 1897 | 11.10 | | 10.55 | | 0.55 | |
| 1898 | 11.05 | | 10.55 | | 0.50 | |
| 1899 | 11.46 | | 9.86 | | 1.60 | |
| 1900 | 12.32 | 11.37 | 9.34 | 10.24 | 2.98 | 1.13 |
| 1901 | 14.12 | | 10.04 | | 4.08 | |
| 1902 | 12.34 | | 8.22 | | 4.12 | |
| 1903 | 13.04 | | 8.62 | | 4.42 | |
| 1904 | 15.03 | | 9.27 | | 5.76 | |
| 1905 | 14.72 | 13.85 | 8.57 | 8.94 | 6.15 | 4.91 |
| 1906 | 14.26 | | 8.64 | | 5.62 | |
| 1907 | 13.89 | | 7.32 | | 6.57 | |
| 1908 | 13.94 | | 8.96 | | 4.98 | |
| 1909 | 13.41 | | 7.65 | | 5.76 | |
| 1910 | 14.87 | 14.07 | 8.25 | 8.16 | 6.62 | 5.91 |
| 1911 | 13.78 | | 7.89 | | 5.89 | |
| 1912 | 14.48 | | 8.15 | | 6.23 | |
| 1913 | 14.83 | | 7.71 | | 7.12 | |
| 1914 | 15.04 | | 7.68 | | 7.36 | |
| 1915 | 14.53 | 14.53 | 7.26 | 7.74 | 7.27 | 6.79 |
| 1916 | 15.66 | | 8.68 | | 6.98 | |
| 1917 | 14.44 | | 7.08 | | 7.36 | |
| 1918 | 15.48 | | 7.31 | | 8.17 | |

conclusions appears to be in part an adherence to the Darwinian idea that all fluctuations are heritable and that continuous selection is therefore always effective in shifting the type; in part a scanty appreciation of the results of other pedigree-culture work; and in part a failure to realize that the unit of selection is the seed and not the ear, combined with a lack of appreciation of the variables which come into play when a system

of breeding by selection through the mother is practised. DAVENPORT and RIETZ (1907), for example, studying the four strains by statistical methods after ten years of selection, use the ears as units of discussion and conclude that "the variability was not sensibly reduced during the

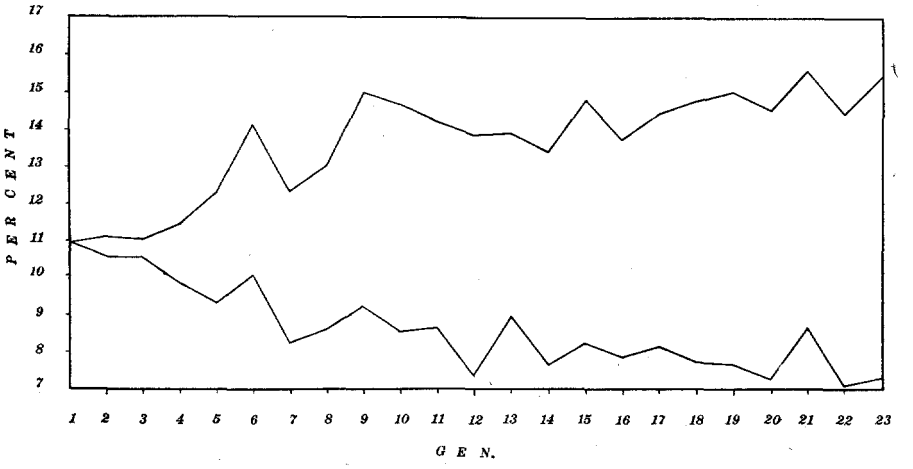


FIGURE 1.—Graphical representation of the results of the ILLINOIS AGRICULTURAL EXPERIMENT STATION in selecting maize for high protein and for low protein.

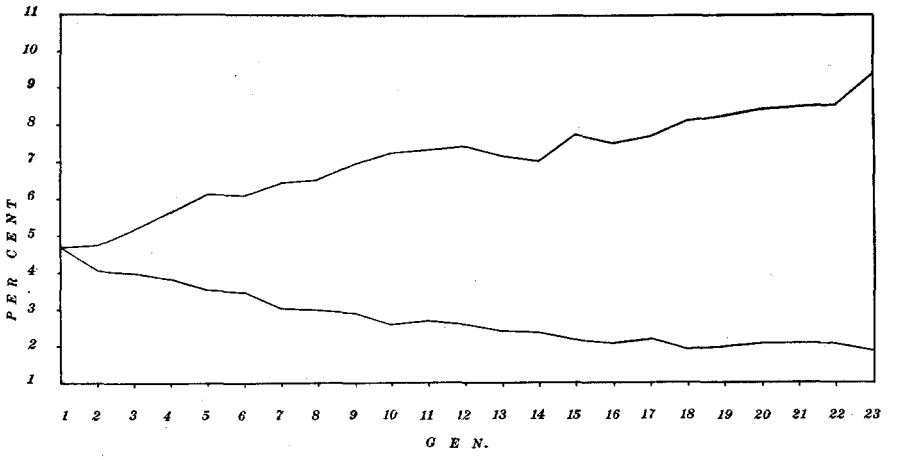


FIGURE 2.—Graphical representation of the results of the ILLINOIS AGRICULTURAL EXPERIMENT STATION in selecting maize for high oil and for low oil.

ten years of rigid selection." Their study of the data, led them to believe that "after great improvement has been secured there is still left abundant variability on which to base future selection, and that if the limits of improvement are ever reached it will be for some reason

out, the theoretical interpretations of the rôle which selection played, have been varied. The conclusions of HOPKINS and SMITH (1903–1917), of DAVENPORT (1908) and of CASTLE (1916), have been similar in that they seem to attribute a peculiar creative power to selection which meets with a certain response on the part of the plant. The reason for these

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conclusions appears to be in part an adherence to the Darwinian idea that all fluctuations are heritable and that continuous selection is therefore always effective in shifting the type; in part a scanty appreciation of the results of other pedigree-culture work; and in part a failure to realize that the unit of selection is the seed and not the ear, combined with a lack of appreciation of the variables which come into play when a system

of each line-bred family in a marked degree, without necessarily reducing the variability of averages of population samples which are obtained by analyzing ears. It is shown in figure 3, for instance, that a normal frequency surface may be constructed in which the variability in one direction—measuring a series of samples—may remain the same, though the variability of the sub-population making up each sample, is very different.

The interpretation noted above has been given up by CASTLE (1919) because of the steadily increasing evidence that recombinations of Mendelian factors account for the results obtained, in a simpler and more helpful manner.

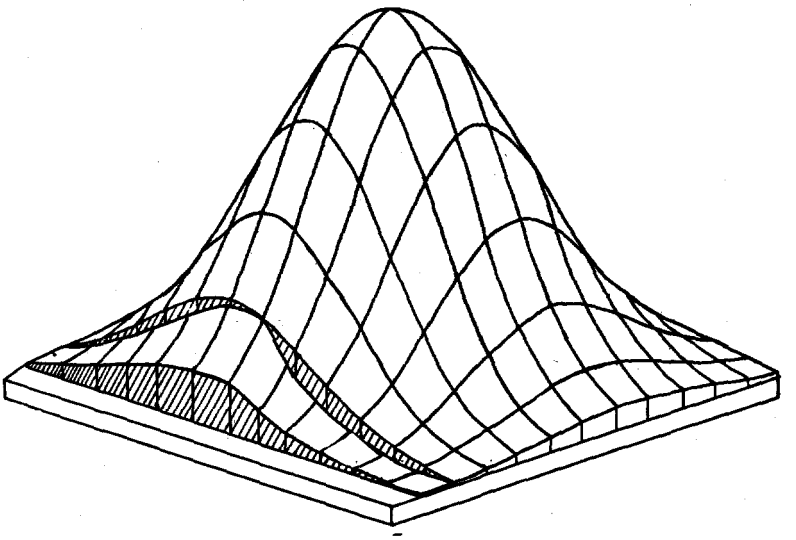

FIGURE 3.—A normal frequency surface showing how the dispersion coefficient may be changed in one direction without affecting it in the other direction.

A Mendelian interpretation of the Illinois results, isolation of various combinations of hereditary factors, was first suggested by EAST (1910), on the general basis that it was more plausible to have an analysis of these facts in keeping with modern genetic interpretations of analogous phenomena, and for the specific reason that the fitted curves showed a retardation in the effects of selection.

This stand was strongly supported by an analysis of the pedigrees of the four Illinois strains after ten years selection, made by SURFACE in 1911. He found that the 24 "High-protein" ears selected for planting in the eleventh generation, all traced back to one original ear, No. 121; of the "Low protein" ears, 20 traced back to No. 106 and 4 to No. 107;

of the "High oil" strain, 12 ears came from No. 111, 4 ears from No. 114 and 8 ears from No. 118; and of the "Low oil" strain 16 ears originated from No. 106 and 8 ears from No. 110. Thus in the eleventh generation all of the 96 ears of the four strains traced back to 8 ears of the original Burr's White, a rather convincing demonstration that the results of selection were mainly the accumulation of hereditary complexes effective in various ways, though of course no one could maintain that mutations had not ensued during this lapse of time.

Other experiments modeled along lines similar to these have been carried on at various agricultural experiment stations since the first report of the Illinois investigations, but so far as we know only one resulted either in new facts or in a new point of attack. We refer to that of PEARL and BARTLETT (1911). In this study a cross was made between a white sugar corn and a yellow dent, and $F_2$ seeds of the four classes, yellow dent, white dent, yellow sugar and white sugar, were analyzed and the results compared with those obtained from seeds of the parental and $F_1$ generations. Moisture, nitrogen, ether extract, ash, crude fiber, pentosans, sucrose, dextrose and starch were determined. No discussion of the relative accuracy of these determinations was made, but it was thought that high moisture and high starch dominated the alternative conditions, low moisture and low starch, while in the remaining constituents the lower percentage dominated the higher percentage. Segregation was obvious in every case. It was not shown, and probably the authors would not now maintain, that single factors determined the difference between "high" and "low" content of any of these complexes; moreover, matters other than simple segregation in the usual sense must be taken into consideration in such a genetic analysis, as we shall show; but the authors deserve great credit for bringing out the fact that the seed and not the ear must be taken as the unit in any such study.

## THE INHERITANCE OF PROTEIN IN MAIZE

### *The problem*

The genetic problems involved in an effort to change the chemical composition of maize by breeding cannot be understood clearly unless the elementary botanical facts connected with seed formation are borne in mind. This would seem to be, and ought to be, an unnecessary observation; yet a careful survey of the statements made by previous investigators leads one to believe that ignorance or carelessness regarding these facts has led to numerous erroneous conclusions.

One should expect the composition of the maize seed to be influenced both by the genetic constitution of the mother plant and by the environment under which it develops, but it should not be forgotten that the grain itself, speaking botanically, is in a sense a young zygote having characters of its own derived from the gametes from which it is formed. Classified according to their origin, however, there are these distinct parts to the seed,—the pericarp, a maternal tissue, the embryo formed by the union of the egg with the first male nucleus, and the endosperm formed by the union of the second male nucleus with the so-called endosperm nucleus—a fusion product of two embryo-sac nuclei. The line of hereditary transmission is confined to the gametes produced by the plant maturing from the embryo, but the composition of the seed is determined largely by the composition of the endosperm which forms about 80 percent of each individual kernel. Now the cytological and the pedigree-culture evidence are in agreement that the above method of seed formation is so rigid in the species under consideration that no one has been able to establish an exception. These experimental methods have also demonstrated (see EAST 1913), first, that from the chromosome standpoint the embryo is a $2x$ body and the endosperm a $3x$ body; second, that the two "male" nuclei on the one hand and the three "female" nuclei on the other hand, have respectively the same genetic composition. If a male nucleus entering into endosperm formation bears a factor through which a particular character develops, therefore, one may rest assured that the "brother" nucleus entering into the formation of the embryo, will bear the same factor. And the same is true of the three "female" nuclei. Nevertheless some complications arise, due to this double-fertilization process, which make the various genetic phenomena involved somewhat difficult to analyse, although the basis upon which such analysis must depend is quite clear.

For example, in the earlier investigations on inheritance of maize-endosperm characters, such characters as the yellow ether-soluble pigment, the blue and red anthocyans of the aleurone cells, and the presence and absence of starch development, it was found that the endosperm could be considered to be identical with the embryo without error. The dominant characters seemed to show the same degree of dominance, a degree approaching perfection, no matter whether they entered from the male or the female side. In other words, a single nucleus contributing certain factors from the male side, seemed to exert the same influence on development as a double ($2x$) nucleus entering from the female side. HAYES and EAST (1915), however, found that this simple behavior was

not characteristic of every character. In the starch differences causing the chief distinction between the floury types and the horny or translucent types, dominance followed the maternal side. The two nuclei coming in from the embryo sac seemed to have a cumulative effect. Corroboration of this phenomenon was recently made by JONES (1919) on another type of starch difference.

The fact that protein is contained in each of these three types of tissue is a further fact that complicates the genetic problems. HOPKINS, SMITH and EAST (1903) found that after four or five years' selection for high protein and for low protein, the high-protein and the low-protein strains had been differentiated physically to such a degree that the embryos and the amount of corneous starch in the high-protein strain were considerably greater than in the low-protein strain. In a single selected low-protein ear the pericarp and remains of the vestigial glumes comprised 6.67 percent, the endosperms 83.73 percent, and the embryos 9.59 percent of the total. Similarly, in a high-protein ear the maternal tissue comprised 7.71 percent, the endosperms 80.37 percent, and the embryos 11.93 percent. The nitrogen was very low in the maternal tissues and probably did not consist largely of proteid nitrogen. The actual percentage of protein in the embryos was high but did not differ very much in the two types, being 19.91 percent in the low-protein ear and 19.56 percent in the high-protein ear. The greatest difference came in the endosperm, where the aleurone layer (probably contaminated with starch) of the low-protein ear contained 19.21 percent protein as compared with 24.58 percent in the high-protein, and the corneous starch (37.15 percent of the seeds by weight) contained 8.12 percent in the low-protein as compared with 10.99 percent in the high-protein ear (44.89 percent of the seeds by weight).

It is not certain what relative changes in the kinds of protein contained were made by the isolation of these strains, but from OSBORNE and CLAPP'S (1908) analyses of ordinary maize and maize from the high-protein plot of the ILLINOIS AGRICULTURAL EXPERIMENT STATION, one would suppose that the proteins of the endosperm had been increased to a greater degree than the proteins of the embryo.

These various facts regarding the origin of the maize seed and the composition of its various parts have been kept in mind, and the problem of changing the protein content has been attacked in various ways. The results obtained will be discussed seriatim.

It is to be understood that all chemical determinations were made by the methods approved by the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

*Variation in the protein content of individual seeds*

The method of work at the ILLINOIS AGRICULTURAL EXPERIMENT STATION was founded on the fact that in certain ears tested, triplicate samples of three rows of seeds taken throughout the length of the ear gave very uniform analyses, though rather marked variations in analysis were noted when ears were sampled at the tip, middle and butt. Under the circumstances under which the Illinois analyses were made this was perhaps to be expected. It is not surprising, for example, to find variations in the chemical constitution of the seeds at the tip, the middle and the butt, for the seeds in these various regions differ in average size, are pollinated successively from butt to tip because of the maturation of the silks in this order, mature at slightly different times, and presumably may be expected to receive somewhat different amounts of nourishment from the parent plant owing to the spike-like mode of development of the ear. For the same reasons, it is to be expected that samples taken throughout the length of the ear will more truly represent the whole ear. Duplicate samples taken in this manner should be similar. At the same time it should be remembered that analyses of duplicate samples taken in this way, tell one nothing concerning the variation shown by individual seeds or the individual potentialities they carry.

For actual use, no better method of selection can be suggested, yet if individual seeds do show a notable variation due to varying zygotic composition, it will depend largely on the heterozygosity or homozygosity of the genes present, whether progress by the mass-selection method will be rapid or slow. In other words, of two ears of 15 percent protein selected for their high protein content, the one might have a coefficient of variation of 7 percent, the other of 16 percent. In mass selection the ear uniformly high in protein would undoubtedly give the best results, for it is likely that there would be 7-percent or 8-percent seeds, both in a phenotypic and a genotypic sense, in the ear with the high dispersion coefficient. The effect of pollen from the plants these seeds would produce can easily be imagined. On the other hand, if self-pollination were practised, the seeds from the more variable ear would hold out the greatest hopes for improvement, for it is probable they would run as high as 19 or 20 percent.

These suppositions can be illustrated from results actually obtained by analysis of individual seeds. In 1907 the ILLINOIS AGRICULTURAL EXPERIMENT STATION kindly sent some ears of their high-protein and low-protein strains to the CONNECTICUT AGRICULTURAL EXPERIMENT

STATION. These strains after the ten years' selection had already become markedly differentiated. The high-protein strain as compared with the low-protein strain had smaller seeds, larger embryos and a much greater percent of corneous starch. A cross was made between two plants, high-protein female by low-protein male, and a series of seeds of the following types were analyzed: (1) seeds from one ear each of selfed high-protein and of selfed low-protein, these being ears from sister plants of those used in the cross, (2) seeds from the ear produced by the immediate cross, i.e., $F_1$ seeds, and (3) seeds from each of two $F_1$ ears bearing $F_2$ seeds. These analyses were made for another purpose at the time, hence some data which might be useful at the present time are lacking; but they will serve our purpose. Protein contents are calculated to dry basis as has always been our practice, but it was impractical in this case to actually dry each sample by the laboratory method. The ears were air-dried in a steam-heated room, and a single moisture determination made for each ear. Moisture determinations made in this way were about 8.3 percent. Since the variation was small, the range being 1.2 percent, it may be assumed that the method was very accurate for our purpose. The samples were taken in spiral fashion around the ear, as fair a method as could be devised. The results are shown in table 8, where the frequency distribution of protein is tabulated in one-half percent classes.

Because of the small number of individuals and large experimental error involved, and because one cannot feel certain that analyses from a single cross represent accurately the conditions usually found in similar crosses, one should be careful not to draw any hard and fast conclusions from these data. The ranges of variation in the various distributions seem to be rather small, and thus corroborate HOPKINS'S conclusions cited previously. The $F_2$ seeds, however, show a somewhat greater range. Turning to table 9 where the statistical constants are shown, one is somewhat surprised to find the rather high variability of the low-protein type. Judging from the appearance of the curves plotted from the figures given, it seems reasonable to suppose that with larger numbers a greater difference in variability between the parents and the F seeds would be found; but, of course, this is a mere surmise. One conclusion, at least, is permissible. It is clear to anyone who has had experience in studying dispersion coefficients of pedigree cultures, that the phenotypic differences between seeds on the same ear are not what would have been expected had the genotypic differences of these seeds been expressed in the composition of the individual grains. In other words, only a very

TABLE 8

*Variations in protein content in individual seeds (dry basis).*

| Source of seeds | Class centers in percent | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 | 14.5 | 15.0 | 15.5 | 16.0 | 16.5 |
| Ear A1, high protein.... | | | | | | | | | | | 1 | 1 | 7 | 13 | 18 | 14 | 11 | 4 | 1 | | |
| Ear B1, low protein..... | 1 | 4 | 8 | 22 | 23 | 8 | 5 | 2 | 1 | | | | | | | | | | | | |
| Ear (A2 × B2), $F_1$ seeds | | | | | | | | | | 2 | 4 | 7 | 13 | 26 | 13 | 5 | 1 | 2 | | | |
| Ear (A2 × B2)1, $F_2$ seeds | | | | | | | | | 1 | 0 | 1 | 8 | 4 | 6 | 8 | 18 | 15 | 3 | 2 | 1 | 1 |
| Ear (A2 × B2)2, $F_2$ seeds | | | | | | | | 1 | 2 | 2 | 5 | 7 | 11 | 17 | 16 | 12 | 7 | 3 | 1 | | |

small portion of the potentialities which a seed may have inherited is expressed in the quality of the individual seed itself by the time it has finished its growth preparatory to the resting period.

The obvious general conclusion from the data thus far considered, therefore, is this: The seeds of maize vary in the transmissible hereditary factors which control the development of the various proteids in the different parts of the seed. These factors, transmitted in the usual manner, must be the basis of all change in composition through breeding. At the same time, there are extraordinary practical difficulties involved. The greater part of the protein is contained in the endosperm, where, though nuclei presumably duplicating those which form the embryo come together, there is the difference that the contribution from the maternal side is twice that of the paternal side. In addition the composition of the seed as a whole is dependent on extremely complex conditions.

TABLE 9

*The statistical constants of the frequency distributions shown in table 8.*

| Source of seeds | Number | Mean | Standard deviation | Coefficient of variation |
|---|---|---|---|---|
| Ear $A_1$, High-protein | 70 | 13.63±0.06 | 0.78±0.04 | 5.72±0.33 |
| Ear $B_1$, Low-protein | 74 | 8.32±0.06 | 0.72±0.04 | 8.65±0.48 |
| Ear ($A_2 \times B_2$), $F_1$ seeds | 73 | 12.91±0.06 | 0.78±0.04 | 6.04±0.34 |
| Ear ($A_2 \times B_2$)1, $F_2$ seeds | 68 | 13.71±0.09 | 1.12±0.06 | 8.17±0.47 |
| Ear ($A_2 \times B_2$)2, $F_2$ seeds | 84 | 12.08±0.08 | 1.08±0.06 | 8.94±0.47 |

First, all external factors affecting growth, play their part; second, the position of the ear, the position of the seed, the size of the seed, and other physiological and morphological factors on which development depends cannot be overlooked; and third, the genetic composition of the plant on which the seed matures, differing as it may from the genetic composition of the seed itself, is of great importance. We cannot differentiate and measure all these influences, but the data already presented are sufficient to show that they exist. When one considers the fact that selection must be founded on the average phenotypic differences shown by fraternities of seeds, and that these phenotypic differences are brought about by such complex conditions, he is constrained to admit that theories as to the effect of selection cannot be based on such material.

There is a possibility, however, of showing some of the effects of particular conditions, and this we shall attempt to do.

TABLE 10

*A comparison between the protein content of self-pollinated (S.) and of open-pollinated (O.P.) ears.*

| Pedigree | | 1912 | 1913 | 1914 | 1915 | 1916 | 1917 | 1918 | Average | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14-30-4-3-7-11-18 | S. | 12.03 | 15.23 | 14.77 | 15.64 | 15.08 | 13.29 | 15.35 | 14.89 | S. |
| | O. P. | — | 13.46 | 14.78 | 14.44 | 13.11 | 13.47 | 15.33 | 14.10 | O. P. |
| 14-30-4-4-2-7 | S. | 12.03 | 15.23 | 14.77 | 16.13 | 14.26 | 14.59 | | 14.99 | S. |
| | O. P. | — | 13.46 | 14.78 | 13.65 | 12.17 | 13.74 | | 13.19 | O. P. |
| 14-30-6-11-3-11 | S. | 12.03 | 15.23 | 15.87 | 16.50 | 16.90 | 15.93 | | 16.30 | S. |
| | O. P. | — | 13.46 | 11.79 | 15.59 | 15.29 | 15.27 | | 14.49 | O. P. |
| 14-30-6-2-13-5 | S. | 12.03 | 15.23 | 15.87 | 16.22 | 14.88 | — | | 15.55 | S. |
| | O. P. | — | 13.46 | 11.79 | 15.44 | 12.76 | 14.20 | | 14.10 | O. P. |
| 14-30-6-4-3-13 | S. | 12.03 | 15.23 | 15.87 | 16.96 | 15.80 | — | | 16.38 | S. |
| | O. P. | — | 13.46 | 11.79 | 15.63 | 10.56 | 14.10 | | 13.10 | O. P. |
| 14-30-9-8-1-6 | S. | 12.03 | 15.23 | 14.51 | 14.47 | 15.78 | — | | 14.92 | S. |
| | O. P. | — | 13.46 | 11.60 | 13.08 | 11.53 | 11.12 | | 12.07 | O. P. |
| 14-30-12-14-1-10 | S. | 12.03 | 15.23 | 14.26 | 16.20 | 14.68 | — | | 15.05 | S. |
| | O. P. | — | 13.46 | 12.13 | 16.31 | 12.09 | 13.81 | | 13.51 | O. P. |
| 14-4-6-16-2-12-47 | S. | 12.03 | 14.58 | 14.16 | 13.53 | 14.25 | 14.05 | 15.27 | 14.31 | S. |
| | O. P. | — | 13.30 | 12.71 | 12.42 | 14.89 | 14.34 | 15.00 | 13.78 | O. P. |
| 14-4-6-4-7-8 | S. | 12.03 | 14.58 | 14.16 | 15.36 | 14.26 | 14.14 | | 14.59 | S. |
| | O. P. | — | 13.30 | 12.71 | 14.65 | 13.79 | 14.96 | | 14.47 | O. P. |
| 14-4-15 | S. | 12.03 | 14.58 | 13.29 | | | | | — | S. |
| | O. P. | — | 13.30 | — | | | | | — | O. P. |
| 14-4-20 | S. | 12.03 | 14.58 | 13.50 | | | | | 13.50 | S. |
| | O. P. | — | 13.30 | 11.97 | | | | | 11.97 | O. P. |
| 14-4-1 | S. | 12.03 | 14.58 | 14.60 | | | | | 14.60 | S. |
| | O. P. | — | 13.30 | 12.87 | | | | | 12.87 | O. P. |
| 14-6-20-10-3 | S. | 12.03 | — | 13.01 | 14.09 | 15.93 | — | — | 14.34 | S. |
| | O. P. | — | — | 11.61 | 12.69 | 15.09 | 16.75 | 16.96 | 13.13 | O. P. |
| 14-22-15-1-29 | S. | 12.03 | 13.42 | 15.86 | 14.47 | | | 15.83 | 14.90 | S. |
| | O. P. | — | 13.47 | 12.60 | 13.04 | | | 14.56 | 13.42 | O. P. |
| 14-8-11 | S. | 12.03 | 14.41 | 13.96 | | | | | 14.19 | S. |
| | O. P. | — | 13.20 | 13.33 | | | | | 13.27 | O. P. |
| 20A-8-5-35-66 | S. | | | 16.09 | 16.49 | 18.01 | 16.93 | 18.69 | 17.32 | S. |
| | O. P. | | | 13.39 | 14.90 | 17.30 | — | 15.27 | 15.22 | O. P. |
| 20A-4-25-47-24 | S. | | | 16.09 | 15.71 | 16.15 | 16.06 | 16.53 | 16.13 | S. |
| | O. P. | — | | 13.39 | 13.90 | 14.43 | — | 16.75 | 15.03 | O. P. |
| 20A-11-10-13 | S. | | | 16.09 | 15.39 | 16.84 | | | 16.12 | S. |
| | O. P. | | | 13.39 | 14.85 | 17.01 | | | 15.93 | O. P. |
| 21-13-9-7-57-43 | S. | | 10.24 | 10.76 | 9.61 | 7.67 | 7.80 | 7.39 | 8.91 | S. |
| | O. P. | | 9.87 | 7.09 | 7.20 | 8.22 | 7.38 | 7.30 | 7.84 | O. P. |
| 21-13-2-11-36 | S. | | 10.24 | 10.76 | 11.18 | 10.01 | — | | 10.60 | S. |
| | O. P. | | 9.87 | 7.09 | 8.15 | 7.36 | 9.98 | | 7.76 | O. P. |
| 30-1-10-8-3 | S. | | | 11.64 | 13.82 | 14.44 | 14.40 | 16.23 | 14.72 | S. |
| | O. P. | | | — | 12.57 | 13.15 | 14.25 | 15.09 | 13.77 | O. P |
| 30-7-5-10-7 | S. | | | 11.64 | 13.38 | 13.03 | 13.06 | 13.99 | 13.37 | S. |
| | O. P. | | | — | 11.91 | 12.28 | 12.04 | 13.85 | 12.52 | O. P. |
| 30-15-4-7 | S. | | | 11.64 | 13.29 | 14.28 | 13.80 | | 13.79 | S. |
| | O. P. | | | | 12.45 | 13.80 | 13.83 | | 13.36 | O. P. |
| Average..... | S. | | 13.58 | 14.28 | 14.65 | 14.57 | 13.45 | 14.91 | 14.24 | S. |
| Average..... | O. P. | | 12.66 | 12.16 | 13.31 | 13.05 | 13.25 | 14.14 | 13.10 | O. P. |
| Percent decrease of O. P. below S. is 8.01. | | | | | | | | Difference | 1.14 | |

*Comparison of the protein content of self-pollinated and of wind-pollinated ears*

As we shall have occasion to speak both of wind-pollinated ears—called open-pollinated ears—and of self-pollinated ears, in several connections, let us first compare the difference in proteid content observed under the two systems. Table 10 lists 23 families which have been grown from self-pollinated seed for varying periods of from 3 to 7 years.

The number in the left-hand column is the pedigree number for the last year grown. It follows a system we have long used. The first number, 14–30–4–3–7–11–18, means that plant 18 was the daughter by self-pollination of ear 11 of the generation before, granddaughter of ear 7, and so on. If a cross had been made, it would have been indicated by the multiplication sign.

Each year a number of plants of each strain were self-pollinated. The resulting ears were analyzed, and the average protein content listed as "S." The number of ears obtained varied from one to twenty, but in general from five to ten may be taken as the number used. In addition a composite sample of the wind-pollinated ears of the same strain was analyzed. These figures are designated "O. P."

It will be noted that there is a rather constant difference in protein content in favor of the selfed ears. In only one instance, family 14–4–6–4–7–8– (1917), does the protein content of the open-pollinated ears exceed that of the self-pollinated ears by an amount greater than might be expected to arise from experimental error. In this case the difference in favor of open-pollination is 0.82 percent. Occasionally a rather large advantage—over 4 percent—is held by the self-pollinated ears, but on the average it is 1.14 percent,—the grand average of the self-pollinated ears being 14.24 percent and that of the open-pollinated ears 13.10 percent. In general then one may figure that the protein content of a self-pollinated ear must be reduced by 8 percent of the total amount found to make it comparable with the protein content of an open-pollinated ear.

Just why the open-pollinated seed runs consistently lower than the self-pollinated seed in protein is not wholly clear. The only constant differentials are the use of the paper bag and the application of the pollen all at one time in self-pollination, and one would hardly expect so great an effect from these seemingly inconsequential factors. There are two other differentials of a variable nature, however, which undoubtedly account for a considerable proportion of the difference. In the first

place the open-pollinated seeds, though related strains have been planted together, may be expected to show more or less heterosis. And, as we shall show later, heterosis increases the weight of the seed and decreases the percent of protein. This phenomenon accounts for a certain range in the proteid differences and for the correlation between self-pollination and high protein; but it does not answer the whole question, for where heterosis is at a maximum in controlled artificial pollinations, as judged by increase in seed weight, the difference in protein content is only about half of that under discussion. Perhaps the remaining difference is due to a relation between composition and the number of seeds produced. In general, though not invariably, the self-pollinated ears contain a less number of seeds, and HAYES and GARBER (1919) have recently noted that there is a high degree (60 percent) of inverse correlation between number of seeds and protein content. Other things being equal, ears with a large number of seeds have a lower protein content than ears with a small number of seeds.

*The immediate effect of pollination on the size and composition of the seed*

By taking advantage of the phenomenon of xenia, so-called, a number of interesting facts connected with the composition of maize have been discovered. The procedure has been to select two varieties which differ in endosperm color, and in which the endosperm color of the $F_1$ seed is not the same as that of either parent. It is obvious that a mixture of approximately equal quantities of pollen may be made from two such plants, A and B, and applied to either plant. Distinguishable selfed and crossed seeds will be obtained on the same ear, maturing under a constant environment.

The effect of crossing on weight of seed in sixteen such mixtures is shown in table 11. Strain A is Illinois Low Protein, a white dent; strain B is Stadtmueller's Leaming, a yellow dent selected for high protein through six generations. Sixteen pairs of plants were selected. The respective mixtures were made and applied to each plant from which the mixture of pollen came. On the resulting ears the selfed and the crossed seeds could be separated easily and weighed. The result was that in each of the 32 comparisons the crossed seeds weighed more than the selfed seeds. The average of A $\times$ B over A was $3.7 \pm 0.23$ cg; and the average of B $\times$ A over B, $5.9 \pm 0.37$ cg. There was a percentage increase of 15.3 in the first case, and 24.2 in the second case.

The difference in actual elaboration of material is even greater than these figures show, for in 25 of the 32 cases listed the water content of

the crossed seeds is lower than the selfed seeds (see table 12). The differences are not striking, it is true, but they are large enough to be significant. The average decrease in water content of A × B from A is 0.57 ± 0.09 percent; and in the case of B × A and B, is 0.25 ± 0.08 percent.

The cause of the smaller amount of moisture in the air-dried crossed seeds, is not clear. It has been our experience that it is impossible to dry out immature seeds as well as seeds that have matured normally

TABLE 11

*The immediate effect of pollination upon the weight of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.*

| Pollen mixture number | Parent plant A | Weight of seeds in centigrams | | | | Parent plant B |
|---|---|---|---|---|---|---|
| | | A | A × B | B × A | B | |
| 1 | 21-13-9-7-57-1 | 27.0 | 32.1 | 30.3 | 22.3 | 14-30-4-3-7-11-4 |
| 2 | -2 | 20.3 | 21.9 | 25.2 | 21.4 | -3 |
| 3 | -3 | 26.0 | 31.1 | 30.9 | 22.5 | -10 |
| 4 | -5 | 22.2 | 24.3 | 31.4 | 25.3 | -2 |
| 5 | -7 | 26.9 | 31.1 | 35.2 | 28.3 | 14-30-4-4-2-7-6 |
| 6 | -10 | 27.8 | 32.4 | 29.9 | 23.7 | 14-30-4-3-7-11-1 |
| 7 | -14 | 28.0 | 30.3 | 39.4 | 29.5 | 14-30-4-4-2-7-3 |
| 8 | -20 | 30.9 | 35.5 | 21.6 | 21.1 | 14-30-6-11-3-11-3 |
| 9 | -24 | 28.5 | 33.0 | 29.1 | 25.5 | 14-4-6-4-7-8-5 |
| 10 | -25 | 24.6 | 29.7 | 36.6 | 30.1 | 14-4-6-16-2-12-8 |
| 11 | -29 | 32.4 | 38.4 | 24.1 | 19.3 | 14-30-4-3-7-11-7 |
| 12 | -31 | 14.7 | 17.3 | 24.3 | 20.5 | -8 |
| 13 | -33 | 16.5 | 18.9 | 23.6 | 18.5 | -9 |
| 14 | -35 | 19.2 | 23.6 | 31.3 | 25.5 | -18 |
| 15 | -36 | 22.3 | 25.1 | 36.4 | 28.9 | 14-30-4-4-2-7-14 |
| 16 | -43 | 20.6 | 22.7 | 34.5 | 27.3 | -2 |
| Average | | 24.2 | 28.0 | 30.2 | 24.4 | |
| Increase | | | 3.7±0.23 | 5.9±0.37 | | |
| Percent increase | | | 15.3 | 24.2 | | |

on the plant. But the fact is that the larger seeds resulting from crosses often appear to mature more slowly than selfed seeds borne on the same ear. Consequently the conclusion that the crossed seeds owe their lack of water to a more rapid and complete maturity is admissible only as a possibility. We are inclined to attribute the matter merely to a difference in the physical constitution of the tissues formed, particularly those of the endosperm. If the pericarp is somewhat more porous and the cell walls within the endosperm somewhat thinner in the crossed seeds, rapidity of drying certainly would be facilitated.

The immediate effect of pollination upon protein content (table 13) is slight. Only 7 instances out of 32 show an increase of protein in the crossed seeds, and there is on the average a decrease that is probably significant; but the decrease is small. The average of A × B is 0.14 ± 0.04 percent less than the average of A; and the average of B × A is 0.60 ± 0.07 percent less than B. When one remembers that the crossed seeds are larger than the selfed seeds, and that the increase in size is greater in B × A than in A × B, it is clear that there is actually more

TABLE 12

*The immediate effect of pollination upon the water content of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.*

| Pollen mixture number | Parent plant A | Percent of water in seeds | | | | Parent plant B |
|---|---|---|---|---|---|---|
| | | A | A × B | B × A | B | |
| 1 | 21–13–9–7–57–1 | 7.20 | 6.90 | 7.05 | 7.70 | 14–30–4–3–7–11–4 |
| 2 | –2 | 7.40 | 6.90 | 6.80 | 7.65 | –3 |
| 3 | –3 | 7.80 | 7.70 | 7.22 | 7.25 | –10 |
| 4 | –5 | 7.80 | 7.33 | 7.13 | 7.60 | –2 |
| 5 | –7 | 7.40 | 6.90 | 7.15 | 7.25 | 14–30–4–4–2–7–6 |
| 6 | –10 | 7.18 | 6.85 | 6.70 | 7.55 | 14–30–4–3–7–11–1 |
| 7 | –14 | 8.27 | 7.55 | 7.60 | 7.10 | 14–30–4–4–2–7–3 |
| 8 | –20 | 8.23 | 6.78 | 6.65 | 7.40 | 14–30–6–11–3–11–3 |
| 9 | –24 | 7.45 | 6.58 | 6.95 | 6.95 | 14–4–6–4–7–8–5 |
| 10 | –25 | 7.30 | 6.83 | 7.10 | 6.45 | 14–4–6–16–2–12–8 |
| 11 | –29 | 7.45 | 7.65 | 7.32 | 7.35 | 14–30–4–3–7–11–7 |
| 12 | –31 | 7.30 | 7.37 | 7.05 | 7.20 | –8 |
| 13 | –33 | 7.53 | 7.65 | 7.45 | 7.30 | –9 |
| 14 | –35 | 8.45 | 7.33 | 7.00 | 7.55 | –18 |
| 15 | –36 | 8.48 | 7.20 | 7.15 | 7.25 | 14–30–4–4–2–7–14 |
| 16 | –43 | 8.40 | 7.05 | 6.50 | 7.20 | –2 |
| Average | | 7.73 | 7.16 | 7.05 | 7.30 | |
| Decrease | | | 0.57±0.09 | 0.25±0.08 | | |

protein produced in the crossed seeds than in the selfed seeds (see table 14). The result of crossing, therefore, is not merely to increase the size of the seed, while the total amount of protein remains the same. The evidence, as far as one may judge from the protein content, is that the increase consists of tissue having practically a normal constitution. At the same time it must be admitted that the increase due to crossing is not uniform in the various parts of the seed, and therefore the increase of one type of protein may be greater than another. This matter is illustrated by the data set forth in table 14.

Five pollen mixtures of the kind illustrated in tables 11, 12 and 13, though not the same ones, were used in obtaining A × B, A, B × A and B seeds in which the weights of pericarp, endosperm, and embryo were determined (table 15). The seeds were soaked in boiling water for about 10 minutes, the parts were separated, and dried to a comparatively constant weight in a steam-heated room by exposure to the air in open sacks for one month. The weights of the 10 to 50 seeds comprising each lot were then averaged. The results serve to explain some of the differences obtained in the reciprocal crosses in protein content.

TABLE 13

*The immediate effect of pollination upon the protein content of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.*

| Pollen mixture number | Low-protein parent plant A | Percent of protein in seeds | | | | High-protein parent plant B |
|---|---|---|---|---|---|---|
| | | A | A × B | B × A | B | |
| 1 | 21-13-9-7-57-1 | 7.54 | 7.25 | 12.64 | 13.27 | 14-30-4-3-7-11-4 |
| 2 | -2 | 7.16 | 7.25 | 11.80 | 13.06 | -3 |
| 3 | -3 | 6.98 | 6.71 | 12.33 | 12.81 | -10 |
| 4 | -5 | 7.46 | 7.55 | 12.59 | 13.19 | -2 |
| 5 | -7 | 7.49 | 7.58 | 14.95 | 15.90 | 14-30-4-4-2-7-6 |
| 6 | -10 | 7.21 | 6.71 | 13.13 | 13.73 | 14-30-4-3-7-11-1 |
| 7 | -14 | 7.23 | 7.10 | 13.59 | 14.53 | 14-30-4-4-2-7-3 |
| 8 | -20 | 8.59 | 8.45 | 16.00 | 15.93 | 14-30-6-11-3-11-3 |
| 9 | -24 | 8.51 | 8.23 | 14.30 | 14.51 | 14-4-6-4-7-8-5 |
| 10 | -25 | 7.01 | 7.31 | 13.72 | 13.63 | 14-4-6-16-2-12-8 |
| 11 | -29 | 10.47 | 9.95 | 11.60 | 12.08 | 14-30-4-3-7-11-7 |
| 12 | -31 | 7.35 | 7.02 | 11.77 | 12.12 | -8 |
| 13 | -33 | 7.30 | 7.45 | 12.03 | 12.88 | -9 |
| 14 | -35 | 6.89 | 6.61 | 13.58 | 14.74 | -18 |
| 15 | -36 | 7.17 | 6.94 | 14.00 | 14.22 | 14-30-4-4-2-7-14 |
| 16 | -43 | 6.89 | 6.99 | 14.11 | 15.23 | -2 |
| Average | | 7.58 | 7.44 | 13.26 | 13.86 | |
| Decrease | | | 0.14±0.04 | 0.60±0.07 | | |
| Percent decrease | | | 1.85 | 4.33 | | |

The average weight of the A × B embryos over the A embryos was only 6.14 percent, as compared with the increase of 28.20 percent by which the B × A embryos exceeded the B embryos. In other words the large embryos characteristic of the high-protein strain were increased only 6.14 percent when crossed with the pollen of the low-protein strain, although the small embryos of the low-protein strain were increased by 28.20 percent. Yet this is somewhat of a distortion of the results due to the difference in size of the embryos in the parent strains. As a matter

of fact there was no significant difference between the size of the embryos in reciprocal crosses though perhaps this result might have been expected because of a possible difference in the metabolic efficiency of the plants of the different strains upon which the seeds matured. But A × B gave embryos averaging 3.11 cg in weight, and B × A yielded embryos averaging 3.00 cg in weight,—as nearly an even result as could be expected.

In the case of the endosperms, where two maternal nuclei unite with one paternal nucleus, the result was different. The large endosperm of

TABLE 14

*The immediate effect of pollination upon the amount of protein in maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.*

| Pollen mixture number | Parent plant A | Amount protein in centigrams | | | | Parent plant B |
|---|---|---|---|---|---|---|
| | | A | A × B | B × A | B | |
| 1 | 21–13–9–7–57–1 | 2.04 | 2.33 | 3.83 | 2.96 | 14–30–4–3–7–11–14 |
| 2 | –2 | 1.45 | 1.59 | 2.97 | 2.80 | –3 |
| 3 | –3 | 1.82 | 2.09 | 3.81 | 2.88 | –10 |
| 4 | –5 | 1.66 | 1.84 | 3.95 | 3.34 | –2 |
| 5 | –7 | 2.01 | 2.36 | 5.26 | 4.50 | 14–30–4–4–2–7–6 |
| 6 | –10 | 2.00 | 2.17 | 3.93 | 3.25 | 14–30–4–3–7–11–1 |
| 7 | –14 | 2.02 | 2.15 | 5.35 | 4.29 | 14–30–4–4–2–7–3 |
| 8 | –20 | 2.65 | 3.00 | 3.46 | 3.36 | 14–30–6–11–3–11–3 |
| 9 | –24 | 2.42 | 2.72 | 4.16 | 3.70 | 14–4–6–4–7–8–5 |
| 10 | –25 | 1.72 | 2.17 | 5.02 | 4.10 | 14–4–6–16–2–12–8 |
| 11 | –29 | 3.39 | 3.82 | 2.80 | 2.33 | 14–4–30–4–3–7–11–7 |
| 12 | –31 | 1.08 | 1.21 | 2.86 | 2.48 | –8 |
| 13 | –33 | 1.20 | 1.41 | 2.84 | 2.38 | –9 |
| 14 | –35 | 1.32 | 1.56 | 4.25 | 3.76 | –18 |
| 15 | –36 | 1.60 | 1.74 | 5.10 | 4.11 | 14–30–4–4–2–7 –14 |
| 16 | –43 | 1.42 | 1.59 | 4.87 | 4.16 | –2 |
| Average | | 1.86 | 2.11 | 4.03 | 3.40 | |
| Percent increase | | | 13.1 | 18.5 | | |

the low-protein type, 27.75 cg, was increased to 32.41 cg by the cross with A; but the endosperm of the high-protein type, 21.55 cg, was increased to 27.27 cg by B pollen. Thus, though the increase of B × A over B was 4.66 cg or 16.79 percent, the increase of A × B over A was 6.02 cg or 27.93 percent. The reciprocals were not alike, as in the case of the embryos. The B × A endosperms exceeded the A × B endosperms by 5.14 cg. Nevertheless, it should be noted that the single nucleus coming from the low-protein (B) increased the endosperm of the seeds borne on A by 6.02 cg, while the single nucleus of the high-protein (A) increased

TABLE 15

*The immediate effect of pollination upon the weight of different parts of the maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.*

| Parent plant A | Parts of seed | Weight in centigrams | | | | Parent plant B |
|---|---|---|---|---|---|---|
| | | A | A × B | B × A | B | |
| 14-30-4-3-7-11-5 | Embryo | 2.68 | 2.57 | 2.63 | 2.10 | 21-13-9-7-57-6 |
| | Pericarp | 2.21 | 2.57 | 1.92 | 2.07 | |
| | Endosperm | 21.74 | 26.79 | 30.35 | 24.85 | |
| | Per. and end. | 23.95 | 29.36 | 32.27 | 26.92 | |
| | Total | 26.63 | 31.94 | 34.91 | 29.02 | |
| 14-30-6-2-13-5-1 | Embryo | 3.04 | 3.10 | 2.29 | 1.82 | 21-13-9-7-57-9 |
| | Pericarp | — | — | — | 1.96 | |
| | Endosperm | — | — | — | 22.11 | |
| | Per. and end. | 26.71 | 29.93 | 29.31 | 24.08 | |
| | Total | 29.75 | 33.04 | 31.60 | 25.91 | |
| 14-30-6-2-13-5-11 | Embryo | 3.30 | 3.37 | 3.10 | 2.26 | 21-13-9-7-57-15 |
| | Pericarp | 2.95 | 2.99 | 1.89 | 1.71 | |
| | Endosperm | 22.46 | 25.32 | 30.19 | 25.96 | |
| | Per. and end. | 25.41 | 28.31 | 32.08 | 27.68 | |
| | Total | 28.71 | 31.69 | 35.19 | 29.94 | |
| 14-30-12-14-1-10-4 | Embryo | 3.05 | 3.18 | 2.15 | 1.92 | 21-13-9-7-57-22 |
| | Pericarp | 1.92 | 2.15 | 2.17 | 1.89 | |
| | Endosperm | 21.46 | 29.08 | 22.01 | 22.15 | |
| | Per. and end. | 23.39 | 31.23 | 24.18 | 24.04 | |
| | Total | 26.44 | 34.41 | 26.34 | 25.96 | |
| 14-30-4-4-2-7-7 | Embryo | 2.62 | 3.33 | 4.83 | 3.64 | 21-13-9-7-57-39 |
| | Pericarp | 2.55 | 2.93 | 2.37 | 2.27 | |
| | Endosperm | 20.55 | 27.91 | 47.12 | 43.68 | |
| | Per. and end. | 23.10 | 30.85 | 49.50 | 45.95 | |
| | Total | 25.72 | 34.18 | 54.33 | 49.60 | |
| Average embryo | | 2.93 | 3.11 | 3.00 | 2.34 | |
| Increase | | 0.18= | 6.14% | 0.66= | 28.20% | |
| Average pericarp | | 2.40 | 2.66 | 2.08 | 1.98 | |
| Increase | | 0.26= | 10.83% | 0.18= | 5.05% | |
| Average endosperm | | 21.55 | 27.27 | 32.41 | 27.75 | |
| Increase | | 6.02= | 27.93% | 4.66= | 16.79% | |
| Average pericarp and endosperm | | 24.51 | 29.93 | 33.46 | 29.73 | |
| Increase | | 5.42= | 22.11% | 3.73= | 12.54% | |
| Average total | | 27.44 | 33.05 | 36.47 | 32.08 | |
| Increase | | 5.61= | 20.40% | 4.39= | 13.68% | |

the endosperm of the seeds borne on B by only 4.66 cg. There is a discrepancy between the effects of the two pollens for which we have no adequate explanation, though some light is thrown on the matter by

the following computations. If one assumes that the heterotic effect would be the same in both crosses and that disregarding this effect the potentiality of each nucleus entering into the formation of the endosperm is realized, then the resulting endosperm of A × B should be one-third of the sum of 21.55 cg plus 21.55 cg plus 27.75 cg, or 23.62 cg, and the resulting endosperm of B × A should be one-third of 27.75 cg plus 27.75 cg plus 21.55 cg, or 25.68 cg. The endosperm actually produced in the cross A × B was 3.65 cg, or 15.5 percent, above the calculated endosperm, and the endosperm actually produced in the cross B × A was 6.73 cg, or 26.2 percent, above the calculated endosperm. The effect of heterosis was presumably greater, therefore, in the case where the low-protein type was the mother.

The increase in the pericarp may be explained as a simple stretching with resulting increase of tissue formation, due to the larger endosperm which had to be covered. Where the increase in endosperm was large, the increase in the pericarp was large; where the increase in endosperm was small, the increase in pericarp was small.

Another rather interesting experiment was made with some of the same pollen mixtures used in obtaining the results set forth in tables 11, 12 and 13. These combinations of pollen from high-protein (variety 14) and from low-protein plants (variety 21) were applied to the silks of a third variety of high-protein plants (20A). One cannot compute the amount of change in seed size, moisture content or protein content when compared with selfed seeds of parent plants C (20A), but a comparison can be made between the effects of the pollen of the low-protein type, 21, and the high-protein type, 14, on the high protein type 20A. Since strain 20A and strain 21 originally came from the same commercial variety of white dent corn, and strain 14 is a yellow dent, it cannot be assumed that the total amount of genetic difference between 20A and 21 is greater or less than the total amount differentiating 20A and 14. For this reason there is no *a priori* justification in predicting a greater or smaller heterotic effect of either pollen on the third strain. The experiments actually did yield a slightly larger seed when the pollen from strain 14 was used. The difference was 1.4 ± 0.38 cg (table 16). If this may be taken to be the result of heterosis, it is a rather important fact, for table 17 shows that there is no significant difference between the moisture content of the two crosses, yet there is a slight but real excess of protein in favor of strain 14,—a difference of 0.26 ± 0.05 percent.

Whether one is justified in generalizing from so few data is problematical. At least it is permissible to point out a possible inference. Illinois

High Protein (20A) and Illinois Low Protein (21), during the process of selection to change the composition, have been differentiated physically in a very marked degree. They must differ by a great many hereditary factors. Stadtmueller's High-Protein Leaming, except for the yellow color of the seeds, resembles Illinois High Protein much more than the latter resembles Illinois Low Protein. Yet the indication of the heterosis test is that Illinois High Protein is genetically more distinct from Stadtmueller's High-Protein Leaming than it is from Illinois Low Protein in certain factors essential to optimum development of the seed. Thus,

TABLE 16

*The immediate effect of pollination upon the weight of maize seed as shown by out-crossed seeds resulting from application of some of the same pollen mixtures used in tables 11, 12 and 13 to a third high-protein variety. Plants grown in 1917.*

| Pollen mixture number | Parent plant C | Weight of seeds in centigrams | | Parent plant A | Parent plant B |
|---|---|---|---|---|---|
| | | C × A | C × B | | |
| 1 | 20A-8-5-35-8 | 20.5 | 24.5 | 21-13-9-7-57-1 | 14-30-4-3-7-11-4 |
| 2 | -3 | 19.7 | 23.7 | -2 | -3 |
| 3 | -4 | 25.4 | 25.0 | -3 | -10 |
| 6 | -11 | 20.3 | 22.9 | -10 | -1 |
| 8 | -24 | 27.3 | 27.5 | -20 | 14-30-6-11-3-11-3 |
| 9 | -26 | 25.9 | 27.7 | -24 | 14-4-6-4-7-8-5 |
| 13 | -6 | 20.2 | 20.1 | -33 | 14-30-4-3-7-11-9 |
| 16 | -13 | 20.1 | 25.8 | -43 | 14-30-4-4-2-7-2 |
| 16 | -15 | 23.9 | 27.5 | -43 | -2 |
| 16 | -18 | 21.6 | 20.9 | -43 | -2 |
| 16 | -21 | 20.2 | 18.9 | -43 | -2 |
| 16 | -30 | 21.7 | 21.2 | -43 | -2 |
| 16 | -37 | 21.6 | 21.0 | -43 | -2 |
| 17 | -7 | 21.3 | 22.8 | -9 | 14-30-6-2-13-5-1 |
| Average | | 22.1 | 23.5 | | |
| Difference | | | 1.4±0.38 | | |

while it is undoubtedly true that selection for high protein in varieties of different origin does bring about a certain phenotypic uniformity and possibly a considerable genotypic uniformity, nevertheless different combinations of genes contributing toward high-protein complexes may be isolated, and through their union a notable heterotic effect obtained without reduction in protein content other than that due to increased size and number of seeds.

Some further information of a character similar to that just discussed, is given in table 18. Mixtures of pollen from pairs of plants designated

A and B were applied to the plants designated A. The comparison was made by self-pollinating plants B. The table shows the calculations of the percent increase in weight of the crossed seeds over the selfed seeds, and the percent of protein in samples of the selfed and the crossed seeds.

The percent increase in weight of seed in crosses of high protein with high protein vary a great deal, all the way from −7.30 to 29.23 percent. In at least two of the examples, the small amount of increase is due to variable time of pollination, since small crossed seeds were found only at

TABLE 17

*The immediate effect of pollination upon weight, water content and percent of protein in maize seed as shown by out-crossed seed resulting from some of the same pollen mixtures as used in tables 11, 12 and 13. High-protein plants pollinated by a mixture of a distinct high-protein and a low-protein strain.*

| Pollen mixture number | Parent plant C | Weight of seeds in centigrams | | Percent water in seeds | | Percent protein in seeds | |
|---|---|---|---|---|---|---|---|
| | | C × A | C × B | C × A | C × B | C × A | C × B |
| 1 | 20A-8-5-35-8 | 20.5 | 24.5 | 7.25 | 7.16 | 16.98 | 17.30 |
| 2 | -3 | 19.7 | 23.7 | 7.40 | 7.25 | 16.61 | 16.98 |
| 3 | -4 | 25.4 | 25.0 | 7.48 | 7.28 | 16.35 | 16.52 |
| 6 | -11 | 20.3 | 22.9 | 7.10 | 7.20 | 17.16 | 17.65 |
| 8 | -24 | 27.3 | 27.5 | 7.67 | 7.63 | 15.70 | 15.70 |
| 9 | -26 | 25.9 | 27.7 | 7.50 | 7.05 | 16.36 | 16.98 |
| 13 | -6 | 20.2 | 20.1 | 7.30 | 7.18 | 13.75 | 13.74 |
| 16 | -13 | 20.1 | 25.8 | 6.85 | 7.00 | 16.58 | 17.00 |
| 16 | -15 | 23.9 | 27.5 | 7.00 | 6.95 | 16.33 | 17.07 |
| 16 | -18 | 21.6 | 20.9 | 6.98 | 6.98 | 12.90 | 12.77 |
| 16 | -21 | 20.2 | 18.9 | 7.10 | 7.00 | 11.98 | 12.24 |
| 16 | -30 | 21.7 | 21.2 | 7.20 | 7.10 | 13.34 | 13.33 |
| 16 | -37 | 21.6 | 21.0 | 7.40 | 6.95 | 13.37 | 13.37 |
| 17 | -7 | 21.3 | 22.8 | 7.05 | 7.44 | 17.69 | 18.03 |
| Average | | 22.1 | 23.5 | 7.23 | 7.16 | 15.36 | 15.63 |
| Difference | | | 1.4±0.38 | 0.07±0.04 | | | +0.26±0.05 |

the tip of some of the ears. But there is also evidence of a real difference in the ratio of increase. For example, the amount of increase when plants of family 14 were pollinated by pollen from plants of family 20A is much greater than when the reciprocal cross was tried. This result is in harmony with the figures obtained when reciprocal crosses were made between families 21 and 14, where the increase was usually greater when the plants of family 14 were used as maternal parents. The explanation would appear to lie in the fact that families 20A and 21 were both originated from Burr's White by selection for high protein and for

low protein respectively. They seem to have retained the power to affect the plants of family 14 somewhat similarly in spite of their present differences in physical appearance.

TABLE 18

*Immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed seed grown upon the same ears (A and A × B seeds from same ears). Plants grown 1916.*

| | Parent plant A | Percent protein in seeds | | | Parent plant B | Percent increase in weight of crossed seeds above selfed |
|---|---|---|---|---|---|---|
| | | A | A × B | B | | |
| High by low | 14-30-9-8-1-3 | 14.84 | 14.63 | 10.63 | 21-13-2-11-18 | 16.94 |
| High by high | 14-4-6-4-7-26 | 13.78 | 13.82 | 16.22 | 20A-4-25-37 | 19.44 |
| | 14-4-6-16-7-27 | 13.14 | 12.59 | 16.63 | -45 | 29.23 |
| | -30 | 12.99 | 13.04 | 16.63 | -45 | 8.90 |
| | -28 | 13.92 | 13.94 | 16.63 | -45 | 25.00 |
| | 20A-4-25-41 | 15.95 | 16.08 | 14.10 | 14-4-6-4-7-23 | 15.29 |
| | -27 | 16.10 | 16.53 | 15.30 | -8 | -7.30* |
| | -31 | 15.61 | 15.56 | 16.49 | 14-30-9-8-1-4 | 17.48 |
| | -37 | 16.22 | 16.27 | 12.90 | 14-4-6-4-7-7 | 6.97 |
| | -45 | 16.63 | 16.41 | 14.44 | -24 | 12.69 |
| | -33 | 16.69 | 16.69 | 16.49 | 14-30-9-8-1-4 | 9.91 |
| | -22 | 16.51 | 17.20 | 16.27 | -6 | -6.86* |
| | 20A-11-10-22 | 15.65 | 15.59 | — | | 6.99 |
| | 20A-4-25-32 | 15.85 | 16.10 | 16.49 | 14-30-9-8-1-4 | 5.36 |
| Average | | 15.31 | 15.37 | 15.72 | | 11.01 |
| Low by high | 21-13-2-11-23 | 10.28 | 10.25 | 12.90 | 14-4-6-4-7-7 | 15.32 |
| | -36 | 7.91 | 7.79 | 14.44 | -24 | 12.57 |
| | -26 (1) | 10.51 | 10.28 | 14.44 | -24 | 15.33 |
| | -24 | 8.23 | 8.36 | 13.06 | -13 | 14.54 |
| | -26 (2) | 11.85 | 11.98 | 14.44 | -24 | 13.65 |
| | 21-13-9-7-22 | 7.54 | 7.39 | 15.85 | 14-30-9-8-1-8 | 16.03 |
| Average | | 9.39 | 9.34 | 14.19 | | 14.57 |

* Crossed seeds at tip of ear.

The increase in size of seed when low-protein plants (family 21) are pollinated with pollen from high-protein plants (family 14) is rather uniform.

The difference in protein content between the crossed and the selfed seeds is not marked. In general it follows the amount contained by the maternal parent.

### *The protein content of different ears borne on the same plant*

In a few instances it has been possible to obtain two selfed ears of similar size on the same plant. Numerous pollinations of this kind were made, but in most cases only one ear developed. As seen by referring to table 19, the difference in protein content is very small with the exception of one plant. Sometimes the upper ear is slightly higher than the lower ear, sometimes the reverse is true. The higher protein content probably follows the ear with the smaller number of seeds. At any rate, position on the plant is not a notable factor in influencing the protein.

TABLE 19

*Difference in protein content of ears produced on the same plant through self-pollination. Plants grown 1917.*

| Plant number | Percent protein in seeds | | | |
|---|---|---|---|---|
| | Top ear | Bottom ear | Difference | |
| 21-13-9-7-57-54 | 7.24 | 6.97* | −0.27 | |
| -58 | 7.74 | 8.09 | +0.35 | |
| -73 | 8.72 | 8.67 | −0.05 | |
| -100 | 11.06 | 10.91 | −0.15 | |
| 21-13-2-11-36 | 7.84 | 7.91 | +0.07 | |
| -26 | 11.85 | 10.51 | −1.34 | |
| Average..... | 9.08 | 8.84 | −0.24 | 2.64% decrease |

* The analysis of this ear was given as 13.79 percent and was not checked as it should have been. This is a difference of 6.53 percent on the same plant. This is nearly twice as large as the greatest difference between any two plants grown the same year in this strain, so it seems that the analysis is wrong. The percent of nitrogen was given as 2.02. In another similar wrong-appearing analysis which was checked, the nitrogen was given as 2.30 when in all probability it should have been 1.30. It therefore seems reasonable to suppose that in this case also the figure should be 1.02 instead of 2.02 and has been so calculated.

### *The immediate effect of pollination on different ears borne on the same plant*

Since two ears borne on the same plant appear to have potentially the same protein contents provided the development of each is similar, the experiments reported in tables 20, 21 and 22 are in a sense repetitions of the experiments reported in tables 13 and 17. There is this point of difference, however: The data for tables 20, 21 and 22 were not obtained by mixing pollen of pairs of plants, applying the mixture to the silks of a single ear, and separating the resulting crossed and selfed seeds by inspection, but by applying foreign pollen to the silks of one ear and self pollen to the silks of another ear on the same plant. Conceivably

there might be a difference in the results of the two experiments. In the mixed-pollen experiments a large number either of crossed or of selfed seeds developing on a single ear might influence the size or composition of the seeds of the other type. In the development of two ears having different male parents on the same plant, such influence—if any—would be expected to be small. Under such an hypothesis, there should

TABLE 20

*Immediate effect of pollination upon the protein content of the seeds as shown by selfed and crossed ears borne upon the same stalks (A and A × B are different ears on same plant). Plants grown 1916.*

| High-protein parent plant A | Percent protein in seeds | | | Low-protein parent plant B |
|---|---|---|---|---|
| | A | A × B | B | |
| 14-30-9-8-1-2 | 16.30 | 12.57 | 9.91* | 21-13-2-11-18 |
| 14-6-20-10-7 | 17.24 | 13.88 | 7.28 | 21-13-9-7-1 |
| 14-4-6-4-7-25 | 14.58 | 14.43 | 11.08 | 21-13-2-11-31 |
| 14-4-6-16-7-15 | 14.00* | 15.43 | 6.97 | 21-13-9-7-37 |
| 7-14 | 14.00* | 12.76 | 6.97 | -37 |
| 2-27 | 14.25* | 15.61 | 7.84 | -40 |
| 14-30-9-8-1-5 | 15.78* | 16.24 | 11.69 | 21-13-2-11-28 |
| 14-6-20-10-9 | 15.93* | 17.30 | 8.79* | 21- -y |
| 20A-11-10-16 | 16.84* | 18.28 | 12.94 | 10-4-3-4-5-2-1 |
| 20A-8-5-43 | 18.01* | 17.55 | 8.56 | 21-13-9-7-5 |
| -42 | 18.01* | 17.21 | 8.56 | -5 |
| -17 | 18.01* | 17.27 | 7.67* | -10 |
| -37 | 18.01* | 16.97 | 7.67* | -10 |
| -41 | 18.01* | 16.94 | 7.67* | -23 |
| Average..... | 16.36 | 15.89 | 8.83 | |
| Low-protein parent plant A | | | | High-protein parent plant B |
| 21-13-2-11-5 | 10.23 | 9.44 | 17.58 | 20A-8-5-10 |
| -38 | 12.32 | 12.81 | 18.44 | -22 |
| Average..... | 11.28 | 11.13 | 18.01 | |

* No selfed ear obtained, average of all selfed ears of the same strain used instead.

be a greater difference between the seeds A and the seeds A × B in the two-ear experiments than in the mixed-pollen experiments.

It does not seem to us that such a conclusion is justified by the facts, though there are a few instances where the difference in protein content between the selfed and the crossed seeds is notably large. The difficulty in the matter is the paucity of evidence. On the plants of table 20,

attempts at self-pollination were successful in only 5 out of 16 cases, owing to a variety of circumstances. No selfed ears being obtained from the plants on which the crosses were, a comparison was made between the percent of protein in the cross-pollinated ears and that of the average of all selfed ears of the same strain. This comparison gave results comparable to those obtained with the mixed pollen. The protein content followed that of the maternal parent, though it lagged somewhat behind. Nevertheless, one could not maintain that a selfed ear obtained on the particular plant used would have had exactly the same value as the average of all the self-pollinated sister ears. Hence a strict comparison is invalid.

TABLE 21

*The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed seed produced on different ears on the same stalk. Plants grown 1915.*

| Low-prot in parent plant A | Percent protein in seeds | | | | High-protein parent plant B |
|---|---|---|---|---|---|
| | A | A × B | B × A | B | |
| 21–13–2–2 | 10.52 | 11.23 | 15.29 | 15.92 | 20A–4–6 |
| 2–12 | 12.19 | 12.33 | 15.37 | — | –2 |
| 2–6 | 9.24 | 8.51 | — | — | –1 |
| 9–10 | 10.51 | 8.59 | 17.50 | — | –9 |
| 2–5 | 12.77 | 12.68 | — | 16.12 | 14–30–6–11–9 |
| 2–8 | 12.35 | 12.13 | — | 16.24 | –10 |
| 2–3 | 11.08 | 11.87 | — | — | 2–6 |
| 9–4 | 10.14 | 11.02 | — | — | 11–5 |
| 9–3 | 10.40 | 11.55 | — | — | 2–1 |
| 9–7 | 7.81 | 7.47 | — | 16.86 | –3 |
| 9–1 | 10.38 | 10.35 | 16.38 | — | 20A–11–2 |
| Average..... | 10.67 | 10.70 | | | |

The five remaining cases comprised three plants where the cross was high protein by low protein, and two cases where it was low protein by high protein. Plant 21–13–2–11–38 crossed with pollen from plant 20A–8–5–28 was the only example of a crossed ear having a higher percent of protein (12.81) than a selfed ear (12.32). In the remaining ears the percent of protein in the crossed ears was less than in the selfed ears, and in two cases the difference is extreme. Plant 14–30–9–8–1–2 selfed had 16.30 percent protein in the ear; but when crossed with pollen from plant 21–13–2–11–18 from a sib averaging 9.91 percent for selfed ears, the crossed seeds contained only 12.57 percent protein. Likewise plant 14–6–20–10–7 yielded a selfed ear containing 17.24 percent protein, but when crossed with pollen from plant 21–13–9–7–1 (7.28 percent protein selfed), the $F_1$ seeds contained only 13.88 percent protein.

These differences in favor of selfed ears were so much greater than in any of the similarly produced ears, that it is difficult to accept them as correct. On the other hand such differences may be obtained at times, partly for the reasons outlined previously and partly because of the possiblity that certain seed factors may have an immediate influence in particular crosses which is very different from their effect in others.

The theory of factor complexes having different effects seems the more reasonable in view of the results tabled in table 21. There comparable figures are listed for 11 experiments. That is, the figures for the protein of a plant of group A, in this case low protein, were always obtained from a selfed ear of that group, and the figures for the cross of the general formula A × B were always obtained from a second ear borne on the same stalk. The difference in protein content between the crossed ears and the selfed ears is on the whole negligible. Furthermore, considering

TABLE 22

*The immediate effect of pollination upon the protein content of maize seed as shown by two ears borne on the same plant one crossed by high the other by low protein. Plants grown 1916.*

| High-protein parent plant A | Percent protein in seeds | | | | Low-protein parent plant B | High-protein parent plant C |
|---|---|---|---|---|---|---|
| | A × B | A × C | B | C | | |
| 20A-4-25-46 | 18.14 | 17.77 | 11.48 | 15.30 | 10-3-7-5-4-2-1 | 14-4-6-4-7-8 |
| 20A-4-25-18 | 16.98 | 17.17 | 9.55 | 16.27 | 21-13-2-11-20 | 14-30-9-8-1-6 |
| Average..... | 17.56 | 17.47 | 10.57 | 15.79 | | |

the data for each pair of ears separately, the crossed ears show an excess in 5 instances and the selfed ears an excess in 6 instances.

In two experiments we were able to obtain a pair of ears on a stalk, the one crossed by high-protein pollen, the other by low-protein pollen (table 22). In the first instance the ear produced by the crossing with the high-protein pollen was slightly higher in protein than the one crossed with low-protein pollen; in the other case the reverse phenomenon occurred.

### *The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed ears grown upon different plants of the same strain*

In connection with the work done to estimate the immediate effect of various matings on the size and protein content of the seed, where environmental factors were largely eliminated, it is interesting to examine

the results listed in table 23. Here, it is true, environmental factors have full play as far as this is possible on uniform plots where the precautions usually taken in comparative field test are observed. Nevertheless averages of selfed ears and of crossed ears yielded figures of just about the comparative values that would have been expected from the previous work.

The first three reciprocal crosses reported were between various selections of Illinois Low Protein (21) and Stadtmueller's High-Protein

TABLE 23

*The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed ears grown upon different plants. Last four pure strains grown 1912, remainder 1913.*

| Pedigree number | Condition of seeds | Number of ears analyzed | Percent protein in populations | |
|---|---|---|---|---|
| | | | Range | Average |
| (21–3) | Selfed | 12 | 8.94–11.24 | 10.24 |
| (21–3 × (14–10)–30) | Crossed | 4 | 9.06–10.83 | 9.72 |
| (14–10)–30 × (21–3) | Crossed | 12 | 12.31–16.71 | 14.69 |
| (14–10)–30 | Selfed | 10 | 13.14–16.22 | 15.23 |
| (21–3) | Selfed | 12 | 8.94–11.24 | 10.24 |
| (21–3) × (14–11)–8 | Crossed | 4 | 9.55–11.05 | 10.12 |
| (14–11)–8 × 21–3 | Crossed | 7 | 13.35–15.15 | 14.21 |
| (14–11)–8 | Selfed | 11 | 12.78–15.92 | 14.41 |
| (21–2) | Selfed | 15 | 7.72–12.57 | 9.41 |
| (21–2) × (14–10) | Crossed | 6 | 8.76–13.37 | 11.42 |
| (14–10) × (21–2) | Crossed | 10 | 8.74–14.37 | 11.66 |
| (14–10) | Selfed | 14 | 8.21–15.94 | 12.19 |
| (14–11) | Selfed | 13 | 8.52–17.86 | 11.85 |
| (14–11) × (20–2) | Crossed | 10 | 7.73–13.28 | 10.92 |
| (20–2) × (14–11) | Crossed | 10 | 10.36–16.89 | 15.10 |
| (20–2) | Selfed | 19 | 11.95–17.10 | 14.87 |

Leaming (14). The fourth cross was between Stadtmueller's High-Protein Leaming and Illinois High Protein (20). Each ear, 168 in all, was hand-pollinated.

In the first two crosses the average protein content of the crossed ears was somewhat lower than the average of the ears of the maternal parent in every case. In the last two crosses the maternal parents were higher than the crosses in two cases. Our conclusions should really be based on the first two crosses, however, for the analysis of the pure strains of the last two crosses was made from ears grown the previous year.

It was thought unnecessary to report the analysis of each individual ear since in no case were more than 15 ears tested, making standard deviations rather untrustworthy. The averages and the protein range of the ears analyzed are sufficient to show that the protein content of the crosses followed that of the mother, and to indicate that the variability of the ears bearing the $F_1$ seeds was no greater than in the selfed strains.

*The protein content of first-hybrid-generation plants bearing second-hybrid-generation seeds*

When one compares the protein content of the seeds borne on plants of the first hybrid generation with that of the pure strains from which they came, naturally there is no chance to eliminate variations due to environment except by growing them under as uniform conditions as possible. There is the further difficulty of comparing the actual populations whose plants furnish the gametes for the cross with the hybrid plants themselves. One of three courses may be pursued. Samples of the true parental populations may be held over a year with resultant loss of vitality in the seeds. Parental populations of one year may be compared with hybrid populations of the next year. Or, self-pollinated daughters of the actual parental populations may be tested at the same time as the hybrid populations. The last course of procedure holds some practical advantages, and is probably not any more inaccurate than the other two because of the uniformity of the inbred parental strains.

The first two tests of this kind were made in 1912, one a cross between two high proteins, the other a low protein by a high protein.

Strain 20–2, Illinois High Protein, ranged from 11.95 percent to 17.10 percent protein in the 19 selfed ears analyzed, with an average of 14.87 percent. It yielded at the rate of 39.7 bushels per acre. This strain was crossed with No. 14, of which two selections grown in 1912 yielded at the rate of 50.1 bushels per acre. The first, 14 (1911 seed), ranged from 8.52 percent to 17.86 percent in the 13 ears analyzed,—an average of 11.85 percent. The second selection, 14 (1910 seed), ranged from 8.21 percent to 15.94 percent (14 ears),—an average of 12.19 percent. The $F_1$ plants, 20–2 × 14–11, yielded at the rate of 55.1 bushels per acre, and ranged from 9.25 percent to 15.02 percent (12 ears),—an average of 11.85 percent. The parental average in protein was thus 13.45 percent, while the average of the $F_1$ plants was 1.60 percent lower.

Illinois Low Protein (21–2), ranging from 7.70 percent to 12.57 percent in protein (average 9.41 percent for 16 ears), and yielding 42.3 bushels per acre, was also crossed with strain 14. The result was an $F_1$ generation yielding 53.3 bushels per acre, with ears ranging from 6.24 percent to 13.03 percent protein (24 ears). The average of the $F_1$ generation, 9.18 percent, was therefore 1.54 percent below the parental average.

In 1915 some further comparisons between $F_1$ generations and parental strains were made based upon analysis of hand-pollinated selfed ears. Cross 14–6 × 21–13 was the union of Stadtmueller's High-Protein Leaming (14.09 percent average) and Illinois Low Protein (10.40 percent

TABLE 24

*Effect of crossing upon protein content as shown by the ears produced by first-generation-hybrid plants from crosses between selected protein strains. Analyses made with self-pollinated ears. Plants grown 1915.*

| Parent strains | | Percent protein; average selfed ears | | | | | Yield, bushels per acre |
|---|---|---|---|---|---|---|---|
| ♀ | ♂ | 1915 ♀ | 1915 ♂ | 1914 ♀ | 1914 ♂ | 1915 $F_1$ | $F_1$ |
| 20A–1 | 14–6 | 15.86 | 14.09 | 16.09 | 13.01 | 13.71 | 90 |
| 20A–2 | 14–6 | 15.86 | 14.09 | 16.09 | 13.01 | 14.01 | 93 |
| 14–30–4 | 14–6 | 15.89 | 14.09 | 14.77 | 13.01 | 14.66 | 46 |
| 14–30–12 | 14–8–11 | 16.20 | 14.49 | 14.26 | 13.96 | 14.02 | 47 |
| 14–6 | 14–30–12 | 14.09 | 16.20 | 13.01 | 14.26 | 13.81 | 80 |
| 14–6 | 20A–1 | 14.09 | 15.86 | 13.01 | 16.09 | 13.94 | 72 |
| Average..... | | 15.33 | 14.80 | 14.54 | 13.89 | | 71 |
| Average of parents | | 15.07 | | 14.22 | | 14.03 | |
| Difference between $F_1$ and average of parents ..... | | From 1914 average, −0.19 = 1.33 percent decrease<br>From 1915 average, −1.04 = 6.90 percent decrease | | | | | |

average). The ears of the $F_1$ plants averaged only 9.49 percent protein but the cross was particularly vigorous, and yielded at the rate of 112 bushels per acre. The remaining $F_1$-generation plants tested this year were all high-protein matings. The results are recorded in table 24. The protein ranges are not given, as they are similar to other homologous cultures reported in the paper. One need only note that with high-protein strains crossed together, the protein in the seeds of the $F_1$ ears is below that of the parents. The yields of the $F_1$ plants are so much greater than those of the inbred strains, however, that the protein per acre is much larger.

TABLE 25

*Effect of crossing upon protein content and yield as shown by the ears produced by first-generation-hybrid plants from crosses between selected protein strains. Analyses made with open-pollinated ears. Plants grown 1917.*

| Pedigree numbers | | Yield bushels per acre | | | Percent protein in seeds O. P. | | |
|---|---|---|---|---|---|---|---|
| ♀ parent | ♂ parent | ♀ | ♂ | $F_1$ | ♀ | ♂ | $F_1$ |
| **High × high** | | | | | | | |
| 20A-4-25-36 | 14-4-6-4-7 | 34.6 | 23.2 | 108.6 | 14.21 | 14.96 | 13.02 |
| 20A-4-25-1 | 14-30-6-4-3 | 63.7 | 25.7 | 112.6 | 11.51 | 14.10 | 10.66 |
| 14-4-6-16-7-28 | 20A-4-25-45 | 59.2 | 67.0 | 107.9 | 13.43 | 14.72 | 13.10 |
| 14-4-6-4-7-26 | 20A-4-25-37 | 23.2 | 55.1 | 110.8 | 14.96 | 13.48 | 12.36 |
| 14-4-6-16-7-27 | 20A-4-25-45 | 45.5 | 67.0 | 124.2 | 12.95 | 14.72 | 12.98 |
| Average..... | | 45.2 | 47.6 | 112.8 | 13.41 | 14.40 | 12.42 |
| Average of parents..... | | | | | 13.91 | | |
| Decrease of $F_1$ below average of parents 10.71 percent..... | | | | | | | −1.49 |
| **High × low** | | | | | | | |
| 20A-4-6 | 21-13-2-2 | — | — | 118.2 | — | — | 8.30 |
| 20A-4-25-18 | 21-13-2-11 | 55.1 | 42.1 | 105.9 | 13.48 | 7.07 | 9.05 |
| 14-30-9-8-1 | 21-13-2-11 | 49.1 | 42.1 | 127.5 | 11.12 | 7.07 | 8.43 |
| 14-30-9-8-1 | 21-13-2-11 | 49.1 | 42.1 | (156.4)* | 11.12 | 7.07 | 8.81 |
| 14-6-20-10-7 | 21-13-9-7-1 | 20.3 | 65.2 | 97.2 | 16.75 | 6.63 | 9.36 |
| Average..... | | 43.4 | 47.9 | 112.2 | 13.12 | 6.96 | 8.79 |
| Average of parents..... | | | | | 10.04 | | |
| Decrease below average of parents 12.45 percent..... | | | | | | | −1.25 |
| **Low × high** | | | | | | | |
| 21-13-9-7-5 | 14-4-6-16-2-7 | 56.3 | 45.3 | 101.6 | 6.70 | 11.91 | 8.41 |
| 21-13-9-7-18 | 14-4-6-16-2-12 | 71.7 | 45.5 | 103.6 | 6.41 | 13.99 | 9.25 |
| 21-13-9-7-10 | 14-4-6-16-2-12 | 76.6 | 45.5 | 122.9 | 6.21 | 13.99 | 8.45 |
| 21-13-9-7-7 | 14-4-6-16-2-12 | 72.8 | 45.5 | 120.2 | 6.46 | 13.99 | 8.09 |
| 21-13-2-11-5 | 20A-8-5-10 | 55.8 | 56.3 | 120.0 | 7.07 | 12.93 | 10.49 |
| Average..... | | 66.6 | 47.6 | 113.7 | 6.57 | 13.36 | 8.94 |
| Average of parents..... | | | | | 9.97 | | |
| Decrease below average of parents 10.33 percent..... | | | | | | | −1.03 |

* Calculated from imperfect stand with few plants, probably too high, not included in average.

As we have seen, the protein content of seed matured under bags after hand-pollination is approximately 8 percent higher than that of ears of the same strains after wind-pollination, although the immediate

effect of the pollen is negligible. This factor should be taken into consideration when the remaining comparisons between $F_1$ and parent strains are studied, for these later analyses were all made upon open-pollinated seed.

Fifteen tests were made in 1917,—five each of high protein by high protein, high protein by low protein, and low protein by high protein. The data obtained are in many ways preferable to the comparisons made between hand-pollinated ears. A larger number of ears were used, reducing the error of random sampling; the ears were of a more uniform size; the seeds were more numerous; and the maturation of the seeds was on the whole better. The results, shown in table 25, are simply a corroboration of those obtained earlier.

TABLE 26

*Protein content of two high-protein types and of first, second and third seed generations of crosses between them. Analyses made with self-pollinated ears.*

| Pedigree number | Seed generation | Year grown | Number analyzed | Range of protein percentage | Average of protein percentage |
|---|---|---|---|---|---|
| 14 | $P_1$ | 1912 | 13 | 8.21–18.95 | 12.02 |
| (20–2) | $P_1$ | 1912 | 18 | 11.95–17.10 | 14.87 |
| (20–2) × 14 | $F_1$ | 1912 | 12 | 9.25–15.02 | 11.85 |
| [(20–2) × 14]–3 | $F_2$ | 1913 | 21 | 12.39–15.89 | 14.22 |
| [(20–2) × 14]–8 | $F_2$ | 1913 | 16 | 13.21–16.10 | 14.83 |
| [(20–2) × 14]–3–14Y | $F_3$ | 1914 | 6 | 14.70–16.63 | 15.84 |
| [(20–2) × 14]–3–14W | $F_3$ | 1914 | 5 | 15.05–16.77 | 15.96 |
| [(20–2) × 14]–3–15Y | $F_3$ | 1914 | 5 | 14.75–16.10 | 15.31 |
| [(20–2) × 14]–3–15W | $F_3$ | 1914 | 6 | 14.53–16.81 | 15.33 |

The similarity of the tests within each quintet, makes it necessary to discuss the averages only. We may note first that the $F_1$ ears are always lower in protein than the average of the parental strains, and that this decrease is rather uniform. In actual percent protein, it is highest in the "high × high" crosses and lowest in the "low × high" crosses, yet the difference is only 0.46 percent. When the difference is reckoned on the mean percent protein, the situation changes. The "high × low" crosses show a decrease of 12.45 percent of the percent of protein carried by the parents, with the other two classes showing 10.71 percent and 10.33 percent respectively. Averaging the results, gives an expectancy of a decrease of a little over 11 percent in protein below the average of the parents in any cross between inbred types. There is, as PEARL and BARTLETT (1911) and HAYES (1914) maintained, a semblance of a domi-

nance of low protein, but the matter is not so easy to interpret. The percent of protein in the $F_1$ ears is about the same whether the low protein is the maternal or the paternal parent, and it stands nearer to the low-protein than to the high-protein parent; but when one considers the crosses between high-protein strains, it is evident that this decrease can be interpreted as an effect of heterosis. Consider the yields of the high-protein strains and their hybrids. The extreme vigor of the hybrids causes a yield of more than double the "pure" types. Thus in spite of the lower protein content, the total amount of protein per acre in the hybrids is twice as large as in the parent strains. If then one increases the percent of protein in the "low × high" and "high × low" crosses by 11 percent of the amount found to correct for heterosis, the percent protein in the hybrid would be somewhat closer to that of the high-protein parent (see also table 26).

*Conclusions regarding the inheritance of protein in maize*

There is some advantage in pausing at this point to bring together the odds and ends of data regarding inheritance of protein in maize, before discussing the remainder of the experiments.

In the first place it is perfectly clear that the external conditions, the factors of environment, have such a marked effect on the protein content of maize that it may be raised or lowered as much as 40 percent above or below the total percent produced under average growing conditions. This conclusion may be drawn from a study of change in direction of the protein curve in the high-protein and the low-protein strains grown by the ILLINOIS AGRICULTURAL EXPERIMENT STATION, from the fluctuations from year to year in our own selected strains, from the difference in protein content between hand-pollinated and wind-pollinated ears belonging to the same strain, and from the protein content of two self-pollinated ears from the same stalk.

No doubt the protein content of maize is affected by each and every environmental factor which has an influence on the development of either the plant as a whole or the seed in particular. For example, some lack of nitrogen might appear to affect the development of the stalk and leaves more than the seeds, and some lack of phosphorus might appear to affect the seeds more than the remainder of the plant, but it seems likely that each plays its part in protein synthesis. These various factors cannot have their influences separated and their individual effects described at present, and it probably would not make matters a great deal clearer if this could be done in the rough manner which would neces-

sarily be inherent in such analysis. The factors of environment work together as do the parts of a machine, and though a greater or smaller degree of efficiency in one part of the machine has its effect, the absence of that part stops the machine. There is one thing that may be emphasized, however. A departure from the optimum temperature and moisture at critical periods of the plant's growth appears to overshadow other features in influencing the constitution of the grain. When some of the other conditions are not at their best a plant produces smaller ears or a less number of seeds without there being any great interference with the normal development of the chemical constituents, but let there come a radical diminution in the available moisture or an extreme temperature change after these organs have been laid down normally, and the effect on development is very great. Nevertheless even under such handicaps, it would seem that nearly the normal amount of protein is developed. The percent of protein is influenced, of course, but it is influenced largely through the diminished elaboration of starch.

Taking these facts as we find them, one can realize what great errors may be made in selection. Seeds due to contain 12 percent protein under a hypothetical "normal" environment, may contain anywhere from 9 percent to 15 percent protein because of the conditions under which they develop; mass selection of desirable phenotypes is therefore of less value than with any other character with which we have had experience.

Admitting that proteid variations in maize are to a great extent due to the modifications imposed by a fluctuating environment, one need only study the work of the ILLINOIS AGRICULTURAL EXPERIMENT STATION to realize what a great rôle heredity plays in the matter. Then comes the important question: Can one estimate the number of differentiating hereditary factors involved and describe the method by which they are inherited? If a precise answer to this question is desired, it must be no. But the situation is not as discouraging as this answer indicates. Some definite conclusions can be drawn which are of real practical value.

The number of hereditary factors affecting protein elaboration by which varieties of maize may differ must be large. This is an indefinite statement, it is true; but what is meant is that the facts will hardly admit the presumption that they may one day be analyzed by the assumption of five or six differentiating determiners. Possibly the main factors involved are some such small number, but apparently there are modifiers that may run into the hundreds.

The evidence in the case is indirect; at the same time, it is valid. In the first place there are the data of OSBORNE showing the complexity of

the protein situation. There are several kinds of proteids. The proteids are very different in their chemical nature. And they are distributed throughout the various tissues which go to make up the seed. Second, the work of the ILLINOIS STATION as well as our own investigations show noteworthy physical differences accompanying change in protein content. Numerous different size relations may be obtained between embryo and endosperm, and between the various tissues making up the endosperm. Size of seed may be thirty times as great in one case as in another. Ear size, number of seeds, shape of seeds, etc., each plays its rôle. Third, the ILLINOIS STATION, starting with a single variety previously brought to a considerable degree of uniformity through selection for physical characters, has been nearly twenty-five years isolating their high-protein and low-protein types without coming to the point where there seems to be no hope of further differentiation.

These facts all point to an involved hereditary complex, a large number of multiple factors affecting protein in the species as a whole. On the other hand, the inexact method of work used at the ILLINOIS STATION, makes it unwise to multiply unduly in our imagination the heterozygous factors involved in their material. Such mass-selection experiments might be carried on for many generations without reaching the end desired when only four or five hereditary factors were under consideration. In fact, the great changes in protein content obtained after only two or three generations of selection in our own experiments because of the control of both parents, lead us to believe in a relatively small number of "main" factors. But the number of subsidiary factors,—factors playing minor rôles,—is by no means small.

The mathematical possibilities involved in recombinations of multiple factors (see EMERSON and EAST 1913) is now a matter of common knowledge. Moreover theory has been corroborated by practical results. Tests have been made on scores of animals and plants and the results reported in numerous scientific papers during the past decade. If such a simple scheme of interpretation could be used for the inheritance of protein, at least an outline of the method of transmission could be made without difficulty. But we are confronted with a much more complicated matter than the cases previously described, due to the protein content of the seed being in part in the embryo and in part in the endosperm, as has already been noted. This is a difficulty inherent in breeding all the cereals, yet it is a difficulty that has been overlooked except for passing mention in one or two papers of the senior author.

The basis of all hereditary transmission, in all such breeding work, is of course the zygote which comes into being with the fusion of two gametes, ♀ + ♂; but at the same time that the zygote is formed the endosperm is laid down by three gametes carrying the same qualities, ♀ ♀ ♂. By successive cell divisions the seed is formed. Now 20 percent of the protein of the maize seed is contained in the ♀ ♂ embryo through which all transfer of hereditary qualities is made, while 80 percent of the protein is found in the ♀ ♀ ♂ endosperm which can have no part whatever in hereditary transmission. One can simplify matters to some degree, however, if he keeps in mind that the size of the embryo and the percent of protein it contains were raised but slightly in the early experiments of the ILLINOIS STATION. The notable variations appeared in the endosperm. The problem, therefore, is the mechanism by which a ♀ ♂ embryo transmits characters which are exhibited in a ♀ ♀ ♂ endosperm. Transmission through the zygote presumably is by the usual methods. Gamete formation is typical. Segregation and recombination occur as in other species, and gametes combine to form zygotes by chance.

It would seem as if no argument need be made in favor of the assumption that the seed is the unit and not the ear. The seed is the new organism, and all of our modern biological evidence leads us to suppose that the seed is formed as described above, and that *many different* hereditary possibilities may be contained in the seeds of a single plant. On the other hand this does not preclude the probability that the genetic constitution of the plant on which the seed matures has a marked influence on its size, shape and composition. In a word the phenotype of the seed may be influenced by the mother no matter what is the genetic composition of the individual seed. The uniformity of the seeds of a single ear in shape and size, the comparative lack of variability in composition of the seeds of a single plant lead to this view.

Keeping these fundamental ideas in mind, what conclusions can be drawn from our data?

Only three facts seem to stand out as important. The chemical composition is influenced by heterosis. This influence on the seed is slight but significant, resulting in a somewhat larger size and concurrent decrease in percent of protein. The influence on the seeds borne by hybrid plants is much greater. The plants themselves being more vigorous than those of the parental strains, the seeds they bear are larger and more numerous, and contain a much smaller percent of protein. Second, the influence of the factors borne by a male gamete are practically without immediate

influence on the seed they help to form. Third, the protein content of the seeds of an $F_1$ hybrid, when corrected for the influence of heterosis, is intermediate between that of the two parents with a tendency to be somewhat closer to that of the high-protein parent.

These facts force us to one of two conclusions. Either the prompt reaction of the two maternal nuclei utilized in the inception of the endosperm has a controlling influence on chemical composition; or, the genetic constitution of the mother plant is the major determining factor. We cannot deny an influence to the immediate reactions within the $3x$ endosperm cells of any seed due to their own individual genetic constitution. There is a demonstrable heterosis as an immediate effect of pollination. There is production of pigments,—at least one ether soluble and at least two water soluble. There is change in the physical character of the starch (horny or floury). Therefore the individual genetic constitution of a seed must effect real changes from the very beginning of the life history. One can hardly call these changes radical, however, when compared with those caused by the genetic constitution of the mother plant. There is no adequate reason for supposing the effect of the two maternal nuclei is more than double the effect of the paternal nucleus, and as far as the change in composition is concerned the latter is almost negligible.

By way of parenthesis it may be said here that this conclusion appears to have considerable theoretical importance. BATESON'S work on the inheritance of pollen color and shape, and the work of EAST on pollen color and self-sterility in Nicotiana have shown the genetic constitution of the mother to be the effective agent. The hereditary factors carried by the gametes seem to have no function during the period of gametic generation. They are passive. The activities of the gametes, their size, shape and color, are determined by the mother's complex.

EAST assumed that the individual constitutions of the gametes were negligible during the haploid generation, that their inheritance was held in abeyance until the formation of the zygote, then to come to the fore to play a rôle in the ontogeny of the organisms.

The data cited in this paper, however, appear to point to a delayed use of individual powers, so to speak, even after the zygote is formed. The individuality of the organism seems to gain momentum as the life history progresses. The genetic constitution by which a seed may differ somewhat from its mother, the inherited individuality which it has received, does not become apparent in the early stages of life. The mother still controls during the time the seed is being matured, para-

sitically as it were, on the body of its parent, and presumably during the early stages of independent life while stored nutriment is still being utilized.

Returning to the subject in hand, let us summarize our conclusions. Besides the practical difficulties arising from the influence of varying factors of environment, maize breeding for seed characters, and the breeding of other cereals as well, is complicated by a most exaggerated lack of correlation between individuality and performance, between phenotype and genotype. One must select by the characters possessed, which are largely influenced by the constitution of the mother plant, yet the characters which the adult plant will possess are determined by the union of nuclei *both* of which may differ widely in potentialities from those possessed by the plant on which it grew.

An example will perhaps make this clear. It is wholly theoretical and diagrammatic. Let us suppose that the differential factors between two plants, a high-protein and a low-protein plant, let us say, are represented by independent factors *A*, *B*, *C* and *D*. The high-protein plant is *AA BB CC DD*; the low-protein plant is *aa bb cc dd*. A cross is made reciprocally. Except for a slight decrease in protein content due to heterosis the composition of the $F_1$ seeds follow the mother plant. Seeds *Aa Bb Cc Dd* from the high-protein mother, are high in protein; seeds of the same genetic constitution, *Aa Bb Cc Dd*, from the low-protein mother, are low in protein. In a general selection experiment (starting with unknown pedigrees) one would undoubtedly breed from the former; one could obtain the same end results by breeding from the latter.

Samples from either of these $F_1$ populations are grown. The average protein content of the ears produced is about the same,—lower than the average of the pure strains entering into combination,—because of heterosis. The seeds vary individually in their protein content, but most of this variation is due to size, position on ear, etc. Only a small proportion of the variation is due to the genetic constitution of the individual seeds themselves. Aside from variations due to the extraneous causes mentioned, the ears are fairly uniform. The protein content has followed the mother. Yet by ordinary recombination the productive capacity is manifold. There are eighty-one ($= 3^4$) actual classes, counting both homozygotes and heterozygotes. And the same troubles ensue in later generations, though in a somewhat lesser degree.

This illustration gives food for thought in connection with cereal breeding. One realizes just why the work carried on by the Experiment Stations in cereal breeding has been so comparatively unproductive.

Presumably scientific methods have yielded no better results than earlier empirical methods. The reason is not far to seek. The later methods have been just as blind, just as empirical as the former. It is also clear why the workers at the ILLINOIS STATION misinterpreted the effects of selection. Selection was endowed with a creative power because of the length of time close selection without pollen control could be carried on without eliminating hope of further progress, and because dispersion indices were not reduced when determined on population averages of seeds (ears). This study, we hope, has done something toward clarifying matters by pointing out the source of the difficulties. But this is not all. There is a method of breeding which may be followed by which results can be obtained in a much shorter time Some of the indefiniteness and blindness of the Illinois method can be eliminated. By its use we have obtained some rather remarkable increases in protein content in a few

TABLE 27

*Yields in the chemical-selection experiments of the Illinois Agricultural Experiment Station, 1913–1918. Bushels per acre.*

| Year | High protein | Low protein | High oil | Low oil | Control variety |
|---|---|---|---|---|---|
| 1913 | 30.2 | 35.5 | 31.0 | 23.9 | 39.6 |
| 1914 | 36.2 | 43.3 | 37.4 | 48.7 | 55.2 |
| 1915 | 42.4 | 57.2 | 45.2 | 49.9 | 53.5 |
| 1916 | 14.6 | 29.6 | 16.7 | 19.8 | 28.2 |
| 1917 | 48.9 | 56.3 | 55.9 | 51.3 | 63.9 |
| 1918 | 38.8 | 47.8 | 46.6 | 58.2 | 62.8 |

generations. Furthermore we were able to keep up, and even to increase the yields of the standard varieties used. In the work at the ILLINOIS STATION, the yields were so greatly reduced through inbreeding that they were unprofitable. Their yields for the last six available years are shown in table 27.

We do not maintain that it is desirable to undertake breeding for high protein, or other chemical constituents, as a practical method of increasing food value or industrial utility, but it can be done in the following manner. Self-pollinate large numbers of plants artificially. Test the seeds produced by each individual as accurately as possible by the progeny-plat method. With the continuation of inbreeding if a large enough series be tested, near-homozygous plants having the ability to produce high-protein seeds will be obtained. Some of the crosses between such types will have a high yield and will retain the power to produce large quantities of protein. To be sure the percent

of protein in the vigorous hybrids will not equal the percent in the purified parent strains. But relatively the percent will be high, and actually the protein per acre will be rather remarkable.

## EXPERIMENTS ON BREEDING FOR HIGH PROTEIN

### *Original experiments on selection*

Selection experiments after the pattern of those conducted at the ILLINOIS AGRICULTURAL EXPERIMENT STATION, were begun in Connecticut in 1906. Seed was selected and a number of ears analyzed from three standard varieties, one a dent, the other two flint. Frequency

TABLE 28

*Frequency distribution of the protein in the ears of certain Connecticut-grown varieties of maize. Analyses on open-pollinated ears.*

| Variety | Year | Class centers in percent of protein | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 | 12.5 | 13.0 | 13.5 | 14.0 | 14.5 | 15.0 |
| Stadtmueller's Leaming... | 1906 seed | | | | 10 | 15 | 6 | 4 | 3 | 2 | | | | | |
| | 1907 seed | | | | | | 4 | 6 | 8 | 13 | 10 | 5 | 0 | 0 | 2 |
| | 1908 seed | | | | | | 1 | 3 | 12 | 13 | 3 | 6 | 1 | 1 | |
| | 1908 crop | 2 | 8 | 12 | 18 | 17 | 20 | 21 | 20 | 13 | 4 | 5 | 1 | 1 | |
| Hopson's Longfellow. | 1906 seed | | | | | | | | | 1 | 25 | 11 | 5 | 5 | 1 |
| | 1907 seed | | | | | | | 5 | 9 | 16 | 11 | 6 | 1 | | |
| Sturges's Hybrid..... | 1906 seed | | | | | | 19 | 14 | 26 | 12 | 12 | 7 | 5 | 1 | |

*Note:* Sturges's Hybrid was produced by crossing a North Carolina Dent with King Philip Flint and selecting toward a twelve-rowed flint type. The corn in 1906 after 8 or 10 years of selection was true to the flint type, and 84 ears out of 96 were twelve-rowed. There were 5 with 10 rows, 6 with 14 rows, and one with 16 rows.

distributions constructed from these data are shown in table 28. They are given merely to throw a little additional light on the amount of protein as found in unselected varieties grown in Connecticut.

No protein selections were made on the variety known as Sturges's Hybrid, which was a twelve-rowed flint. Hopson's Longfellow, an eight-rowed flint, was selected again in 1907, and Stadtmueller's Leaming was grown for three years. The results, omitting details, are found in the table.

It was soon found, from discoveries made in an extended investigation on heredity in maize, that this method of procedure was hopeless. It had no scientific basis, and carried with it no prospects of the production

of a variety which would elaborate a high percentage of protein and at the same time give large yields of grain. This work was discontinued, therefore, until 1912 when the experiments on heredity and on the effects of inbreeding and cross-breeding had proceeded far enough to give some idea of the correct methods to pursue. Two lines of breeding were then started, the one a series of selections in self-fertilized lines, the other a series of selections in alternately crossed and selfed lines. They will be described in order.

### *Selections in self-fertilized lines*

Selections in self-fertilized lines were made on four varieties,—Stadtmueller's Leaming, Burwell's Flint, Illinois High Protein and Illinois Low Protein. No great amount of work was done on any one variety, for the expense of such an investigation is considerable and the available resources were small. Nevertheless the data show conclusively that by breeding successively from self-fertilized ears, strains high in protein can be obtained in a relatively short time.

### Stadtmueller's Leaming

Table 29 shows the results obtained between 1912 and 1918 on Stadtmueller's Leaming. The data can be followed easily by referring to the number of the "mother ear" planted. Twenty-seven self-pollinated ears were obtained from planting variety No. 14. These were analyzed. The percent protein varied from 8.21 to 17.86, with an average of 12.03. From the ears highest in protein of this population, five lines were started. The first family in the table descended from ear 14–6. The next selection was ear 14–6–20. The second selection was ear 14–6–20–10. From the population produced by this ear in 1916, two ears were grown. One was ear 14–6–20–10–3, grown in 1917; the other was ear 14–6–20–10–15, grown in 1918.

Passing down the table, the second selection from the population of 1912 was ear 14–30. In 1915, the seeds from two sister ears of 1914 were planted. These are numbered 14–30–4–3 and 14–30–4–4. Thus two lines branch off from ear 14–30–4 in that year, and the ancestors of ear 14–30–4–4 can be followed by referring to the family tabled above.

The major extreme in the population of 1912 was an ear containing 17.86 percent protein. If a larger number of ears had been analyzed, an ear still higher in protein might have been expected. It is clear then that a commercial variety unselected for high protein may contain

TABLE 29

*Selection of Stadtmueller's Leaming for high protein in self-fertilized lines.*

| Subject matter | 1912 | 1913 | 1914 | 1915 | 1916 | 1917 | 1918 |
|---|---|---|---|---|---|---|---|
| Mother ear planted | No. 14 | | 14–6 | –6–20 | –20–10 | –10–3 | –10–15 |
| Protein in mother ear | — | | 17.86 | 16.21 | 15.92 | 17.30 | 16.59 |
| Range S.-P. population | 8.21–17.86 | | 9.87–16.21 | 11.82–15.92 | 13.47–17.30 | — | — |
| No. S.-P. ears analyzed | 27 | | 4 | 11 | 10 | — | — |
| Ave. S.-P. population | 12.03 | | 13.01 | 14.09 | 15.93 | — | — |
| Ave. O.-P. population | — | | 11.61 | 12.69 | 15.09 | 16.75 | 17.07 |
| Mother ear planted | Ditto | 14–30 | –30–4 | –4–3 | –3–7 | –7–11 | –11–18 |
| Protein in mother ear | | 15.80 | 15.59 | 14.93 | 16.18 | 16.47 | 14.74 |
| Range S.-P. population | | 13.14–16.22 | 13.74–15.52 | 14.17–16.68 | 13.39–16.47 | 12.08–15.05 | 14.10–16.75 |
| No. S.-P. ears analyzed | | 10 | 8 | 11 | 10 | 13 | 9 |
| Ave. S.-P. population | | 15.23 | 14.77 | 15.64 | 15.08 | 13.29 | 15.35 |
| Ave. O.-P. population | | 13.46 | 14.78 | 14.44 | 13.11 | 13.47 | 15.33 |
| Mother ear planted | Ditto | Dit o | Ditto | –4–4 | –4–2 | –2–7 | |
| Protein in mother ear | | | | 15.52 | 16.64 | 15.82 | |
| Range S.-P. population | | | | 15.37–16.64 | 12.40–15.92 | 12.45–15.90 | |
| No. S.-P. ears analyzed | | | | 4 | 7 | 8 | |
| Ave. S.-P. population | | | | 16.13 | 14.26 | 14.59 | |
| Ave. O.-P. population | | | | 13.65 | 12.17 | 13.74 | |
| Mother ear planted | Ditto | Ditto | –30–6 | –6–2 | –2–13 | –13–5 | |
| Protein in mother ear | | | 16.22 | 16.03 | 16.16 | 15.90 | |
| Range S.-P. population | | | 15.18–16.29 | 14.49–17.39 | 13.30–15.90 | — | |
| No. S.-P. ears analyzed | | | 4 | 6 | 5 | — | |
| Ave. S.-P. population | | | 15.87 | 16.22 | 14.88 | — | |
| Ave. O.-P. population | | | 11.79 | 15.44 | 12.76 | 14.20 | |
| Mother ear planted | Ditto | Ditto | Ditto | –6–4 | –4–3 | –3–13 | |
| Protein in mother ear | | | | 15.96 | 17.45 | 16.30 | |
| Range S.-P. population | | | | 15.65–17.72 | 15.04–16.30 | — | |
| No. S.-P. ears analyzed | | | | 6 | 7 | — | |
| Ave. S.-P. population | | | | 16.96 | 15.80 | — | |
| Ave. O.-P. population | | | | 15.63 | 10.56 | 14.10 | |
| Mother ear planted | Ditto | Ditto | Ditto | –6–11 | –11–3 | –3–11 | |
| Protein in mother ear | | | | 16.29 | 18.33 | 16.90 | |
| Range S.-P. population | | | | 15.31–18.33 | — | — | |
| No. S.-P. ears analyzed | | | | 7 | 1 | 1 | |
| Ave. S.-P. population | | | | 16.50 | 16.90 | 15.93 | |
| Ave. O.-P. population | | | | 15.59 | 15.29 | 15.27 | |

TABLE 29 (continued)

| Subject matter | 1912 | 1913 | 1914 | 1915 | 1916 | 1917 | 1918 |
|---|---|---|---|---|---|---|---|
| Mother ear planted | No. 14 | 14–30 | −30–9 | −9–8 | −8–1 | −1–6 | |
| Protein in mother ear | — | 15.80 | 15.63 | 15.49 | 14.59 | 16.27 | |
| Range S.-P. population | 8.21–17.86 | 13.14–16.22 | 13.28–15.49 | 12.35–17.46 | 14.67–16.49 | — | |
| No. S.-P. ears analyzed | 27 | 10 | 11 | 8 | 7 | — | |
| Ave. S.-P. population | 12.03 | 15.23 | 14.51 | 14.47 | 15.78 | — | |
| Ave. O.-P. population | — | 13.46 | 11.60 | 13.08 | 11.53 | 11.12 | |
| Mother ear planted | Ditto | Ditto | −30–12 | −12–14 | −14–1 | −1–10 | |
| Protein in mother ear | | | 16.09 | 15.63 | 14.11 | 16.77 | |
| Range S.-P. population | | | 12.67–15.63 | 13.73–18.64 | 12.23–16.77 | — | |
| No. S.-P. ears analyzed | | | 6 | 9 | 10 | — | |
| Ave. S.-P. population | | | 14.26 | 16.20 | 14.68 | — | |
| Ave. O.-P. population | | | 12.13 | 16.31 | 12.09 | 13.81 | |
| Mother ear planted | Ditto | −14–22 | −22–15 | | | −15–1 | −1–29 |
| Protein in mother ear | | 14.83 | 15.43 | | | 15.46 | 15.14 |
| Range S.-P. population | | 11.81–15.43 | — | | | 12.42–15.66 | 14.75–16.39 |
| No. S.-P. ears analyzed | | 16 | 2 | | | 13 | 13 |
| Ave. S.-P. population | | 13.42 | 15.86 | | | 14.47 | 15.83 |
| Ave. O.-P. population | | 13.47 | 12.60 | | | 13.04 | 14.56 |
| Mother ear planted | Ditto | 14–8 | −8–11 | | | | |
| Protein in mother ear | | 14.75 | 15.92 | | | | |
| Range S.-P. population | | 12.78–15.92 | 13.16–14.61 | | | | |
| No. S.-P. ears analyzed | | 11 | 6 | | | | |
| Ave. S.-P. ears analyzed | | 14.41 | 13.96 | | | | |
| Ave. O.-P. population | | 13.20 | 13.33 | | | | |
| Mother ear planted | Ditto | 14–4 | −4–6 | −6–4 | −4–7 | −7–8 | |
| Protein in mother ear | | 14.73 | 15.48 | 14.62 | 16.50 | 15.30 | |
| Range S.-P. population | | 11.90–15.77 | 13.65–14.68 | 14.25–16.95 | 12.90–15.30 | 13.51–14.68 | |
| No. S.-P. ears analyzed | | 14 | 10 | 11 | 17 | 11 | |
| Ave. S.-P. population | | 14.58 | 14.16 | 15.36 | 14.26 | 14.14 | |
| Ave. O.-P. population | | 13.30 | 12.71 | 14.65 | 13.79 | 14.96 | |
| Mother ear planted | Ditto | Ditto | Ditto | −6–16 | −16–2 | −2–12 | −12–47 |
| Protein in mother ear | | | | 14.68 | 15.62 | 15.82 | 14.75 |
| Range S.-P. population | | | | 12.20–15.62 | 11.90–15.82 | 13.63–14.75 | 14.71–15.88 |
| No. S.-P. ears analyzed | | | | 10 | 10 | 10 | 21 |
| Ave. S.-P. population | | | | 14.04 | 14.25 | 14.05 | 15.27 |
| Ave. O.-P. population | | | | 12.42 | 14.89 | 14.34 | 15 00 |

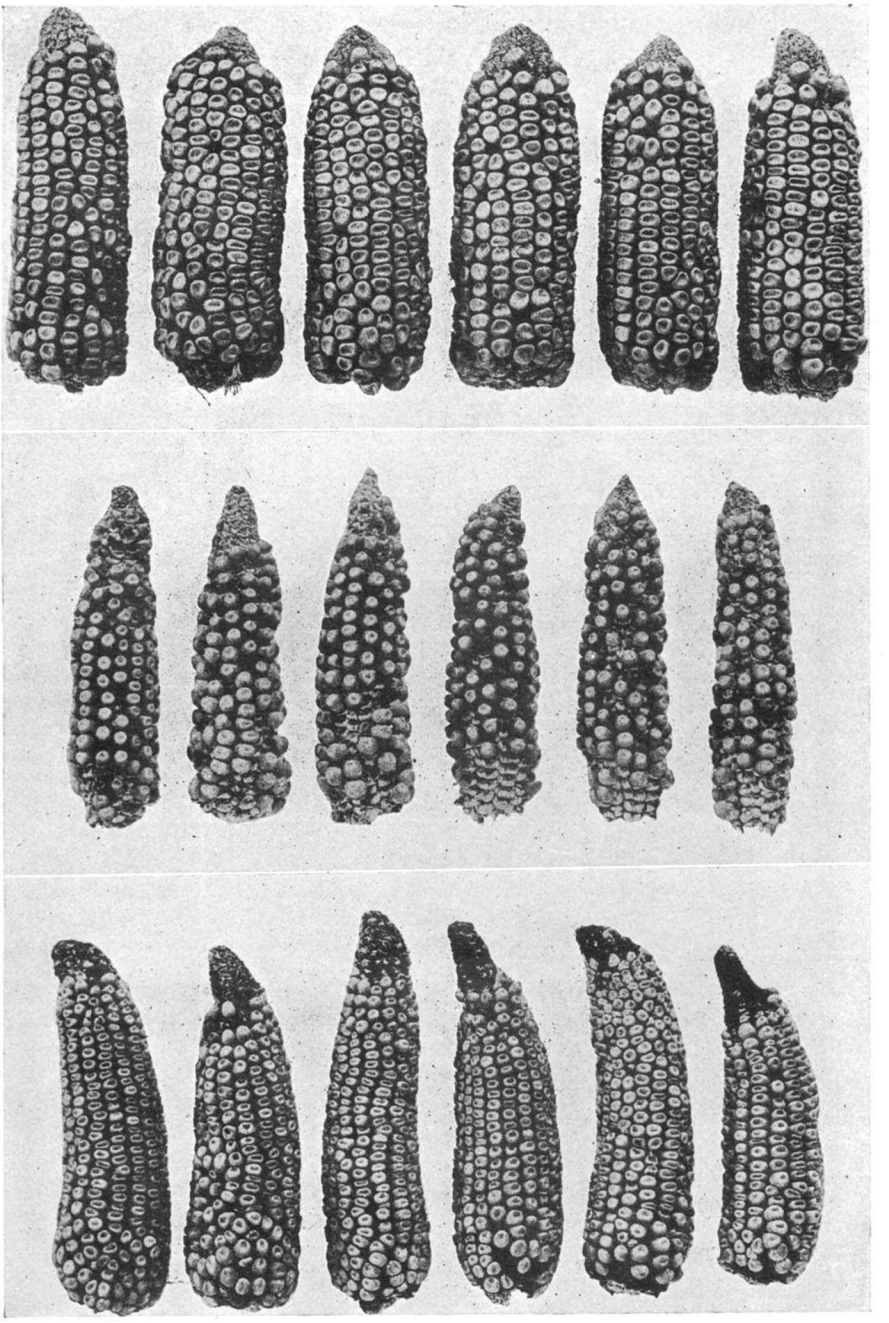

FIGURE 4.—Self-fertilized lines of Stadtmueller's Leaming selected for high protein. Above 14–4; center 14–22; below 14–30.

ears which are very high in protein. And when one considers the fact that these analyses are made on populations of seed in which the parentage of the individual cannot be controlled, there is reason to believe that almost any commercial variety contains hereditary factors which when brought together in a homozygous condition will produce ears as high in protein as those the ILLINOIS STATION has secured after nearly a quarter of a century of mass selection from open-pollinated ears.

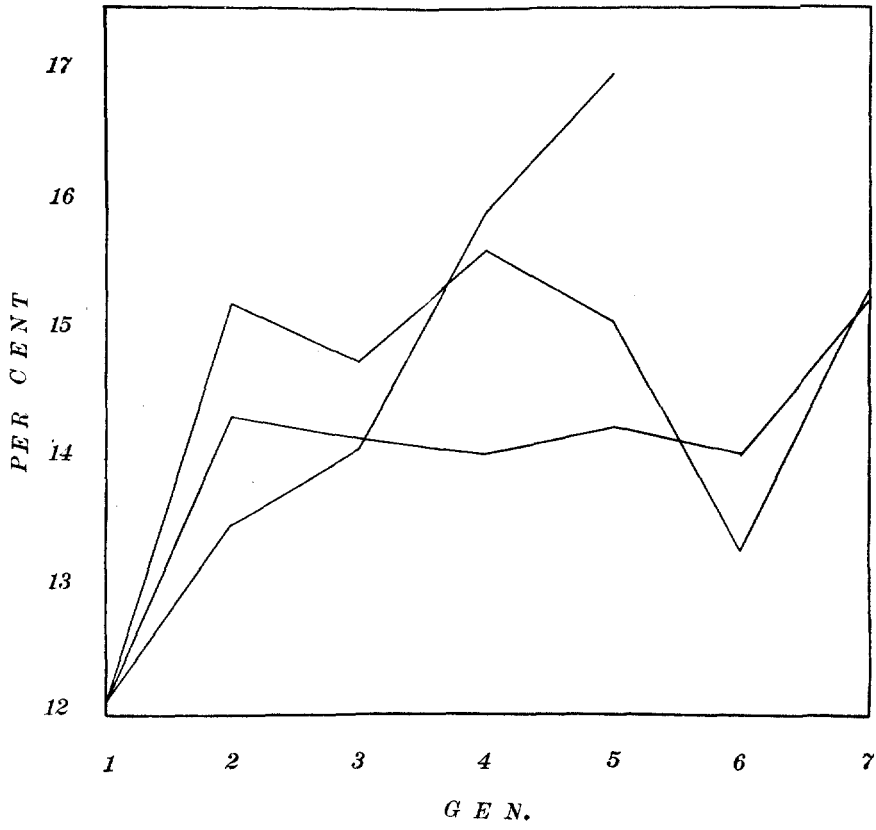


FIGURE 5.—Graphical representation of the results of selecting Stadtmueller's Leaming for high protein in self-fertilized lines.

The highest percentage of protein obtained in any one year's crop at the ILLINOIS STATION was 15.66 in 1916. In 1918 family 14–6–20–10–15 of Stadtmueller's Leaming contained 17.07 percent protein. This amount came as the result of five years of selection. Since the fluctuations from year to year are so wide, one could not rest assured that this strain would continue to produce quite as high a percentage of

protein as this; but the fact that it produced 15.09 percent of protein in 1916, and that a sister ear this crop produced 16.75 percent in 1917, makes the quality of performance of this family rather certain.

Perhaps the next best average selection is found in the family ending in ear 14–30–6–11–3–11. The last three years the crops averaged 15.59, 15.29 and 15.27 percent respectively. Some other families did almost as well, but to these two must be given the prizes for protein production. Reference must be made to table 29, if one really wishes to make a comparative study of the strains. Further description in the text is superfluous if this is done; if not, any description is likely to be inadequate.

## Illinois High Protein

Selections from the Illinois High-Protein strain were grown at various times between 1906 and 1914 from seed kindly sent to us by Professor L. H. SMITH, but not until 1914 were regular analyses made of the ears produced. In that year a mixture of a small quantity of seed from each of a number of ears from the Illinois High-Protein crop of 1913 was grown. Only a few self-fertilized ears were obtained, but of these nine were analyzed. They averaged 16.09 percent, and ranged from 14.97 percent to 16.64 percent. A mixed sample of the open-pollinated ears contained 13.39 percent, which does not indicate a particular aptness for protein production in the Connecticut conditions.

Three selections were grown, two of which were carried on for five years. Table 30 shows the results.

The selfed strains from this material, (including Illinois Low Protein) were better from a developmental standpoint than any of the others included in the experiments. They also reached a comparatively static condition of uniformity more quickly. This was to have been expected, however, for the Illinois strains having been selected through the mother plants rather closely for sixteen generations must have made some approach toward homozygosity in their various characters. Nevertheless it is interesting to note that the experience of the strain during the years of selection in Illinois had not eliminated all possibilities of improvement. In other words, they still exhibited some heterozygosity. This fact may be shown in three different ways. First, there was a reduction in vigor due to the more intense inbreeding of self-fertilization. Second, the two families 20A–4 and 20A–8 are so different from one another that they can easily be distinguished either by the plants or by the ears. No. 20A–8 is smaller in size of plant, earlier to flower, and produces more

abundant pollen. The seeds are more corneous and are scarcely dented at all. Third, the advance in protein content from selection in one of the self-fertilized lines is remarkable. The ratio of selective elimination was very small compared to that of the ILLINOIS STATION, for we did not have the facilities for analyzing large numbers of ears; yet, there is no question but that progress in the isolation of a high-protein strain sped more rapidly after selection by this method was begun. Family 20A-8 was different in this respect from family 20A-4. The curve of the latter family shows little change from year to year. Presumably it was more nearly homozygous in the beginning.

TABLE 30

*Selections of Illinois High Protein for high protein in self-fertilized lines.*

| Subject matter | 1914 | 1915 | 1916 | 1917 | 1918 |
|---|---|---|---|---|---|
| Mother ear planted | 20A | 20A-8 | -8-5 | -5-35 | -35-66 |
| Protein in mother ear | — | 16.64 | 18.32 | 18.56 | 18.60 |
| Range S.-P. population | 14.97-16.64 | 15.32-18.32 | 17.52-18.97 | 13.83-18.60 | 16.35-20.49 |
| No. S.-P. ears analyzed | 9 | 10 | 11 | 10 | 23 |
| Ave. S.-P. population | 16.09 | 16.49 | 18.01 | 16.93 | 18.69 |
| Ave. O.-P. population | 13.39 | 14.90 | 17.30 | — | 15.27 |
| Mother ear planted | Ditto | 20A-11 | -11-10 | | |
| Protein in mother ear | | 16.34 | 16.57 | | |
| Range S.-P. population | | 13.94-16.57 | 15.51-18.56 | | |
| No. S.-P. ears analyzed | | 12 | 11 | | |
| Ave. S.-P. population | | 15.39 | 16.84 | | |
| Ave. O.-P. population | | 14.85 | 17.01 | | |
| Mother ear planted | Ditto | 20A-4 | -4-25 | -25-47 | -47-24 |
| Protein in mother ear | | 16.27 | 16.23 | 16.91 | 16.31 |
| Range S.-P. population | | 14.90-16.23 | 14.93-16.91 | 15.36-16.63 | 15.60-17.91 |
| No. S.-P. ears analyzed | | 10 | 12 | 4 | 22 |
| Ave. S.-P. population | | 15.71 | 16.15 | 16.06 | 16.53 |
| Ave. O.-P. population | | 13.90 | 14.43 | — | 16.75 |

## Illinois Low Protein

The same argument may be made in the case of Illinois Low Protein. The two families raised came from a single open-pollinated ear of 1912. The two lines separate in 1915. Family 21-13-2-11-36 reaches the year 1917 with a protein content of 9.98 percent, but family 21-13-9-7-57-43 in 1918 had only 7.30 percent protein. The latter family was a rather constant low-protein performer, and if it had been possible to analyze a large number of ears one can hardly doubt a still more rapid

drop. The low limit of 6.41 percent obtained in a self-fertilized ear of 1918 is an indication of what might have been expected (see table 31).

It may be worth while to note here that these particular families of Illinois Low Protein were characterized by deficient pollen production. One line was lost because pollen sufficient for producing self-fertilized ears could not be obtained.

TABLE 31

*Selection of Illinois Low Protein for low protein in self-fertilized lines.*

| Subject matter | 1913 | 1914 | 1915 | 1916 | 1917 | 1918 |
|---|---|---|---|---|---|---|
| Mother ear planted.... | 21 | 21–13 | –13–2 | –2–11 | –11–36 | |
| Protein in mother ear.. | — | 9.24 | 10.16 | 8.62 | 7.91 | |
| Range S.-P. population. | 8.94–11.24 | 10.00–11.50 | 8.62–14.18 | 7.91–12.32 | — | |
| No. S.-P. ears analyzed. | 12 | 6 | 11 | 15 | — | |
| Ave. S.-P. population.. | 10.24 | 10.76 | 11.18 | 10.01 | — | |
| Ave. O.-P. population.. | 9.87 | 7.09 | 8.15 | 7.36 | 9.98 | |
| Mother ear planted.... | Ditto | Ditto | –13–9 | –9–7 | –7–57 | –57–43 |
| Protein in mother ear.. | | | 10.80 | 7.81 | 6.68 | 6.89 |
| Range S.-P. population. | | | 7.81–12.81 | 6.68–10.15 | 6.89–11.06 | 6.41–9.28 |
| No. S.-P. ears analyzed. | | | 11 | 16 | 20 | 20 |
| Ave. S.-P. population.. | | | 9.61 | 7.67 | 7.80 | 7.39 |
| Ave. O.-P. population.. | | | 7.20 | 8.22 | 7.38 | 7.30 |

## Burwell's Flint

It has always been the impression among maize-breeders that flint varieties in general average somewhat higher than dent varieties in protein. In fact both the average of all flint varieties and the maximum for flint varieties are somewhat greater than the average and the maximum for dent varieties in JENKINS and WINTON'S (1892) compilation. Moreover our own analyses of Hopson's Longfellow show considerably more protein than those of Stadtmueller's Leaming (table 28). Mindful of this fact selection in self-fertilized lines was undertaken with a standard Connecticut variety known as Burwell's Yellow Flint.

The results (table 32) were not as satisfactory as one might wish. In a series of 19 self-fertilized ears, daughters of self-fertilized ear No. 30, there was only a range of from 7.40 percent to 13.68 percent protein. This was not very promising material. The average of 11.64 percent in self-fertilized ears, probably a percent or two higher than those of the open field, was really lower than that of any flint we have analyzed. But as a means of demonstrating the practicability of the method of breeding, one variety was as good as another, and as this variety was above the average in productiveness, it was used.

Three lines were split off in 1915 and continued for four years. Each of the families yielded to selection but not in the same degree. The banner selection was family 30–1 in which the protein content rose continuously at almost a uniform rate. Based on self-fertilized ears, the average protein content rose from 11.64 percent to 16.23 percent, a gain of 4.59 percent of actual protein content or over 40 percent of the protein originally contained in the variety. Since the range of protein in the 10 selfed ears analyzed in this strain in 1918 was low, and since the maximum was greater than had been found previously in the variety, the prospect of obtaining a really efficient protein producer in Burwell's Flint is thus fairly good.

TABLE 32

*Selection of Burwell's Flint for high protein in self-fertilized lines.*

| Subject matter | 1914 | 1915 | 1916 | 1917 | 1918 |
|---|---|---|---|---|---|
| Mother ear planted | 30 | 30–1 | –1–10 | –10–8 | –8–3 |
| Protein in mother ear | — | 13.68 | 14.67 | 16.51 | 15.36 |
| Range S.-P. population | 7.40–13.68 | 12.74–14.67 | 12.08–16.51 | 12.96–15.56 | 15.81–17.00 |
| No. S.-P. ears analyzed | 19 | 10 | 9 | 10 | 10 |
| Ave. S.-P. population | 11.64 | 13.82 | 14.44 | 14.40 | 16.23 |
| Ave. O.-P. population | — | 12.57 | 13.15 | 14.25 | 15.09 |
| Mother ear planted | Ditto | 30–7 | –7–5 | –5–10 | –10–7 |
| Protein in mother ear | | 13.48 | 14.36 | 14.14 | 13.78 |
| Range S.-P. population | | 11.39–14.36 | 11.70–14.14 | 12.24–13.78 | 13.10–15.31 |
| No. S.-P. ears analyzed | | 10 | 10 | 10 | 13 |
| Ave. S.-P. population | | 13.38 | 13.03 | 13.06 | 13.99 |
| Ave. O.-P. population | | 11.91 | 12.28 | 12.04 | 13.85 |
| Mother ear planted | Ditto | 30–15 | –15–4 | –4–7 | |
| Protein in mother ear | | 13.24 | 14.28 | 15.85 | |
| Range S.-P. population | | 12.59–14.28 | 12.68–15.85 | 13.17–14.97 | |
| No. S.-P. ears analyzed | | 10 | 10 | 8 | |
| Ave. S.-P. population | | 13.29 | 14.28 | 13.80 | |
| Ave. O.-P. population | | 12.45 | 13.80 | 13.83 | |

### *Conclusions regarding selection for high protein in self-fertilized lines*

MENDEL'S original paper showed that the result of self-fertilization without selection on any allelomorphic pair *Aa* is to reduce the number of heterozygotes so that in the *n*th generation the ratio is $1:2^n - 1$. Equal fertility for all plants and random mating of gametes is of course assumed.

EAST and HAYES (1912) generalized this expression, for independent inheritance showing that the probable number of homozygotes and of any particular class of heterozygotes is expressed in the formula

$[1 + (2^r + 1)]^n$, where $r$ is the segregating generation and $n$ is the number of allelomorphic pairs. JENNINGS (1916) stated the same formula somewhat differently.

In 1917 JENNINGS considered the numerical results of breeding when genes are linked, and found that while the formulae are complex, the general result in self-fertilization is to decrease the number of heterozygotes and to increase the number of homozygotes.

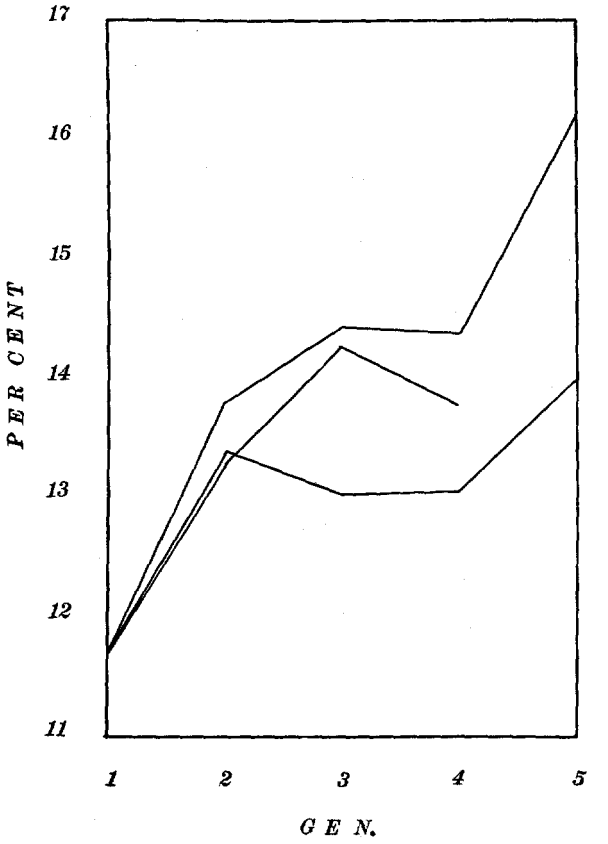


FIGURE 6.—Graphical representation of the results of selecting Burwell's Yellow Flint for high protein in self-fertilized lines.

More recently JONES (1918), by studying the variability of various characters in maize in successive self-fertilized generations, has demonstrated that these mathematical generalizations actually hold in practice.

The data presented here are merely a corroboration of these results on another character complex. There are indeed many complications in the inheritance of protein, as has been emphasized in the preceding pages.

Nevertheless, it can be stated without hesitation that methods of self-fertilization will give high-protein strains of maize in shorter time than any other procedure. We need only call attention, therefore, to one or two practical points in connection with the strains thus produced.

In the first place, there is no direct correlation between protein content and heterosis. In fact, the correlation, if any, is negative. In other words, inbreeding for protein does not have to overcome the obstacles which stand in the way of breeding for yield in that yield is a function of vigor and vigor dependent to some extent on heterosis. On the other hand, inbreeding reduces the vigor, and with it the size of the ear, the size of the seeds and the number of the seeds per ear. Since there is an inverse correlation between size and number of seeds and protein content, the percent of protein actually found in our inbred strains is higher than may be expected in high-yielding strains with the same potential protein production. The same statement holds for such closely bred strains as those produced by the long-continued selection experiments of the ILLINOIS AGRICULTURAL EXPERIMENT STATION, though there the vigor has not been depressed as it would have been by a like number of generations of self-pollination. The facts being as they are, however, we must take them into consideration in any practical method of breeding for high protein, for in order to have any desirability whatever in agricultural practice a strain of maize or any other crop must have a high yield. Without the power of yielding high returns, no commercial variety can survive, be its particular qualities what they may. To try and surmount these difficulties, several experiments were undertaken where the strains under observation were alternately selfed and crossed.

### *Selected matings between high-protein plants*

One of the major difficulties in producing high-protein strains by inbreeding is the lack of a practical method for making rigid selections based upon large numbers. In other words straight selection on a small scale does not begin to exhaust the possibilities inherent in such a variable cross-fertilized plant as maize. Theoretically, one should test out all the extreme individuals in a very large population; but this is impracticable. A partial solution of the problem was found, however, in a plan by which selected high-protein plants were crossed together.

This plan is based upon the plausible assumption that since the various inbred high-protein strains differ in their morphological features, similar protein percentages may be due to different genetic constitutions. The

TABLE 33

*Effects of crossing and selection upon protein content 1914. Analyses of cross-pollinated ears between inbred strains of high-protein corn. Plants grown 1914.*

| Plant number ♀ | × | Plant number ♂ | Percent protein | Crossed ears planted 1915 |
|---|---|---|---|---|
| 14-4-1-6 | × | 14-30-9-7 | 14.66 | |
| -10 | × | 14-6-2 | 13.23 | |
| -8 | × | 14-30-12-8 | 14.67 | |
| -4 | × | 14-6 | 13.42 | |
| -3 | × | 14-6-1 | 14.09 | |
| -9 | × | 14-30-4-16 | 14.07 | |
| -12 | × | 14-8-11-2 | 13.75 | |
| -15-12 | × | 14-8-11-4 | 13.32 | |
| -5 | × | 14-6-7 | 12.89 | |
| 30-4-16 | × | 14-4-1-9 | 12.10 | |
| -6 | × | (20 × 14)-3-14W-1 | 12.90 | |
| -12 | × | 14-6-13 | 15.54* | E |
| -6-8 | × | 14-6 | 13.93 | |
| -9-3 | × | 14-6-3 | 13.80 | |
| -7 | × | 14-4-1-6 | 11.53 | |
| 12-10 | × | 14-6-17 | 13.10 | |
| 12-8 | × | 14-4-1-8 | 12.55 | |
| -16 | × | 14-8-11-6 | 15.31* | F |
| -6 | × | 14-6 | 15.02 | |
| 14-6-3 | × | 14-30-9-3 | 13.78 | |
| -6 | × | 14-30-12-6 | 11.89 | |
| -9 | × | 14-8-11-5 | 12.99 | |
| -8 | × | 14-4-1-3 | 12.77 | |
| -2 | × | 14-4-1-10 | 12.31 | |
| -1 | × | 14-4-1-3 | 15.38 | |
| -7 | × | 14-4-15-15 | 12.59 | |
| -5 | × | 14-30-6-8 | 14.83 | |
| -17 | × | 14-30-12-10 | 10.71* | G |
| -15 | × | 20 | 13.33 | |
| -16 | × | 20 | 9.08* | D |
| -22 | × | (20 × 14)-3-14-12 | 12.17 | |
| -4 | × | (20 × 14)-3-14-1 | 13.15 | |
| 14-8-11-5 | × | 14-6-9 | 14.84 | |
| 20 | × | 14-6-15 | 17.98* | A |
| | × | 14-6-16 | 18.48* | B |
| (20 × 14)-3-14-1 | × | 14 | 15.27 | |
| -1 | × | 14-30-4-6 | 16.37* | C |

* Selected for growing 1915.

procedure was simply to cross different selected high-protein lines, to self-pollinate the first-generation plants, and to select again from the progenies which represent segregating generations. Cross matings were

made between individual plants of several such second-generation families in as large numbers as possible, using each plant both as a male and as a female. Since it had already been determined that the immediate effect of pollination was small, the analysis of the seed was used to indicate the value of the individual in reciprocal crosses. Thus the crosses selected for continuation by self-fertilization were those in which both parents were high-protein performers.

The results from the first year's work carried on in this manner are set forth in table 33. From among the families of Stadtmueller's Leaming (14) and Illinois High Protein (20) which had been selected for high protein in self-fertilized lines during three or more years, thirty-seven crosses were made. The protein contents of the ears produced ranged from 18.48 percent to 9.08 percent,—the minor extreme possibly being

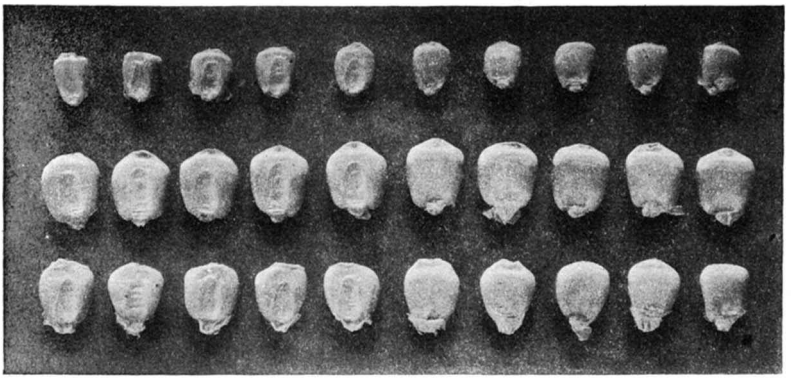

FIGURE 7.—Above, Illinois High Protein; below, Illinois Low Protein; center, $F_1$ generation.

an error of analysis. The results of the analyses are practically the same as they would have been had self-fertilized ears of the mother plants listed been analyzed. Nevertheless, since reciprocal pollinations were made in several cases, it is clear that we were dealing with individuals which had the ability to store up rather large quantities of protein. In 1915, seven of these ears were planted.

Table 34 presents the results of analyzing ten selfed ears from the vigorous plants produced by these high-protein extremes. On the whole there is not much choice in the various lots. Lot C with ears ranging from 16.32 percent to 13.99 percent is the best. Its average protein content was 15.33 percent, not a bad average, though 1 percent lower than the parent ear.

TABLE 34

*Effects of crossing and selection upon protein content. Analyses of first-generation crosses between high-protein strains. Plants grown in 1915.*

| Pedigree number | Desig-nation | Range of percent protein in seeds of individual selfed $F_1$ plants — Highest ear | | | | | | | | | Lowest ear | Aver-age | Percent protein of parent ear |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 × (14–6–15) | A | 15.86* | 14.81 | 14.36 | 14.26 | 14.22 | 14.17 | 13.71 | 13.45 | 12.81 | 12.46 | 14.01 | 17.98 |
| 20 × (14–6–16) | B | 16.50* | 14.81 | 14.69 | 14.44 | 13.59 | 13.29 | 13.04 | 13.04 | 12.07 | 11.61 | 13.71 | 18.48 |
| (20 × 14)–3–14W–1) × (14–30–4–6) | C | 16.32* | 16.14 | 15.78 | 15.52 | 15.47 | 15.14 | 15.04 | 14.99 | 14.88 | 13.99 | 15.33 | 16.37 |
| (14–6–16) × (20) | D | 16.38* | 15.29 | 15.08 | 14.69 | 14.21 | 14.10 | 14.02 | 12.64 | 12.41 | 10.57 | 13.94 | 9.08 |
| (14–30–4–12) × (14–6–13) | E | 15.92* | 15.48 | 15.08 | 15.17 | 14.94 | 14.63 | 14.49 | 13.86 | 13.68 | 13.39 | 14.66 | 15.54 |
| (14–30–12–16) × (14–8–11–6) | F | 15.13* | 14.89 | 14.62 | 14.59 | 13.95 | 13.69 | 13.38 | 13.36 | 13.34 | 13.23 | 14.02 | 15.31 |
| (14–6–17) × (14–30–12–10) | G | 15.55* | 14.57 | 14.54 | 14.52 | 13.96 | 13.55 | 13.52 | 13.28 | 12.42 | 12.20 | 13.81 | 10.71 |

* Selected for growing 1916.

TABLE 35

*Effect of crossing and selection upon protein content. Analyses of cross-pollinated ears grown on $F_2$ plants from the highest $F_1$ ears. Plants grown in 1916.*

| Plant number ♀ ♂ | Percent protein | Plant number ♀ ♂ | Percent protein | Plant number ♀ ♂ | Percent protein | Plant number ♀ ♂ | Percent protein | Plant number ♀ ♂ | Percent protein | Plant number ♀ ♂ | Percent protein | Plant number ♀ ♂ | Percent protein |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A2 × B2 | 14.16 | B1 × C3 | 16.65 | C3 × B1 | 14.73 | D2 × A3 | 16.55 | E2 × D6 | 14.26 | F2 × D6 | 17.10 | G1 × F2 | 12.50 |
| A4 × D2 | 14.77 | B2 × A4 | 18.57* | C7 × B7 | 14.64 | D4 × A6 | 11.44 | E4 × D6 | 13.30 | F3 × D6 | 15.74 | G2 × F2 | 12.10 |
| A5 × C7 | 14.88 | B3 × C7 | 16.78 | C10 × B7 | 11.07 | D6 × A5 | 9.76 | E6 × D6 | 13.58 | F4 × D6 | 15.49 | G3 × F2 | 14.86 |
| A6 × D2 | 14.15 | B4 × C10 | 13.90 | C11 × B7 | 10.75 | D9 × B10 | 9.78 | E8 × D4 | 9.28 | F5 × A14 | 10.93 | G15 × F15 | 10.74 |
| A8 × D2 | 12.13 | B6 × C42 | 16.82 | C13 × B20 | 14.99 | D10 × B10 | 9.43 | E16 × C22 | 14.26 | F13 × A8 | 14.28 | G17 × F15 | 13.58 |
| A10 × D2 | 15.41* | B7 × A4 | 16.22 | C22 × B20 | 11.45 | D12 × A5 | 9.34 | | | F15 × A8 | 12.68 | G19 × F15 | 10.25 |
| A11 × D2 | 14.74 | B9 × A4 | 17.70 | C23 × B20 | 18.16* | D15 × A4 | 15.28 | | | F17 × A8 | 15.05 | G21 × F15 | 14.88 |
| A13 × D4 | 15.75 | B10 × A23 | 15.75 | C27 × B7 | 13.39 | D18 × A11 | 15.21 | | | F19 × A8 | 16.46 | G31 × F29 | 10.73 |
| A14 × D18 | 11.81 | B12 × A23 | 12.83 | C28 × B22 | 12.21 | D21 × A11 | 16.26 | | | F21 × A8 | 10.74 | G39 × F31 | 12.10 |
| A15 × D4 | 17.43 | B16 × D15 | 16.38 | C42 × B22 | 12.01 | D22 × A11 | 14.09 | | | F22 × A8 | 12.66 | G40 × F31 | 15.08 |
| A17 × D18 | 11.12 | B20 × D15 | 18.35* | | | D24 × A11 | 15.15 | | | F23 × A8 | 15.79 | | |
| A18 × D18 | 13.43 | B22 × C42 | 11.19 | | | D25 × A15 | 11.67 | | | F27 × C27 | 13.14 | | |
| A19 × D18 | 14.56 | B27 × A25 | 17.00 | | | D26 × A15 | 12.61 | | | F29 × O.P. | 12.02 | | |
| A22 × B2 | 15.23* | B28 × A25 | 16.04 | | | D27 × A15 | 16.35* | | | F31 × G32 | 13.23 | | |
| A23 × D18 | 14.66 | B38 × C42 | 12.62 | | | D29 × G19 | 15.60 | | | | | | |
| A25 × D18 | 13.39 | B41 × A46 | 16.15* | | | D30 × A18 | 12.79 | | | | | | |
| A26 × B2 | 16.21* | | | | | D31 × G31 | 15.92 | | | | | | |
| A28 × D21 | 15.81* | | | | | D34 × A4 | 16.72 | | | | | | |
| A36 × D18 | 17.85* | | | | | D35 × A36 | 12.13 | | | | | | |
| A38 × D30 | 16.69 | | | | | D39 × A36 | 14.79 | | | | | | |
| A41 × D18 | 16.07 | | | | | D41 × A4 | 16.14 | | | | | | |
| A46 × D30 | 15.71 | | | | | D43 × A41 | 9.72 | | | | | | |
| A47 × D35 | 16.46 | | | | | | | | | | | | |
| A48 × D35 | 17.35 | | | | | | | | | | | | |
| Average | 14.99 | Average | 15.81 | Average | 13.34 | Average | 13.49 | Average | 12.94 | Average | 13.95 | Average | 12.68 |
| Parent A | 15.86 | Parent B | 16.50 | Parent C | 16.32 | Parent D | 16.38 | Parent E | 15.92 | Parent F | 15.13 | Parent G | 15.55 |

* Selected for growing 1917 based upon the protein content of both parents.

The major extreme of each lot was planted in 1916, and a series of crosses made between the resulting plants (see table 35). The protein content was determined on 101 of these crosses. As mother plants, lot B proved to be the best,—the average for the ten ears tested being 15.81 percent. The second highest average came from lot A used as mothers, —an average of 14.99 percent based on 24 ears. Two ears having over 18 percent protein were obtained, and ears with over 17 percent were numerous. Those ears marked with an asterisk were planted the spring of 1917.

Several ears were selfed from each of these ten selections. The analytical results are shown in table 36. The columns of the table are in descending order of the protein content of the parent ears. If now we note the order of the average protein content of the offspring we find it is thus: 1, 5, 4, 10, 2, 6, 7, 3, 8, 9. In other words the plus half of the parents produced four-fifths of the plus half of the offspring, and the minus half of the parents likewise produced four-fifths of the minus half of the offspring. The parent-offspring correlation is so high that one cannot doubt the great influence of heredity on the result. The general result is that three strains of corn have been produced after seven years' work, one averaging 16.31 percent, the second averaging 15.42 percent, and the third averaging 15.05 percent in protein. They have considerable vigor, give fair yields of grain, and at the same time are as high in protein as most of the straight self-fertilized families. In these strains, moreover, there is a reserve of genetic variability out of which it is possible theoretically to carry the percent of protein to a still higher level.

The two ears highest in protein content from family (C23 × B20) were planted in 1918 (see table 37). From the plants of lot (C23 × B20) −8, the fifteen selfed ears obtained averaged 17.68 percent protein. The average was thus higher than the parent ear by a slight margin. The majority of the ears were over 18 percent in protein, and one reached the extraordinary figure of 20.14. Here, then, was a fairly high-yielding partially inbred strain which in the single year of 1918 probably averaged over 16 percent in protein in the ordinary field run of ears.

The second lot of plants, daughters of lot (C23 × B20) −6 was not quite as good as the other, still an average for fifteen selfed ears of 16.39 percent protein would have been considered exceptional had the sister strain not been in existence. Four of the fifteen ears were over 18 percent in protein.

Two other lots of seed were grown as checks. No. 170 was a seed mixture taken from the three highest ears of *each* of the ten selections

TABLE 34

*Effects of crossing and selection upon protein content. Analyses of first-generation crosses between high-protein strains. Plants grown in 1915.*

| Pedigree number | Desig-nation | Range of percent protein in seeds of individual selfed $F_1$ plants — Highest ear | | | | | | | | Lowest ear | | Aver-age | Percent protein of parent ear |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 × (14–6–15) | A | 15.86* | 14.81 | 14.36 | 14.26 | 14.22 | 14.17 | 13.71 | 13.45 | 12.81 | 12.46 | 14.01 | 17.98 |
| 20 × (14–6–16) | B | 16.50* | 14.81 | 14.69 | 14.44 | 13.59 | 13.29 | 13.04 | 13.04 | 12.07 | 11.61 | 13.71 | 18.48 |
| (20 × 14)–3–14W–1) × (14–30–4–6) | C | 16.32* | 16.14 | 15.78 | 15.52 | 15.47 | 15.14 | 15.04 | 14.99 | 14.88 | 13.99 | 15.33 | 16.37 |
| (14–6–16) × (20) | D | 16.38* | 15.29 | 15.08 | 14.69 | 14.21 | 14.10 | 14.02 | 12.64 | 12.41 | 10.57 | 13.94 | 9.08 |
| (14–30–4–12) × (14–6–13) | E | 15.92* | 15.48 | 15.08 | 15.17 | 14.94 | 14.63 | 14.49 | 13.86 | 13.68 | 13.39 | 14.66 | 15.54 |
| (14–30–12–16) × (14–8–11–6) | F | 15.13* | 14.89 | 14.62 | 14.59 | 13.95 | 13.69 | 13.38 | 13.36 | 13.34 | 13.23 | 14.02 | 15.31 |
| (14–6–17) × (14–30–12–10) | G | 15.55* | 14.57 | 14.54 | 14.52 | 13.96 | 13.55 | 13.52 | 13.28 | 12.42 | 12.20 | 13.81 | 10.71 |

* Selected for growing 1916.

TABLE 37

*Effect of crossing and selection upon protein content. Analyses of selfed plants of the progenies of two ears highest in protein grown 1917 and of sib-pollinated plants from two mixtures of high-protein ears grown 1916. Plants grown in 1918.*

| Plant number | Percent protein | Plant number | Percent protein |
|---|---|---|---|
| (C23 × B20)-8-3 | 20.14 | (C23 × B20)-6-7 | 18.54 |
| -8 | 19.28 | -13 | 18.41 |
| -2 | 19.11 | -6 | 18.30 |
| -15 | 19.04 | -10 | 18.18 |
| -7 | 18.53 | -8 | 17.89 |
| -6 | 18.47 | -3 | 17.41 |
| -5 | 18.46 | -9 | 17.28 |
| -12 | 18.03 | -15 | 16.71 |
| -9 | 17.94 | -5 | 16.14 |
| -11 | 17.64 | -11 | 15.63 |
| -13 | 17.04 | -4 | 15.21 |
| -10 | 16.88 | -1 | 14.39 |
| -4 | 15.76 | -14 | 14.18 |
| -14 | 15.06 | -2 | 13.82 |
| -1 | 13.87 | -12 | 13.74 |
| Average self..... | 17.68 | | 16.39 |
| Parent ear..... | 17.59 | | 17.25 |

| Plant number | Percent protein | Plant number | Percent protein |
|---|---|---|---|
| 170*-90 | 17.83 | 171†-40 | 18.93 |
| -110 | 17.24 | -90 | 18.77 |
| -120 | 17.17 | -50 | 18.36 |
| -140 | 16.87 | -130 | 17.56 |
| -70 | 16.55 | -120 | 17.35 |
| -30 | 16.34 | -20 | 16.82 |
| -10 | 16.29 | -140 | 16.66 |
| -50 | 15.77 | -60 | 16.61 |
| -80 | 15.64 | -80 | 16.43 |
| -60 | 15.62 | -150 | 15.80 |
| -100 | 15.82 | -30 | 15.56 |
| -130 | 14.58 | -110 | 15.05 |
| -40 | 13.21 | -70 | 14.44 |
| -20 | 12.79 | -10 | 12.50 |
| | | -100 | 12.46 |
| Average sib-pollinations... | 15.84 | | 16.22 |

* 170 = mixture of three highest ears of 10 selections grown in 1917.

† 171 = mixture of one ear each of the 3 highest of 10 selections grown in 1917.

grown in 1917. The average protein content was 15.84 percent. The second lot, No. 171, was a seed mixture taken from one ear each of the three *highest* of the ten selections of 1917. The average protein content was 16.22. Thus again heredity shows its ruling hand. Selections from the plants having the higher protein contents, produced plants giving the higher protein contents.

### *Conclusions regarding breeding for high protein*

For one acquainted with that vast reservoir of genetic variability—the maize plant—emphasis as to its breeding possibilities has an empty

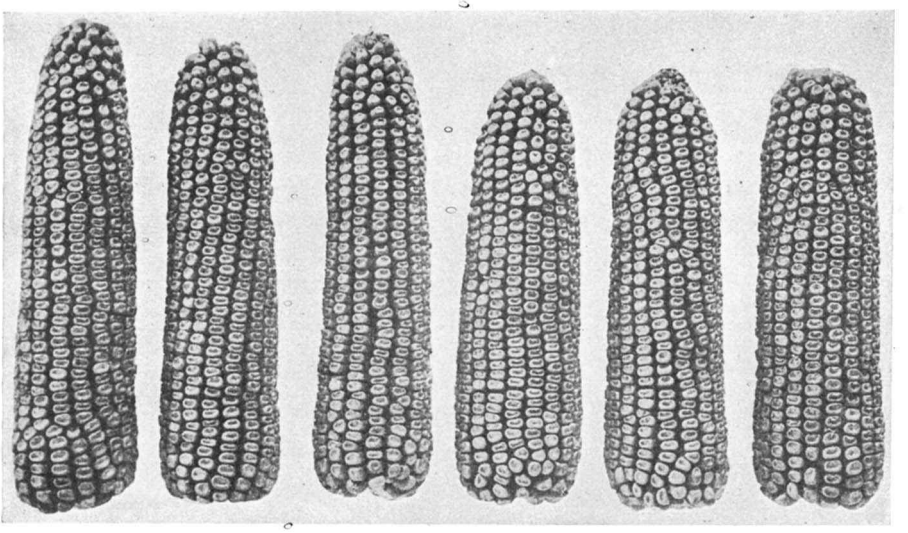

FIGURE 8.—Ears of $F_1$ hybrid plants (14–30 × 14–6), a cross between two families of Stadtmueller's Leaming selected for high protein. Average protein content, selfed ears, 14.66 percent.

sound. It is sufficient to say that no one knows the limits of progress when breeding to increase or to decrease any one of its characters. What we have to say regarding breeding for high protein, therefore, concerns breeding methods rather than breeding limits.

High-protein maize can be secured in the shortest possible time and with a minimum expenditure of effort only when selection is based upon an accurate control of the true biological units and when the germ-plasm contributed by each sex is given due consideration and equal opportunity of expression. In practice the basis of such a method is self-fertilization.

The results obtained from continued selection for high protein in self-fertilized lines depend almost exclusively upon the heredity of the original

plants chosen as progenitors. Unless adequate possibilities for recombination are thus present, no amount of selection can create the qualities sought, since there is no evidence of frequent mutation. Obviously the chances for success do not depend wholly upon the number of individuals used, and the rigidity of selection. External conditions must be such as will bring out the highest expression of the desired character, and correlation between personal characteristics and genetic constitution must be fairly high; but given these conditions, progress depends upon the magnitude of the operations at the beginning rather than at the end.

In reality this statement is but a rephrasing of old genetic postulates, and their application to the specific problem of breeding maize for high protein. But what of the result? A high percentage of protein may be produced by this method with certainty and rapidity; yet high percentage composition does not insure high production per unit of area. There is a certain amount of antagonism between high yield and high protein; and even if this were not the case, selection for one character alone would tend to be at the neglect of the other. Merely as a matter of probability it would be more difficult to secure a high proportion of certain ingredients together with high yield than it would be to secure either alone. But the truth is that inbred strains showing the highest percent protein are weak and unproductive. As a rule high-protein strains are less vigorous than strains not so selected, and crosses between them generally give lower yields than other crosses. It may well be, therefore, that high-protein maize can be secured only at the expense of maximum total production. Whether it is worth while to produce special types of maize with increased proportions of certain ingredients in spite of their reduced yields, need not be discussed here.

Such results as are possible can be obtained most easily, we think, by combining strains obtained by self-fertilization and selecting again from the recombinations obtained. Protein content is due to a large number of inherited factors; and various strains having the same percentage composition probably differ in respect to the factors inherited, so that there is a real chance for progress in their union. Since at the same time such a method increases productiveness by means of heterosis it thus serves two purposes.

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